

ABSTRACT

BECKER, SID MARTIN. In Vivo Electroporation of Skin and Biological Tissue: Theoretical Model Development and Numerical Investigation of Associated Thermo-Electrical and Structural Responses and Enhanced Mass Transport. (Under the direction of Dr. Andrey V. Kuznetsov.)

Electroporation is an approach used to enhance the transport of large molecules to the cell cytosol in which a targeted tissue region is exposed to a series of electric pulses. The cell membrane, which normally acts as a barrier to large molecule transport into the cell interior, is temporarily destabilized due to the development of pores in the cell membrane.

Consequently agents that are ordinarily unable enter the cell are able to pass through the cell membrane. Similarly, electroporation of the skin enhances enhance transdermal transport by temporarily destabilizes the structure of the outer skin layer, the stratum corneum, by creating microscopic pores through which agents, which ordinarily are unable to pass into the skin, are able to pass through this outer barrier.

In this thesis, transient finite volume models of *in vivo* parallel plate electroporation of homogenous tissue and the composite layers of the skin are developed and used to further develop the understanding between the underlying relationships between the physical parameters involved with tissue electroporation and the thermal-electric response.

To study the localized effects of skin electroporation, a model of thermally induced electroporation pore development is developed in which stratum corneum lipid phase transitions are modeled as a series of melting processes. The results confirm that applied voltage to the skin results in high current densities within the less resistive regions of the stratum corneum.

In Vivo Electroporation of Skin and Biological Tissue: Theoretical Model Development and
Numerical Investigation of Associated Thermo-Electrical and Structural Responses and
Enhanced Mass Transport

by
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A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

Mechanical Engineering

Raleigh, North Carolina

2007

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Dedication

For Bernie, the little brother I look up to.
You are my hero and best friend. I love you, and am proud of you.

Biography

Sid Becker was born in West Germany in 1972. He began his education at North Carolina State University in 1990, but took a prolonged break from the University in the years 1992-2000 during which he was a painter, carpenter, landscaper, French Quarter bartender, waiter, cook, and finally a grocery store shelf stocker at which point he decided that maybe it wouldn't be such a bad idea to see if the University was still an option. During his first semester in graduate school two years later he met Dr. Andrey Kuznetsov, who helped to convince Sid that a doctorate was a good direction to take.

Acknowledgments

I would like to thank my boss, advisor, and friend, Dr. Andrey Kuznetsov for his guidance and patience. He is a prolific writer and by his fearless research methods, he has shown me that much of science is learned by the presentation of research.

I thank my friends at the Westminster group for helping me take it one day at a time.

I am glad for the friendships of my fellow students: Dan Cassidy who taught me how to model melting processes, gave me a big fish tank and his teaching notes, and has been otherwise very good to me, Hemanth Kolera-Gokula who taught me about his culture and was the first to finish inspiring the rest of us, Bhargav Ranganath whose witty and perceptive observations of American culture spread his good cheer and natural enthusiasm when things got difficult, and Sean Danby with whom, no matter how bad the circumstances, I was always able to return to a solid K-5 humor level. Last I would like to thank Michael Lane who has been a very good friend to me: he spent hours with me on the telephone listening and talking about life, invited me into his home for Christmas, and shared his insights into the fundamental tie between generations, perceptions, and design.

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1 Introduction

1.1 Targeted drug delivery

Traditional systemic drug delivery methods often result in low efficacy and unintentional treatment of healthy tissue. Oral intake may result in drug denaturation in the body's gastrointestinal tract: drugs consisting of water soluble molecules may degrade in the digestive tract prior to reaching intended locations of treatment. Injection of drugs into the circulatory system results in a lower selectivity level of the region of drug influence. For drugs with a high toxicity, for example those used in chemotherapy, systemic delivery is associated with a high risk of unintentional damage to healthy tissues. Targeted drug delivery refers to any method of drug administration designed to ensure and enhance a drug's transfer rate to specific location (rather than the entire body as in systemic delivery). A great deal of success has been clinically demonstrated using non-traditional methods of targeted drug delivery involving application of electric currents to tissue in order to increase drug efficacy. This thesis concerns the numerical modeling of one such delivery method, electroporation.

1.2 Electroporation

Electroporation in its most general sense is an approach used to enhance the delivery of large molecules to the cell cytosol in which the cell is exposed to a series of intense electrical pulses. The cell membrane, which normally acts as a barrier to large molecule transport into the cell interior, is temporarily destabilized due to the development of pores in the cell membrane [1-5]. Consequently agents that are ordinarily unable enter the cell are able to pass through the cell membrane.

1.3 Skin electroporation

The thin ($10 - 20 \mu m$) outermost layer of the skin, the stratum corneum (SC), acts as a barrier inhibiting transdermal drug delivery. The SC is composed of flat dead cell shells, called corneocytes, which are interconnected by a lipid lamellar bilayer structure in a crystalline-gel phase. The barrier function of the SC is primarily associated with this lamellar lipid structure in the extracellular space of the SC [6, 7]. Skin electroporation has been shown to greatly increase the success of transdermal delivery of large molecules [8-10]. During skin electroporation, the skin is exposed to a series of intense electric pulses in order to change the structure of the SC by creating microscopic aqueous pores [11-13]. The breakdown of the SC barrier allows for increased molecular transport and radically reduces the electrical resistance of the skin [11], [14-17]. Although the primary effects of electroporation are these microscopic transient pores, under certain pulse parameters secondary effects occur resulting from Joule heating and the associated highly localized regions of elevated temperatures. At temperatures around $70^{\circ}C$ the SC lipid lamellar structure is compromised [18-22] and subsequently the barrier function of the SC is dramatically reduced [19, 23]. It is recognized that localized Joule heating associated with electroporation is likely to contribute to increased permeability of the SC by lipid chain melting [9], [24-27].

1.4 Thesis chapter format

This thesis has been written following the historical progress representing the five stages of the underlying research. Each of these five stages resulted in a different publication, and the chapters have been designed in a similar manner to that of the journal articles. The overall result of this format is that each of the thesis chapters 2-6 may be read independently without the reader having to reference previous sections. In the second chapter, a numerical model of simple homogenous tissue plate electroporation is developed and presented. The effects of

pulse parameter and electrode spacing on transient temperature, electrical and potential thermal damage are investigated. Subsequent chapters focus on more complex skin electroporation. In Chapter 3 a composite model representing the different layers of the skin is implemented into the thermal-electrical model. Thermal damage and the electrical-thermal solutions are presented and compared are made between composite and homogenous tissue models. The fourth chapter presents and justifies a theoretical method of reducing thermal damage associated with skin electroporation. The fifth and sixth chapters are devoted to small scale effects of skin electroporation. Chapter 5 introduces a numerical model that captures localized thermal-electric effects of skin electroporation; specifically it incorporates a model of thermal lipid phase transformation in the development of an electroporation pore. The sixth chapter incorporates mass transport in a developing electroporation pore by including the Nernst-Planck equation and substantiates using chemical enhancers on the skin in order to lower the *SC* lipid phase transition temperatures.

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2 Numerical modeling of *in vivo* plate electroporation thermal dose assessment

Electroporation is an approach used to enhance the transport of large molecules to the cell cytosol in which a targeted tissue region is exposed to a series of electric pulses. The cell membrane, which normally acts as a barrier to large molecule transport into the cell interior, is temporarily destabilized due to the development of pores in the cell membrane.

Consequently agents that are ordinarily unable enter the cell are able to pass through the cell membrane. Of possible concern when exposing biological tissue to an electric field is thermal tissue damage associated with joule heating. This chapter explores the thermal effects of various geometric, biological, and electroporation pulse parameters including the blood vessel presence and size, plate electrode configuration, and pulse duration and frequency. A three-dimensional transient finite volume model of *in vivo* parallel plate electroporation of liver tissue is used to develop a better understanding of the underlying relationships between the physical parameters involved with tissue electroporation and resulting thermal damage potential.

2.1 Introduction

Electroporation is a technique in which the cell membrane is altered in order to increase permeability into the cell cytosol by exposing a cell or group of cells to an electric field in which the transmembrane potential exceeds a critical threshold [1-5]. There are many interesting and useful applications of this phenomenon such as gene therapy, cell and tissue manipulation (fusion), protein insertion, and drug delivery. In dealing with *in vivo* biological tissues exposed to an electric field it is important to consider the temperature rise associated with joule heating. The severity of this temperature rise is dependant upon electric pulse input parameters and tissue electrical properties. In some cases, such as electrochemotherapy, pulse parameters are such that there are negligible changes in temperature. Electrochemotherapy incorporates electroporation with chemotherapeutic drugs such as bleomycin, and has been shown to be an effective technique in treating tumors *in vivo* [6,7]. Clinical electroporation pulse parameters are typically: 4-8 short (99 μ s), intense (1-1.3 kV/cm) pulses at 1Hz frequency [6, 8-13]. Due in part to the short pulse duration the resulting temperature rises and associated thermal damage to the tissue are negligible. For large molecule electroporation such as electro gene and DNA transfer longer pulses are required to utilize electrophoretic effects [3, 14 – 16]. Pulse parameters in some such cases may result in noticeable tissue heating. In [17] an *in vivo* study is conducted of electrogenetransfer to rat liver cells, in which it is shown that the highest rate of gene transfer occurs at locations on the border between necrotized and surviving cells. In [18] it is shown that the amount of gene transfer is strongly tied to the amount of heat generated (below the threshold of tissue damage). It appears that for large molecule *in vivo* electroportation,

insight into some of the parameters affecting thermal damage could be helpful to experimentalists.

An innovative application of electroporation is proposed in [19] in which irreversible electroporation (which requires longer, more intense pulses than reversible electroporation) is used to induce non-thermal killing of cancerous tissue. In that paper, a numerical study is conducted exploring electroporation parameters that maximize irreversible electroporation while minimizing thermal damage in order to isolate cell killing by electroporation from cell killing by heating.

There are many physical influences on the temperature distribution of *in vivo* electroporation such as the presence of a blood vessel, external convective influences, metabolic heat generation, perfusion influences, and electrode geometry and spacing. In [20] one and two dimensional finite element models are implemented in order to gain useful insight of the temperature related effects of these influences. This chapter uses a transient three dimensional finite volume model to further investigate relevant parameter influences on the temperature distribution and thermal tissue damage.

2.2 Model geometry

In electroporation electric pulses are delivered to a target area either by plate or pin electrodes. A great deal of interest has been shown in the effects of electrode configuration and placement [21, 22]. This chapter considers internal parallel plate electroporation in which an incision is made and the electrodes are in direct contact with the targeted tissue (assumed here to be rat liver). Figure 2.1 shows the geometric representation of the model configuration. A small section of liver tissue is raised and two thin electrodes are positioned on either side of the target region (which is near the axial center of the raised section). The

ends of the electrodes extend away from the body. A small blood vessel runs axially through the center of the raised tissue. Table 2.1 lists the model dimensions. The coordinate system origin lies at the target region center (the blood vessel center) for the x and y axes, and for the z axis, along the axial upwind side of the target region entrance. In this model the blood vessel's circular circumference is represented by a polygon of 16 equal sides, while the electrode plates are represented by rectangular bars.

Table 2.1 Model dimensions

Symbol	Description	Value (mm)
R	Blood vessel radius	0.1-0.5
W	Electrode spacing	4-12
H	Electrode face dimension, Target region height	10
TH	Electrode thickness	1
LF	Electrode length above target region	100
LZM	Upwind axial length (pre-target region)	35
LZP	Downwind axial length (post-target region)	65
LYM	Vertical depth below target region	20
LXM	Horizontal width past target region	20

2.3 Mathematical model

2.3.1 Thermal energy

In representing the energy equation of the system, the system is divided into three sub domains: vessel, tissue, and electrode plate.

To represent the tissue domain, the Pennes' bioheat equation [23] is used along with an added source term:

$$\rho_t c_t \frac{\partial T}{\partial t} = \nabla \cdot (k_t \nabla T) - W_b c_b (T - T_a) + q''' + Q(x, y, z, t) \quad (2.1)$$

where the subscript t refers to tissue parameters, ρ is the density, c is the specific heat, k is the thermal conductivity, T is the temperature, T_a is the arterial temperature, Q is the heat generated from the induced electric field, q''' is the metabolic volumetric heat generation, W_b is the blood perfusion term, t is the time, and x , y , and z are the Cartesian coordinate directions.

Vascular lock is a phenomenon characterized and termed in [24] in which blood perfusion is obstructed after the application of an electric pulse: after the pulse is administered, blood perfusion is temporarily (depending on pulse severity) stopped. Following these results, in this model blood perfusion is neglected in the target region. Note that Eq. (2.1) is now recast as:

$$\rho_t c_t \frac{\partial T}{\partial t} = \nabla \cdot (k_t \nabla T) - A[W_b c_b (T - T_a)] + q''' + Q(x, y, z, t) \quad (2.2)$$

$$\text{where } A = \begin{cases} 1 & \text{outside target region} \\ 0 & \text{otherwise} \end{cases}$$

For the blood vessel domain, the following energy equation is used:

$$\rho_b c_b \left(\frac{\partial T}{\partial t} + w \frac{\partial T}{\partial z} \right) = \nabla \cdot (k_b \nabla T) + Q(x, y, z, t) \quad (2.3)$$

where the subscript b refers to blood parameters, and w is axial blood velocity.

The effects of pulsation resulting from heartbeat were neglected, the flow is assumed one dimensional and an averaged blood velocity is used (as in [25]). Three representative blood vessel radii were used and the corresponding average blood velocities were based on data given in [25] which is presented in Table 2.2. The vessel wall is assumed to have the same property values as the surrounding tissue.

Table 2.2 Average blood velocity

Radius R (mm)	Average blood velocity w (mm/s)
0.1	34
0.3	60
0.5	80

The electrode plate section is represented by the transient conduction equation:

$$\rho_e c_e \frac{\partial T}{\partial t} = \nabla \cdot (k_e \nabla T) \quad (2.4)$$

where the subscript e refers to electrode plate parameters.

The electrodes are modeled as 1mm x 10 mm x 100 mm stainless steel plates. Commonly in parallel plate electroporation a conductive gel is used between the electrodes and the target region, thus in this model electrical and thermal contact resistance at the electrode- tissue interface is neglected.

The physical properties of the tissue, steel and blood are assumed to be constant and are presented in Table 2.3.

Table 2.3 Thermophysical properties

Symbol	Description	Value	Units
k_t, k_b	Tissue and blood thermal conductivity	0.5	W/mK
k_e	Electrode thermal conductivity	16	W/mK
ρ_t, ρ_b	Tissue and blood density	1060	kg/ m ³
ρ_e	Electrode density	7850	kg/ m ³
c_t	Tissue heat capacity	3750	J/kgK
c_b	Blood heat capacity	3640	J/kgK
c_e	Electrode heat capacity	450	J/kgK

2.3.2 Boundary and initial conditions

Initially the temperatures of the tissue and blood region domains are assumed to be equal to T_a , while the electrodes are assumed to have the same temperature as the surroundings,

T_∞ :

$$T(x, y, z, 0) = \begin{cases} T_a & \text{tissue and blood vessel domain} \\ T_\infty & \text{electrode domain} \end{cases} \quad (2.5)$$

The temperature of all faces of the domain not exposed to the ambient air is assumed to be equal to T_a :

$$\begin{aligned} T(x, y, z_{\min}, t) &= T(x, y, z_{\max}, t) = T_a \\ T(x, y_{\min}, z, t) &= T(x_{\min}, y, z, t) = T(x_{\max}, y, z, t) = T_a \end{aligned} \quad (2.6)$$

For the tissue domain, a convective flux boundary condition is imposed onto all sides exposed to the ambient air:

$$q'' = h(T - T_\infty) = -k \left. \frac{\partial T}{\partial n} \right|_{\text{surface}} \quad (2.7)$$

where q'' is the heat flux convected from the exposed face, h is the convection coefficient, T_∞ is the ambient air temperature, and n is the normal direction to the surface.

For the electrode plate, the edge away from the tissue domain is treated as an insulated fin tip:

$$q'' = 0 = -k \left. \frac{\partial T}{\partial y} \right|_{\text{tip}} \quad (2.8)$$

Boundary condition (2.7) is imposed onto all remaining sides of the electrode exposed to the ambient air.

2.3.3 Electric field power generation

During electroporation the induced electric field causes a temperature rise due to joule heating. The power supplied is found as follows. The electric potential is solved from the Laplace equation:

$$\nabla \cdot (\sigma \nabla \phi) = 0 \quad (2.9)$$

where ϕ is the electric potential and σ is the conductance of the target area. This equation is solved using the following boundary conditions: at one electrode the electric potential is zero, while at the other electrode the potential is the same as the applied voltage:

$$\phi\left(-\frac{W}{2}, y, z\right) = 0, \quad \phi\left(\frac{W}{2}, y, z\right) = V_{app} \quad (2.10)$$

where W is the distance between the electrodes.

All other boundaries of the target area are assumed electrically insulated:

$$\frac{\partial \phi}{\partial n} = 0 \quad (2.11)$$

where n represents the normal direction to the insulated face.

It has been shown that when exposed to an intense electric pulse the electrical resistance of the skin drops dramatically in a time period of microseconds [26-28], and in [28] regions of high temperatures were identified representing localized changes in the structure of the stratum corneum by electroporation. In this model the plate electrodes are placed internally directly on the surface of the rat liver, thus the effects of skin electrical properties are neglected. In [29,30] it is explained that an increase in current density about the electrode perimeter often occurs as a result of low electrode impedance coupled with large tissue resistivity. This chapter neglects such edge effects. It is also assumed that the electrical

properties of blood and tissue are uniform and equal so that the solution to Eq. (2.9) with boundary condition (2.10) and (2.11) is:

$$\phi(x) = \frac{x}{W} V_{app} + \frac{1}{2} V_{app} \quad (2.12)$$

The power due to joule heating is then defined as:

$$Q = \sigma |\nabla \phi|^2 \quad (2.13)$$

where σ is the electrical conductivity. Substituting Eq. (2.12) this is represented in the model as:

$$Q(x, y, z, t) = \begin{cases} \sigma \left(\frac{V_{app}}{W} \right)^2 & \text{inside the target region} \\ 0 & \text{outside the target region} \end{cases} \quad (2.14)$$

The remaining system parameter values used to solve Eqs. (2.2), (2.3), (2.14) and conditions (2.5) - (2.7) are given in Table 2.4.

Table 2.4 Parameter values

W_b	Blood perfusion term	1	kg/m ³ s
q'''	Metabolic heat generation	33800	W/ m ³
T_a	Arterial blood temperature	37	°C
T_∞	Ambient temperature	25	°C
h	Convection coefficient	10	W/ m ² K
σ	Tissue and blood electrical conductivity	0.286	S/m

2.3.4 Damage evaluation

Thermal damage occurs when tissue temperature is elevated over a period of time. In [30] a thermal dose estimate is proposed to quantify thermal damage in part so that damage may be compared between different time-at-temperature situations. In this model, the level of

damage is described in a time-temperature relation as in [30] in which this relation is represented in minutes of exposure at 43°C by:

$$EM_{43} = \int R^{(43-T)} dt \quad (2.15)$$

where $R = \begin{cases} 0.25 & T < 43 \\ 0.5 & T > 43 \end{cases}$ and T is the temperature. In this model the thermal dose is

calculated beginning with the onset of the first applied electric pulse.

2.4 Discretization and solution procedure:

Equations (2.2-2.4) are discretized using a cell centered finite volume approach and are solved implicitly using a global 5 ms time step and a variable rectangular grid consisting of 236,120 - 444,920 cells (depending on plate spacing and vessel radius). Interface conductivity is represented by a harmonic mean similar to that described in [31], while a resistor representation is used between the cell center and the convection boundary condition (2.7). An upwind scheme is adopted to represent advection in the blood vessel domain.

Temperature is solved for by an implicit point iterative method. Since it is expected that the dramatic temperature changes occur not over the entire solution domain, but locally near the target region, a convergence measure analogous to the L_∞ norm is used between iterations.

The convergence criterion is defined as $\delta = |T_{i,j,k}^{m+1} - T_{i,j,k}^m|$ where m is iteration and

convergence is reached when $\delta < 10^{-5}$. The value of the convergence parameter, δ , was decreased until the maximum local percent difference (between solutions of decreasing δ value) was less than 1%. Computations were performed on a 3.0 GHz Intel Xeon processor. Typical CPU times were 18-25 hours.

2.5 Results and discussion

Symmetry about the y - z plane is utilized, so in most of the following figures the solution is only presented for the positive x half-plane. Unless otherwise specified, the following results were found using plate spacing, W , of 8 mm and a vessel radius, R , of 0.3 mm. Times referenced to in the figures begin immediately after the last electroporation pulse cycle completion, while the thermal dose equation (2.15) is solved beginning with the first electroporation pulse cycle.

2.5.1 Temperature profiles

Geometric and transient temperature profile results are presented in Figure 2.2 and 2.3. To better isolate the cooling process a single 400 ms pulse of amplitude 250 V/cm is modeled. In this case a 0.6 mm diameter blood vessel is incorporated down the axial direction of the center of the target region.

Figure 2.2 represents target region temperature profiles fifteen seconds after the pulse has been administered for the three vertical cross sections: blood vessel entrance, midpoint, and exit ($z = 0, 5$, and 10 mm respectively); these planes are perpendicular to the axial direction. The decreasing horizontal contour lines along the top of the target region show convective influences through the top of the tissue, while the decreasing vertical contour lines along the right outer side of the target region show conductive influences from the tissue to the fin. Evidence of heat conducted to the lower tissue region is seen in the temperature rise there. Locations far away from the heated region are unaffected reflecting both perfusion and the low tissue thermal conductivity. The effects of heat convection by the blood vessel are also

evident. As the blood flows into the target region it is heated by the surrounding tissue. At the entrance and halfway into the target region, the blood vessel cools the surrounding tissue, as seen in the lower temperatures near the vessel wall. Further down the region, however, the vessel heats the surrounding tissue as it carries the heat introduced in the upstream regions. It is evident that thermal convection by the vessel plays a significant role in the transient cooling process after electroporation pulses have been administered. It is for this reason that the vessel centerline ($y = 0$) is used as the reference plane to present the results in the following Figures 2.3-2.9.

The transient temperature profiles in the x - z plane at $y = 0$ are presented in Figure. 2.3. Initially there is a large temperature gradient about the target region boundary. For the 1 Hz pulse cycle frequency, it should be noted that after the heat is added during the 400 ms pulse, a 600 ms period remains during which heat is removed from the target region primarily by conduction to the electrode and advection by the vessel. This is reflected by the streamwise and inward shift of the temperature profile at the end of the pulse cycle ($t = 0$). At later times the target area is cooled and higher temperatures concentrate on the downstream side and away from the electrode plates. From $t = 0$ to $t = 5$ s the temperature drop in the target region is dramatic, followed by a slower cooling rate. This slower rate reflects heat conducted from regions above and below the reference plane which are at further distances from the vessel. At $t = 90$ seconds this portion of the tissue model has nearly returned to the arterial blood temperature ($T_a = 37^\circ\text{C}$). For this reason the thermal damage is evaluated at $t = 90$ s (calculated beginning with the first pulse until 90 s after the end of the last pulse cycle).

2.5.2 Blood vessel effects

As seen in the previous results, the blood vessel convection has a large influence on the target region tissue cooling. Thus the effect of varying blood vessel radius on target region thermal damage is investigated in Figure 2.4. Here $8\ 250\ V/cm$, 50 ms pulses are administered at a 1 Hz frequency. In the absence of a vessel's convective cooling, the damage profiles are symmetric about the axial center of the target region ($z = 5\ mm$). Heat leaves the reference region primarily by conduction to the electrodes. No damage occurs outside the target region boundaries. For a very small diameter blood vessel most of the damage profile symmetry (about $z = 5\ mm$) is retained, although it is a bit skewed in the streamwise direction. For the intermediate and large diameter blood vessels, the effect is much more pronounced. Increasing the vessel radius to 0,3 mm, the damage is much reduced.. Recalling the temperature lines for an equivalent blood vessel (see Figure 2.3) the locations and magnitudes of the thermal damage contour lines are not unexpected. This size vessel also brings the lower level thermal damage contour lines outside the target region boundary. The large diameter blood vessel has again reduced the thermal damage, this time to negligible magnitudes. Similar blood vessel thermal effects are found in [25].

Although the variation in damage between the case with no vessel and the very small diameter vessel seems slight, a more careful comparison is made in Figure 2.5. Here the two solutions are plotted on the same axis. It is noted that near the vessel there is a significant shift in the damage contour lines for the small diameter vessel. This shift takes up a more significant proportion for higher magnitudes of thermal damage.

2.5.3 Effect of plate spacing

The effect of varying the electrode plate spacing is investigated in Figure 2.6. In this figure, 8 $250V/cm$, 50 ms pulses are administered at a 1 Hz frequency for four different electrode plate spacing. Here the damage increases as plate spacing increases. This is a result of two main factors. First since the voltage is held constant, more energy is added with increased target region volume. Therefore, there is more energy for the mechanisms of conduction and convection to remove from the target region with larger plate spacing. The second factor is made by recalling that the vascular lock assumption is made inside the target region (perfusion in the target region is neglected) so that the target region is cooled only by heat moving across its boundary. This implies that as the target region volume to surface area ratio increases, cooling decreases, resulting in an increase in thermal damage. The corresponding volume to surface area ratios are given in Table 2.5.

Table 2.5 Volume to surface area ratios

Electrode spacing	Volume to surface area ratio
<i>4 mm</i>	<i>1.85 mm</i>
<i>8 mm</i>	<i>3.45 mm</i>
<i>12 mm</i>	<i>4.84 mm</i>
<i>16 mm</i>	<i>6.06 mm</i>

2.5.4 Pulse duration

The effects of varying the pulse duration and number of pulses is investigated here. The amount of total energy introduced to the target region by the electric pulse is kept constant by varying pulse duration and number of pulses. During the exposures,

$n \times t_p \times Q(x, y, z, t) = \text{constant}$ where n is the number of pulses and t_p is the pulse duration.

Figure 2.7 shows the thermal damage evaluated at 90 seconds after last pulse administered

for four (n, t_p) combinations for 250 V/cm , 1 Hz pulses. Thermal dose magnitudes drop dramatically as the pulse delivery is divided into more cycles. Figure 2.8 shows the temperature profiles at the end of the last pulse cycle. Recalling the modified Pennes' bioheat equation, Eq. (1), and neglecting conduction, convection, metabolic heat generation, and blood perfusion, the temperature rise may be approximated as:

$$T = T_{initial} + n \times t_p \times \frac{Q(x, y, z, t)}{\rho c} \quad (2.17)$$

For the presented solutions this value corresponds to:

$$T = 37^\circ\text{C} + 0.4\text{s} \times \frac{0.286\text{S/m} \cdot (25000\text{ V/m})^2}{1040\text{kg/m}^3 \cdot 3750\text{J/kgK}} = 55.16^\circ\text{C} \quad (2.18)$$

For a single pulse this approximation more accurate for the maximum temperature, while the multiple pulse figures show the maximum temperature contours to have lower magnitudes. This is an effect of the major cooling mechanisms (conduction across the boundaries and advection along the vessel) acting on the target region between pulses during the electroporation cycles.

2.5.5 Pulse frequency

The effects of varying the pulse frequency for 8 50 ms, 250 V/cm pulses are shown in Figures 2.9 and 2.10. Here the frequencies are decreased from 4 Hz to 0.5 Hz. The thermal dose profiles in Figure 2.9 show dramatic drop in the magnitude of thermal dose profiles as the frequency is decreased. This is again attributed to cooling during pulse applications: the decrease in frequency corresponds to an increase in time between pulse applications. For the 0.5 Hz frequency, the same energy is added to the target region, but over a 16 s period as compared to a 2 s period for the 4 Hz frequency, allowing for longer periods of time during which thermal energy is conducted and advected across the target region boundaries. The

longer cooling periods between pulses are reflected in the lower temperature profiles for lower frequencies depicted in Figure 2.10.

2.6 Conclusions

A parallel plate electroporation process was modeled in which blood vessel, geometric, and electroporation pulse parameter effects on thermal dose magnitudes were investigated. In the presence of a blood vessel the target region tissue was found to have lower thermal damage, and for larger vessels ($R > 0.3$ mm) this effect was more pronounced. The vessel carried heat from the target region, cooling the tissue as it entered the target region and heating the tissue as it exited the target region. The effect of spacing between the electrode plates was investigated. Increased plate spacing increases thermal damage: as the volume to surface area ratio increases, conduction and convection have relatively less area across which to move more thermal energy. The results of dividing a long pulse into shorter pulses of equal intensity and frequency were explored. It was shown that increasing the time over which energy is introduced into the system results in lower maximum target region temperatures and significantly decreased thermal dose values. Similarly it was found that decreasing pulse frequency results in lower thermal damage and lower maximum temperatures.

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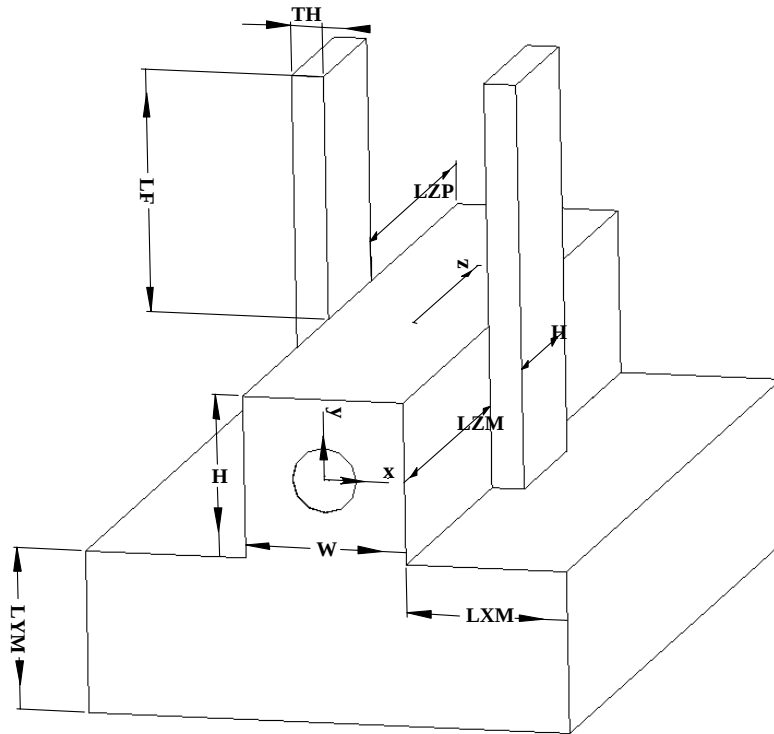


Figure 2.1 Model schematic

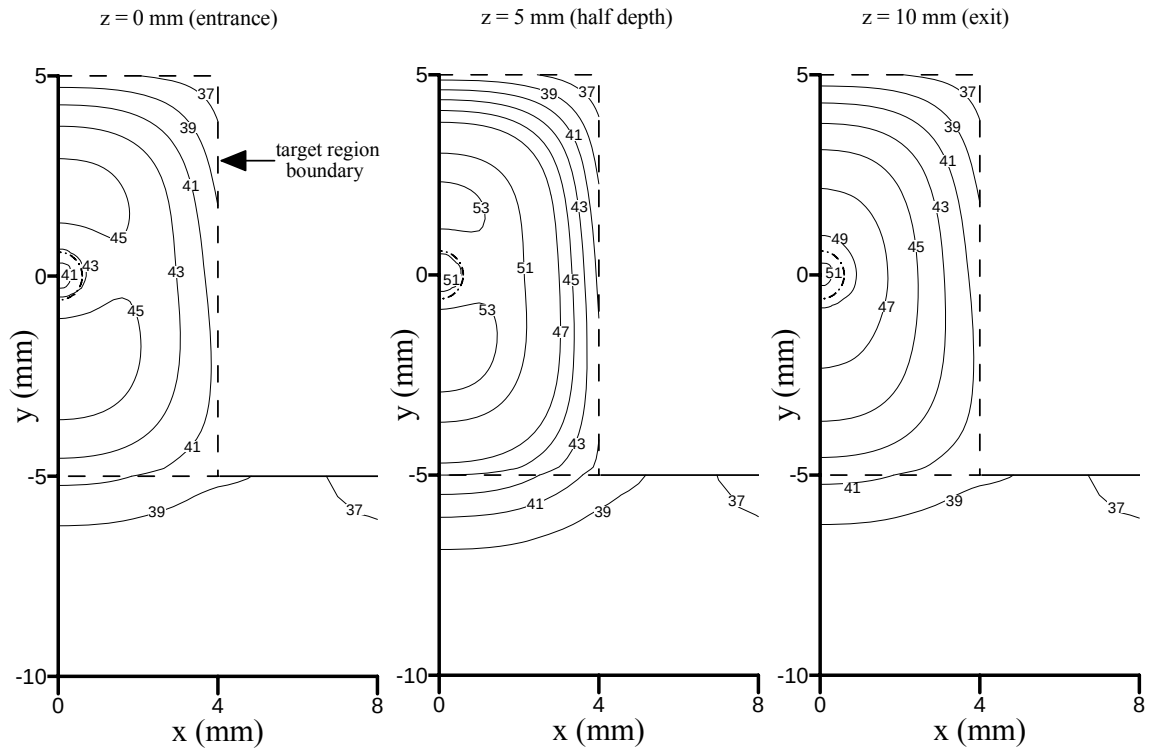


Figure 2.2 x - y plane temperature profiles: varying axial positions at $t = 15s$

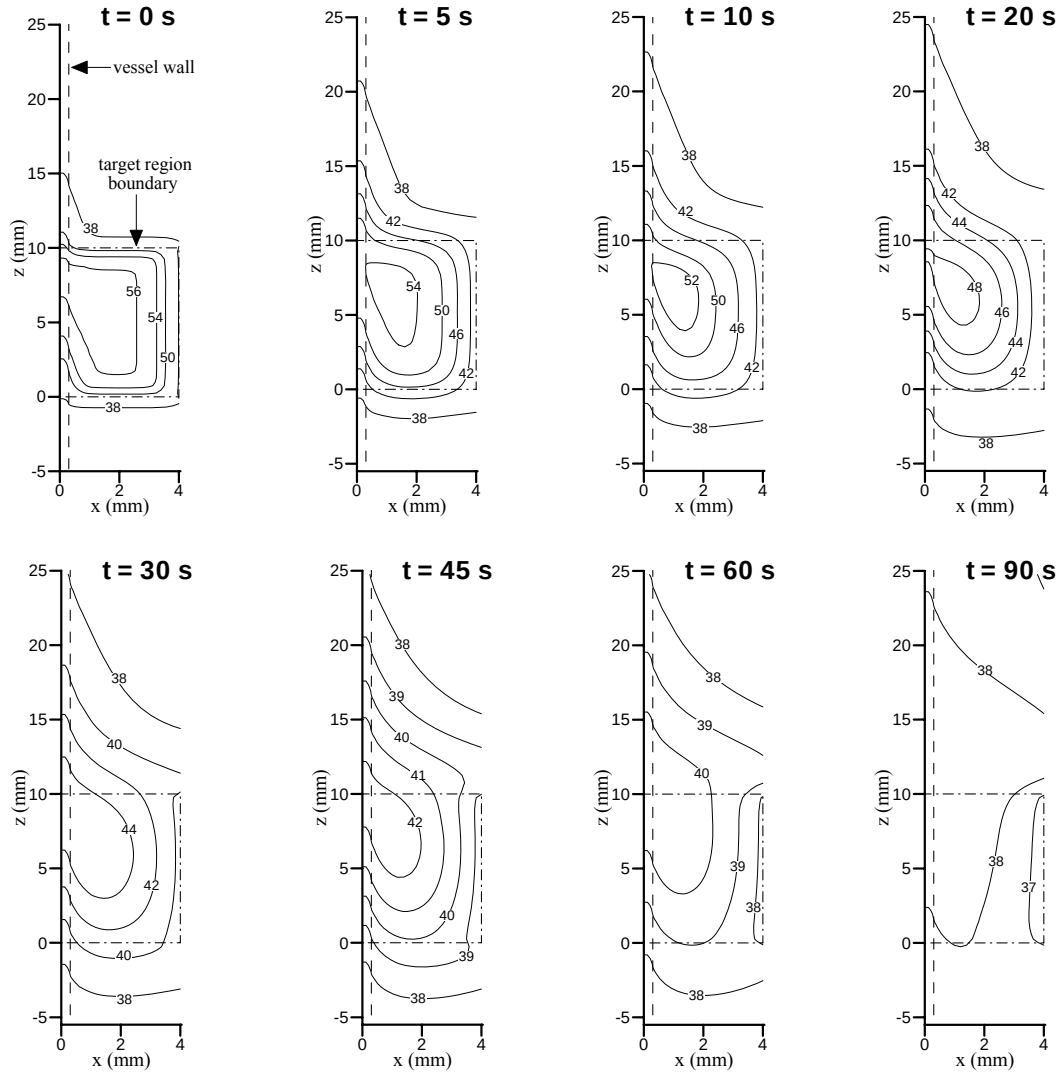


Figure 2.3 Transient temperature profiles at $y = 0 \text{ mm}$ (vessel centerline); t is time after last pulse cycle

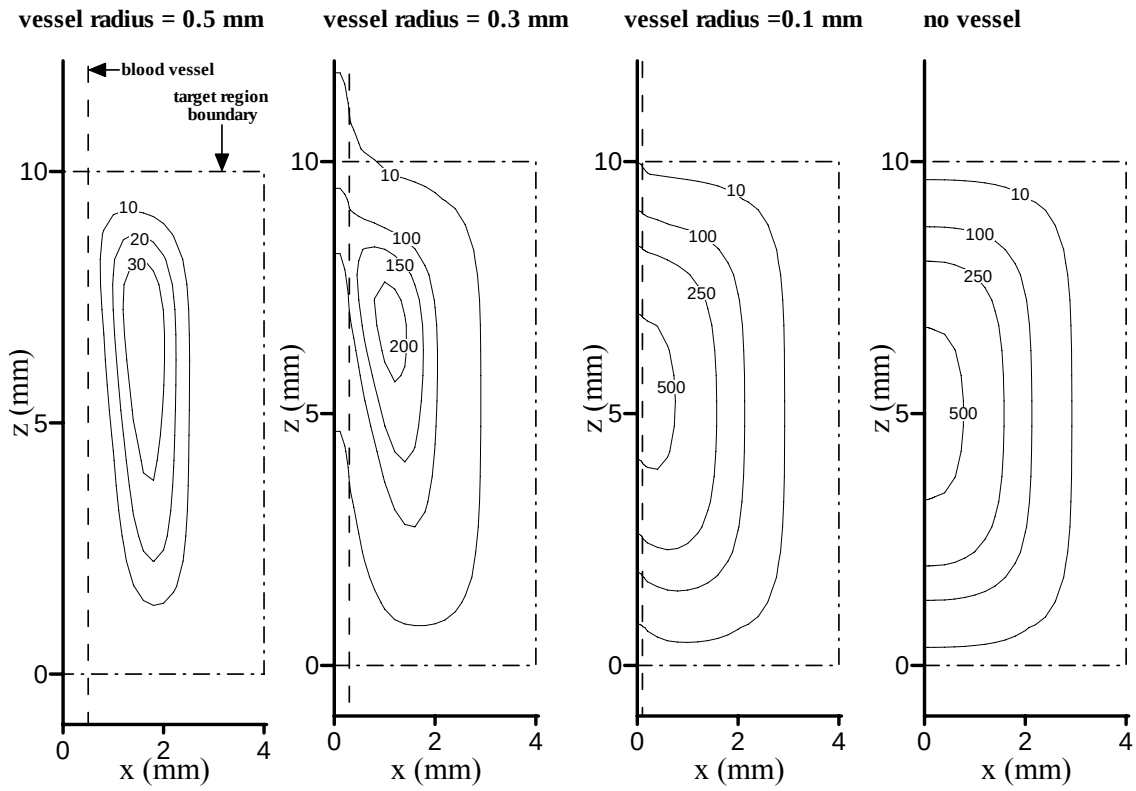


Figure 2.4 EM_{43} at $t = 90$ s: Effect of blood vessel radius

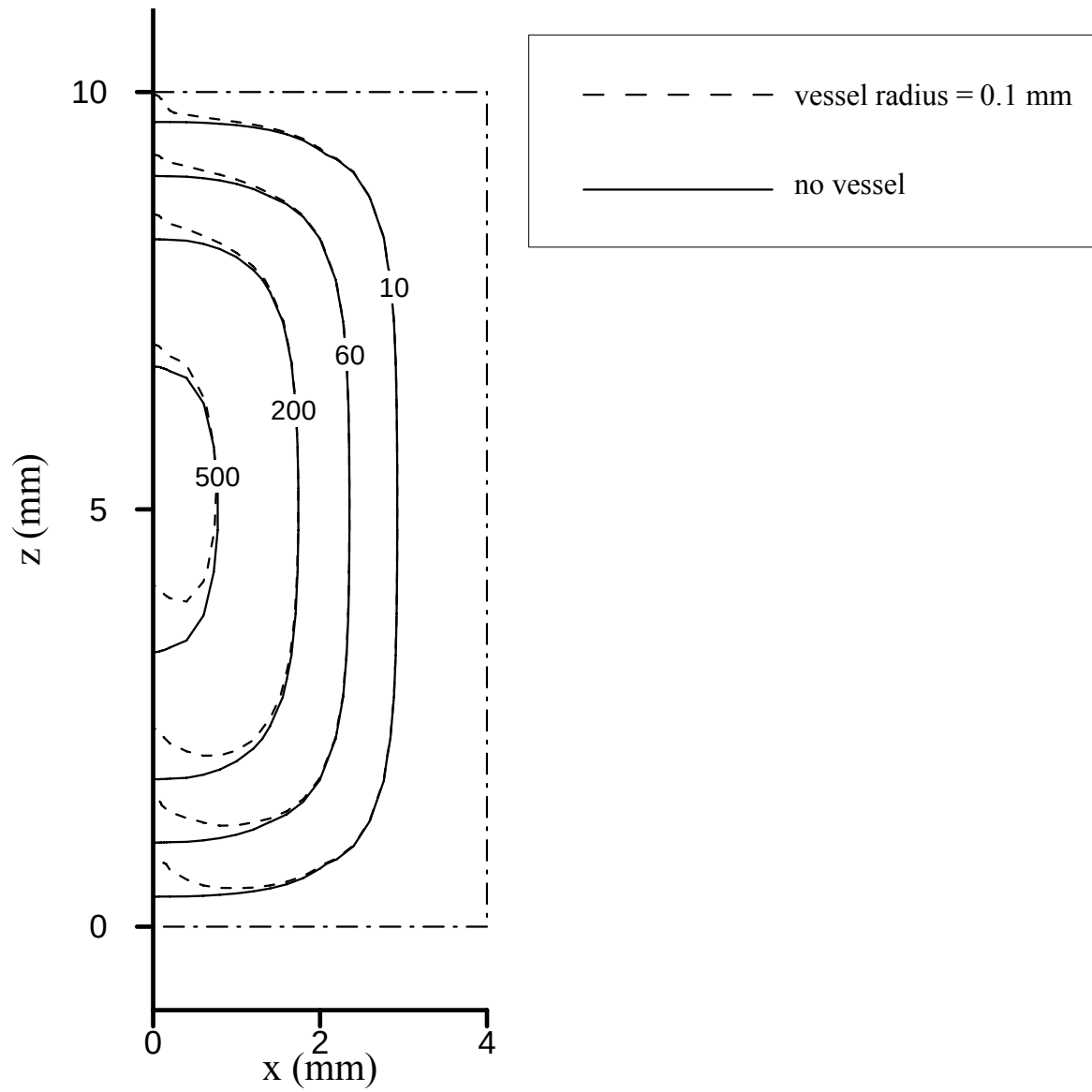


Figure 2.5 EM_{43} at $t = 90s$ (after last pulse cycle): Small vessel effect

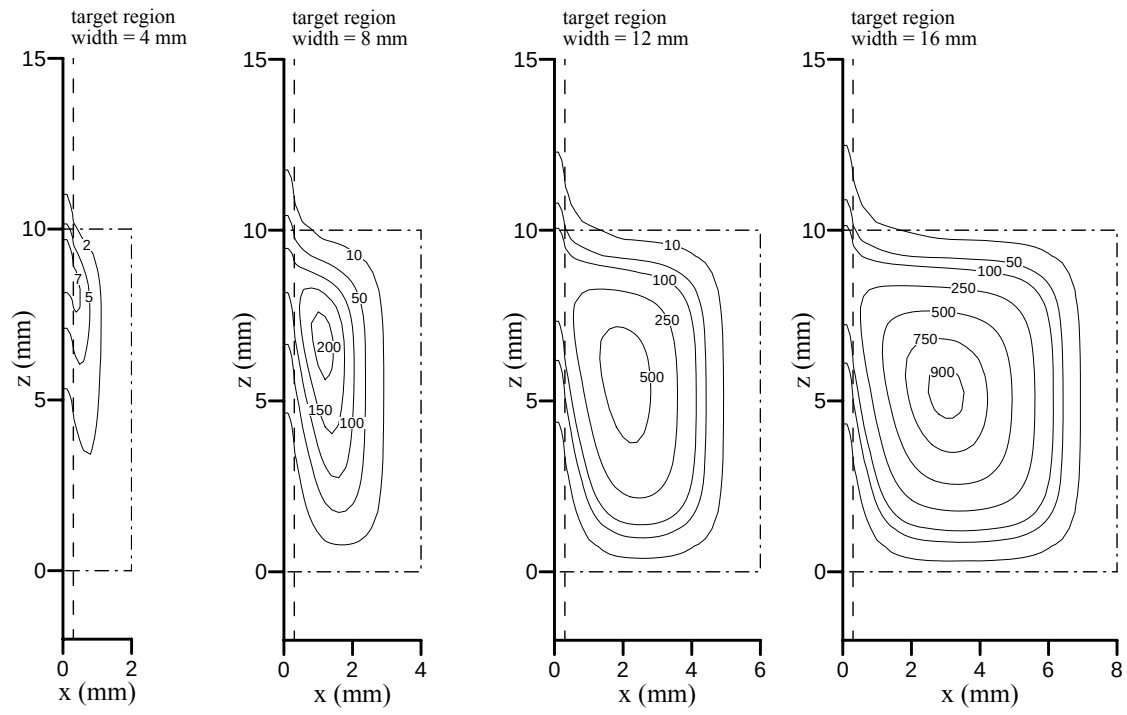


Figure 2.6 EM_{43} at $t = 90s$ (after last pulse cycle): Effect of electrode plate spacing

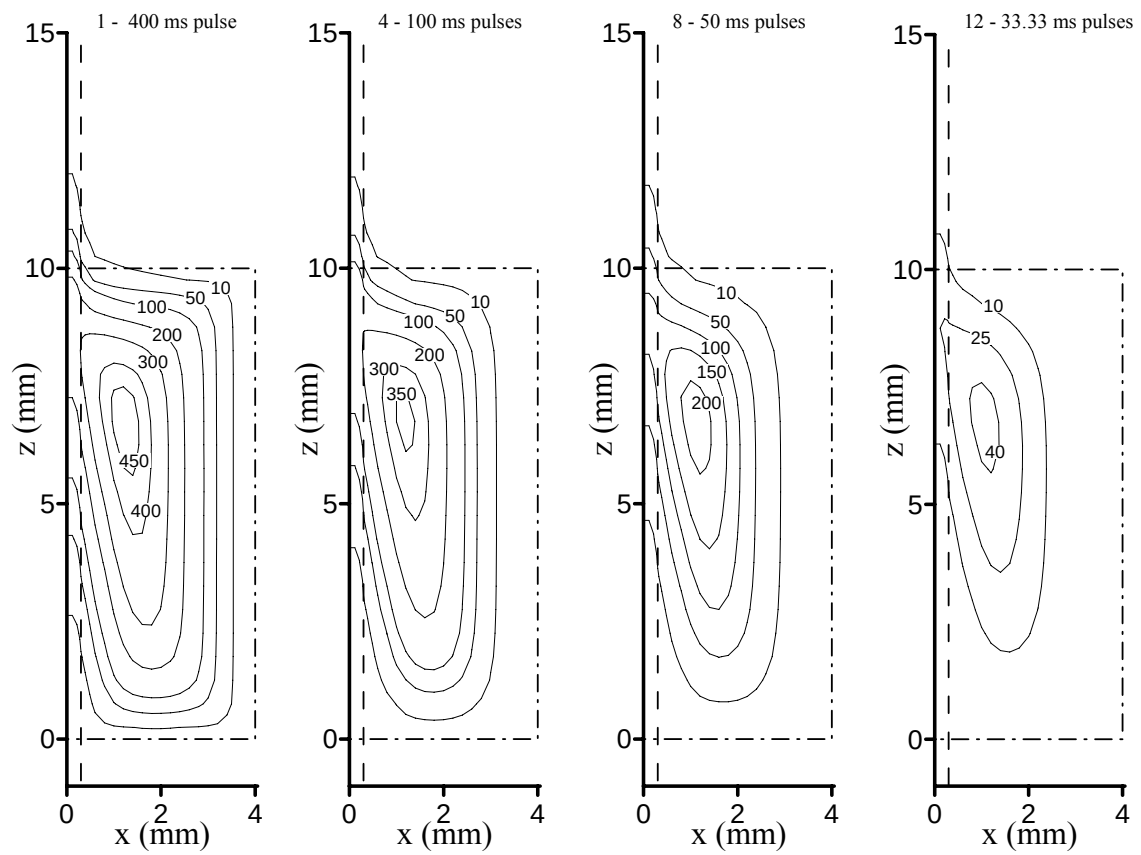


Figure 2.7 EM_{43} at $t = 90s$ (after last pulse cycle): Effect of pulse duration- number of pulses administered

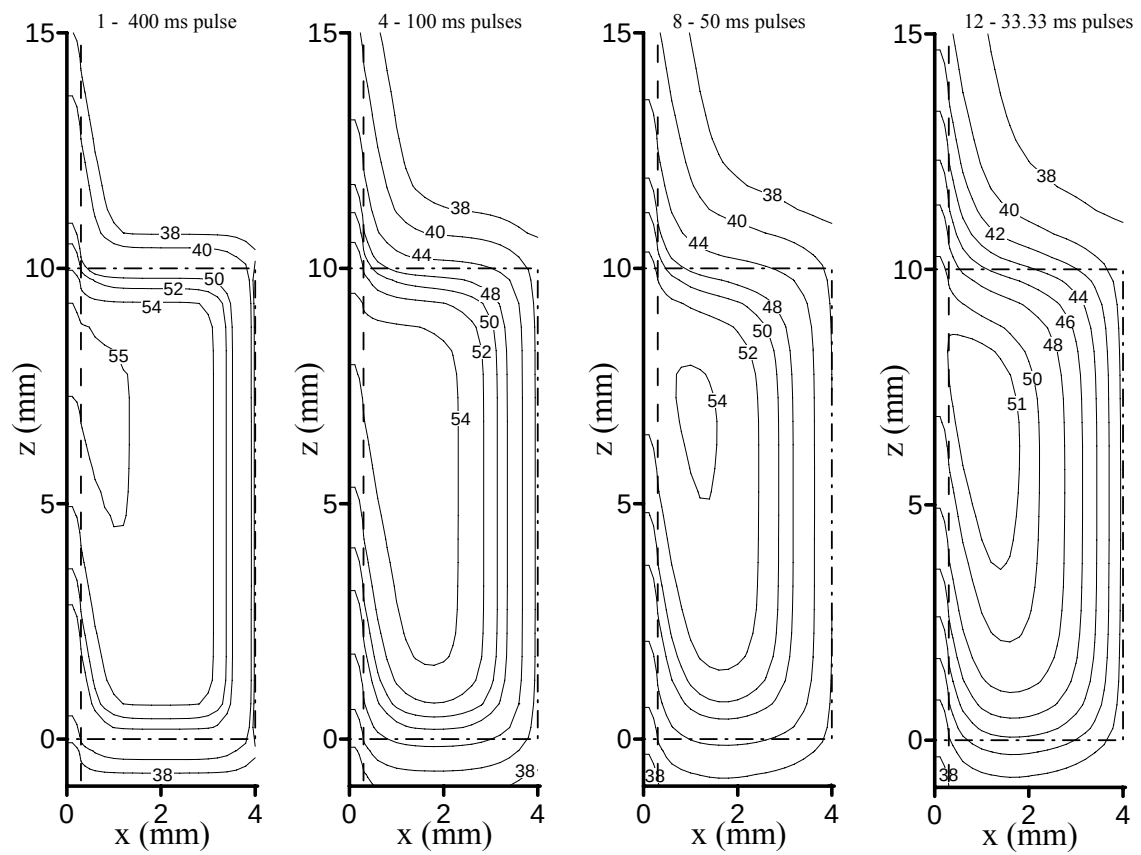


Figure 2.8 Temperature at $t = 0s$ (after last pulse cycle): Effect of pulse duration- number of pulses administered

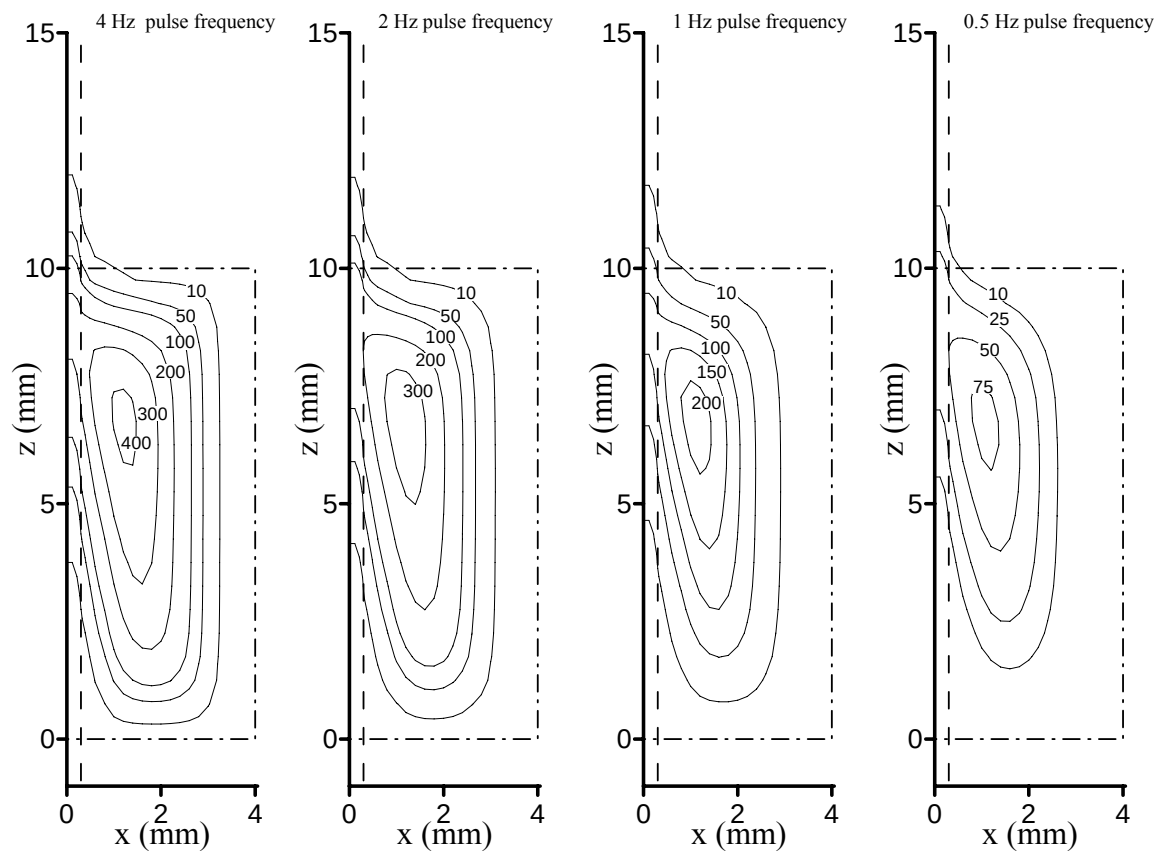


Figure 2.9 EM_{43} at $t = 90s$ (after last pulse cycle): Effect of pulse frequency

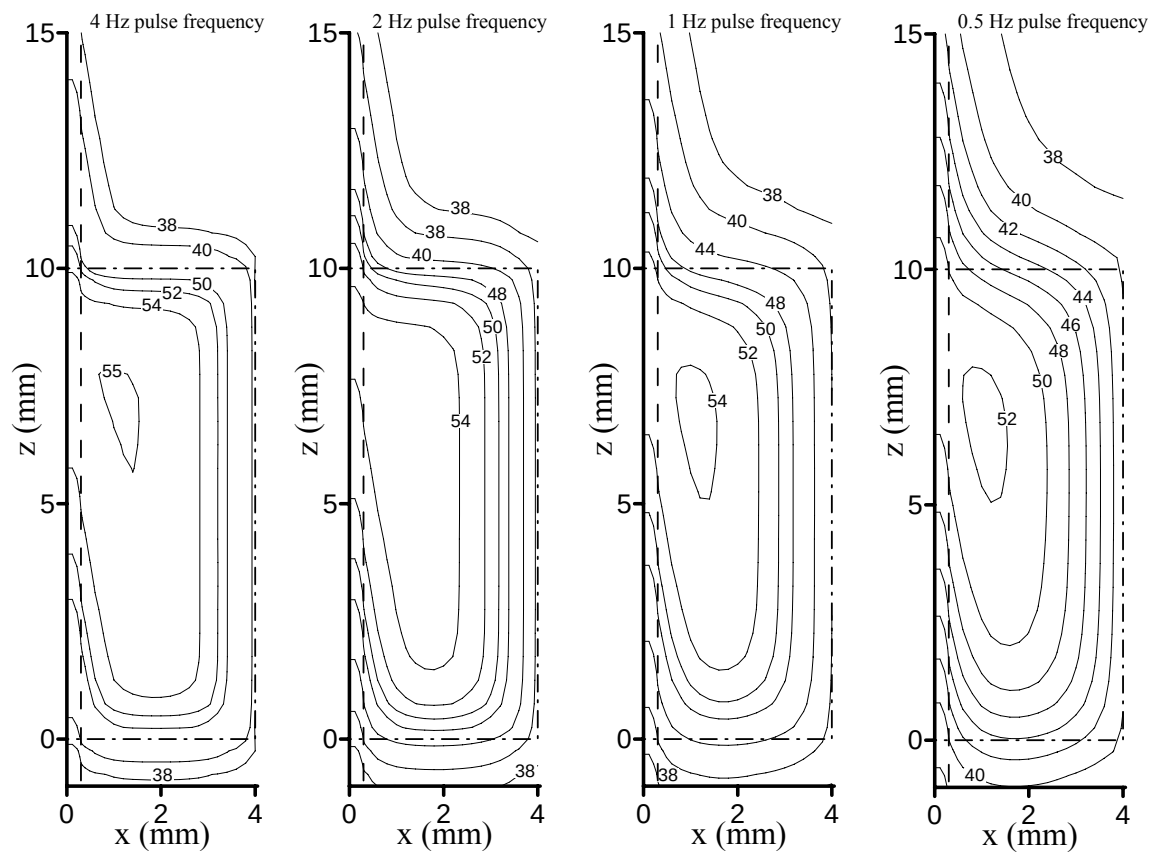


Figure 2.10 Temperature at $t = 0s$ (after last pulse cycle): Effect of pulse frequency

3 Numerical assessment of thermal response associated with *in vivo* skin electroporation: the importance of the composite skin model

Electroporation is an approach used to enhance transdermal transport of large molecules in which the skin is exposed to a series of electric pulses. The structure of the transport inhibiting outer layer, the stratum corneum, is temporarily destabilized due to the development of microscopic pores. Consequently agents that are ordinarily unable to pass into the skin are able to pass through this outer barrier. Of possible concern when exposing biological tissue to an electric field is thermal tissue damage associated with Joule heating. This chapter shows the importance of using a composite model in calculating the electrical and thermal effects associated with skin electroporation. A three-dimensional transient finite-volume model of *in vivo* skin electroporation is developed to emphasize the importance of representing the skin's composite layers and to illustrate the underlying relationships between the physical parameters of the composite makeup of the skin and resulting thermal damage potential.

3.1 Introduction

Electroporation is a technique in which the cell membrane is altered in order to increase permeability into the cell cytosol by inducing an electric field in which the transmembrane potential exceeds a critical threshold [1-5]. The lipid bilayer matrix of the thin ($10 - 20 \mu m$) outermost layer of the skin, the stratum corneum (*SC*), acts as a barrier inhibiting transdermal drug delivery. It has been shown that electroporation changes the structure of the *SC* creating aqueous pores [6-8]. The breakdown of the *SC* barrier allows for increased molecular

transport and radically reduces the electrical resistance of the skin [6, 9-12]. Experimentally it is common practice to use short high voltage ($100\mu s$ $1000V/cm$) pulses to permeabilize the skin and longer low voltage (100 ms $400 V/cm$) pulses to assist molecular transport by electrophoretic forces [13]. Joule heating resulting from short duration pulses has been shown to be negligible [3], and it is the long duration pulses in particular that contribute to Joule heating.

In dealing with *in vivo* biological tissues exposed to an electric field it is important to consider the temperature rise associated with Joule heating. In electroporation the severity of this temperature rise is proportional to pulse length and magnitude and is strongly tied to tissue electrical properties. Experimental results show that transdermal molecular flux rates increase with increased pulse duration and magnitude [14-17]. Experiments involving homogenous tissue electroporation have shown that the success of large molecule gene transfer is strongly tied to the amount of heat generated [18, 19]. Extensive and novel numerical studies have been conducted for homogenous tissue cases in which factors influencing thermal responses of electroporation are explored [20, 21]. In a preliminary study the relationship between the thermal response of homogenous *in vivo* plate electroporation and electroporation parameters is investigated [22]. The purpose of this chapter is to model thermal effects associated with *in vivo* electroporation of the human skin by accounting for variations in physical parameters of the skin's composite layers and underlying tissue. These composite findings are then compared to the results of homogenous tissue electroporation.

3.2 Model geometry

This chapter considers external parallel plate electroporation in which the electrodes are in direct contact with the skin. Figure 1 shows the geometric representation of the tissue

modeled in this study. A small section of tissue is raised and two thin electrodes are positioned on either side of the target region (which is at the axial center of the raised section). The ends of the electrodes extend away from the body. In two cases of this study, the advective cooling effects of a blood vessel are addressed. In these, a small blood vessel runs axially through the center of the raised tissue. Table 3.1 lists the model dimensions used. The coordinate system origin lies at the target region center (the blood vessel center) for the x and y axes, and for the z axis, along the axial upwind side of the target region entrance. When the presence of a blood vessel is addressed, the blood vessel's circular circumference is modeled by a polygon of 16 equal sides. The electrode plates are represented by rectangular bars that act as cooling fins to the electroporated tissue.

Table 3.1 Model dimensions

Symbol	Description	Value (mm)
R	Blood vessel radius	0, 0.3
W	Electrode spacing	10
H	Electrode face dimension, Target region height	10
TH	Electrode thickness	1
LF	Electrode length above Target region	100
LZM	Upwind axial length (pre-target region)	15
LZP	Downwind axial length (post-target region)	15
LYM	Vertical depth below Target region	9.805
LXM	Horizontal width past Target region	8.5

A representative composite model is used to depict the skin and underlying tissue layers of the human volar forearm (see Fig.2). The outer skin layer, the epidermis, is without

vasculature. The previously mentioned outermost layer of the epidermis, the *SC*, is electrically insulative and acts as a protective barrier to the skin preventing molecule transport. The inner skin layer is the highly vascular dermis. The dermal skin blood flow exceeds metabolic requirements and the excess blood flow primarily regulates body temperature [23]. Below the dermis is a thermally insulative fat layer that acts to conserve body heat. The innermost layer is the subcutaneous tissue layer which in this study is modeled as muscle tissue. Figure 2 shows the composite layer model used to represent the skin and underlying tissue.

3.3 Mathematical model

3.3.1 Thermal energy

In finding the thermal solution, the system is divided into three sub-domains: vessel, composite tissue, and electrode plate.

To represent the composite tissue domain, the Pennes' bioheat equation [24] is used along with an added source term:

$$\rho_m c_m \frac{\partial T_m}{\partial t} = \nabla \cdot (k_m \nabla T) - \omega_m c_b (T - T_a) + q_m''' + Q_J \quad (3.1)$$

where ρ is the density, c is the specific heat, k is the thermal conductivity, T is the temperature, T_a is the arterial temperature, Q_J is the Joule heat generated from the induced electric field, q''' is the metabolic volumetric heat generation, ω_m is the non-directional volumetric blood flow associated with perfusion, t is the time, and x , y , and z are the Cartesian coordinate directions. The subscript m refers to one of the four composite tissue layers: stratum corneum (*SC*), epidermis (*ED*), dermis (*D*), subcutaneous fat (*F*), and subcutaneous muscle tissue (*T*).

Vascular lock is a phenomenon characterized and termed in [25] and clinically supported in [26-28] in which blood perfusion is obstructed after the application of an electric pulse: after the pulse is administered, blood perfusion is temporarily (depending on pulse severity) obstructed. In this study we model electrical obstruction of perfusion as total obstruction by neglecting blood perfusion in the target region after the first pulse application. Note that Eq. (3.1) is now recast as:

$$\rho_m c_m \frac{\partial T_m}{\partial t} = \nabla \cdot (k_m \nabla T_m) - A [\omega_m c_b (T_m - T_a)] + q_m''' + Q_J \quad (3.2)$$

where $A = \begin{cases} 1 & \text{outside target region} \\ 0 & \text{inside target region} \end{cases}$

Two cases addressed in this study involve the convective effects associated with the presence of a small blood vessel in the center of the composite tissue domain. To represent the blood vessel domain, the following energy equation is used:

$$\rho_b c_b \left(\frac{\partial T_b}{\partial t} + w \frac{\partial T_b}{\partial z} \right) = \nabla \cdot (k_b \nabla T_b) + Q_J \quad (3.3)$$

where the subscript b refers to blood parameters, and w is the axial blood velocity.

The effects of pulsation resulting from heartbeat are neglected. A representative blood vessel radius of 0.3 mm is used with the corresponding average blood velocity of 6 mm/s based on data given in [29]. The vessel wall is assumed to have the same property values as the surrounding tissue. The biological composite tissue and blood properties are presented in Table 3.2.

Table 3.2 Composite tissue and blood properties

			<i>SC</i>	<i>ED</i>	<i>D</i>	<i>F</i>	<i>T</i>	<i>b</i>
thickness	<i>mm</i>	δ	0.015 [23]	0.035 [23]	1.1 [34]	1.2 [34]	-	-
thermal conductivity	<i>W/mK</i>	<i>k</i>	0.2 [30]	0.209 [33]	0.293 [33]	0.23 [31]	0.5 [31]	0.572 [31]
density	<i>kg/m³</i>	ρ	1500 [31]	1110 [31]	1116 [31]	850 [32]	1040 [31]	1060 [31]
heat capacity (specific)	<i>J/kgK</i>	<i>c</i>	3600 [32]	3600 [32]	3800 [32]	2300 [32]	3800 [32]	3840 [31]
perfusion	<i>kg/m³s</i>	ω	0	0	2.33 [35]	0.545 [35]	0.695 [35]	-
metabolic heat generation	<i>W/m³</i>	q'''	0	0	200 [32]	5 [32]	800 [32]	-
Electrical conductivity	<i>S/m</i>	σ	10 ⁻⁵ [31] Pre-breakdown	0.01	0.015	0.02 [31]	0.41 [31]	0.62 [31]

The electrode plate section is represented by the transient conduction equation:

$$\rho_e c_e \frac{\partial T_e}{\partial t} = \nabla \cdot (k_e \nabla T_e) \quad (3.4)$$

where the subscript *e* refers to electrode plate parameters. Electrode property values are given in Table 3.3.

Commonly in parallel plate electroporation a conductive gel is used between the electrodes and the target region, thus in this model electrical and thermal contact resistance at the electrode-tissue interface are neglected.

3.3.2 Boundary and initial conditions

Initial temperature distributions of the tissue and blood region domains are calculated from the steady state forms of Eqs. (3.1) and (3.3) without the induced electric field:

$$0 = \nabla \cdot (k_m \nabla T_m) - \omega_m c_b (T_m - T_a) + q'''_m \quad (3.5)$$

$$\rho_b c_b w \frac{\partial T_b}{\partial z} = \nabla \cdot (k_b \nabla T_b) \quad (3.6)$$

Blood perfusion within the target region is not neglected in Eq. (3.5)

Initially electrodes are assumed to have the same temperature as the surroundings, T_∞ :

$$T_e(x, y, z, 0) = T_\infty \quad (3.7)$$

The subcutaneous muscle tissue temperature of lower face of the composite tissue domain is assumed to be equal to the arterial blood temperature, T_a :

$$T_r(x, y_{\min}, z, t) = T_a \quad (3.8)$$

The remaining boundary faces not exposed to the ambient environment are assumed thermally insulated:

$$\begin{aligned} \frac{\partial}{\partial z} T_m(x, y, z_{\min}, t) &= \frac{\partial}{\partial z} T_m(x, y, z_{\max}, t) = 0 \\ \frac{\partial}{\partial x} T_m(x_{\min}, y, z, t) &= \frac{\partial}{\partial x} T_m(x_{\max}, y, z, t) = 0 \end{aligned} \quad (3.9)$$

The blood vessel inlet temperature is assumed to be equal to T_a while axial thermal conduction at the outlet is neglected:

$$T_b(x, y, z_{\min}, t) = T_a \quad (3.10)$$

$$\frac{\partial}{\partial z} T_b(x, y, z_{\max}, t) = 0 \quad (3.11)$$

Along the outer surface of the composite domain (the SC), a convective flux boundary condition is imposed onto all sides exposed to the ambient air:

$$q'' = h(T_{SC} - T_\infty) = -k \left. \frac{\partial T_{SC}}{\partial n} \right|_{surface} \quad (3.12)$$

where q'' is the heat flux convected from the exposed face, h is the convection coefficient, T_∞ is the ambient air temperature, and n is the normal direction to the surface.

A convective flux boundary condition is imposed onto all electrode sides exposed to the ambient air:

$$q'' = h(T_e - T_\infty) = -k_e \left. \frac{\partial T_e}{\partial n} \right|_{surface} \quad (3.13)$$

For the electrode plate, the face of the end away from the tissue domain is treated as an insulated fin tip:

$$q'' = 0 = -k_e \left. \frac{\partial T}{\partial y} \right|_{tip} \quad (3.14)$$

The convection coefficient, h , of boundary conditions (3.12) and (3.13) is modeled to result from the natural convection of air, and it is solved from:

$$h = \frac{Nu \cdot k_f}{L} \quad (3.15)$$

where Nu is the average Nusselt number over the face, k_f is the fluid film thermal

conductivity, and L is a characteristic length scale defined as : $L = \begin{cases} \text{Vertical} : H_s \\ \text{Horizontal} : \frac{A_s}{P_s} \end{cases}$ where

H_s is the vertical surface height, A_s is the horizontal surface area, and P_s is the horizontal surface periphery.

Correlations described in [36] are used to evaluate the averaged Nusselt number, Nu , as follows:

For vertical surfaces:

$$Nu = \left\{ 0.825 + \frac{0.387 \cdot Ra^{1/6}}{\left[1 + (0.492/Pr)^{9/16} \right]^{8/27}} \right\}^2 \quad (3.16)$$

For horizontal surfaces:

$$Nu = 0.54Ra^{1/4} \quad (3.17)$$

Pr is the Prandtl number and Ra is the Rayleigh number which is defined as:

$$Ra = \frac{g\beta(T_s - T_\infty)L^3}{\alpha\nu} \quad (3.18)$$

where g is gravity, β is the coefficient of volumetric thermal expansion, α is the thermal diffusivity, ν is the kinematic viscosity, and T_s is the surface temperature. The surface temperature value used in Eq. (3.18) is obtained by averaging the temperature over the surface. The values of the fluid properties k_f , Pr, β , α , and ν are evaluated at the air film temperature, T_f , which is defined as:

$$T_f = \frac{(T_s + T_\infty)}{2} \quad (3.19)$$

3.3.3 Electric field power generation

The electrical conductivity of the SC is several orders of magnitude lower than the underlying composite layers. Current passing through the skin is believed to follow two parallel pathways: one is through the cell matrix composing the SC and the other through skin appendages such as hair follicles and sweat glands [37]. With the electroporation induced permeabilization of the SC matrix, the current experiences much lower resistance within the SC. Experimentally it has been shown that when exposed to a high voltage electric field the electrical resistance of the skin drops dramatically in a time period of microseconds [6, 9, 10]. To model the breakdown of the SC electrical resistance, the

conductivity of the *SC* is prescribed a pre-breakdown value of $\sigma_{SC} = 10^{-5} \text{ S/m}$ during the first 5 *ms* of the first electroporation pulse, and thereafter is increased to a value of $\sigma_{SC} = 10^{-3}$ or 10^{-2} S/m (depending on case study). This single 5 *ms* 400 V/cm pulse has the power equivalent of 8 100 μs 1000V/cm pulses which have comparable parameters to those of experimental SC permeabilization pulses. Experimentally it has been shown that permeabilization associated with pulses of high voltage and short duration result in a high pore density [15]. This chapter makes the assumption that for a high density of electroporation pores and given the length scale at which the physical model is presented, the electrical properties of the *SC* may be averaged. Since it is beyond the scope of this study to model the local pathways through the *SC*, in this chapter uniform conductivities are used within composite layers. In [37] it is shown that even at moderate voltages the capacitive charging time associated with non-Ohmic behavior of the skin is very short and may be neglected at times greater than 1 *ms*. This behavior is neglected in this study. Epidermal and dermal electrical conductivities given in Table 3.2 have been chosen to reflect electrical resistance drop with increased depth into the skin.

During electroporation the induced electric field causes a temperature rise due to Joule heating. The power supplied is found as follows. The electric potential is solved from the Laplace equation:

$$\nabla \cdot (\sigma_m \nabla \phi) = 0 \quad (3.20)$$

where ϕ is the electric potential and σ_m is the composite electrical conductivity. This equation is solved using the following boundary conditions: at one electrode the electric

potential is zero, while at the other electrode the potential is the same as the applied voltage,

V_{app} :

$$\phi\left(-\frac{W}{2}, y, z\right) = 0, \quad \phi\left(\frac{W}{2}, y, z\right) = V_{app} \quad (3.21)$$

where W is the distance between the electrodes. This study uses an applied voltage value of $V_{app} = 400 \text{ V}$ and a plate spacing of $W = 1 \text{ cm}$.

All other boundaries of the target area are assumed electrically insulated:

$$\frac{\partial \phi}{\partial n} = 0 \quad (3.22)$$

where n represents the normal direction to the insulated face.

The power due to Joule heating is then defined as:

$$Q_J = \sigma_m |\nabla \phi|^2 \quad (3.23)$$

where σ_m is the tissue electrical conductivity. Obviously there is no Joule heating outside the target region or at times during which the electric pulses are not applied.

In [38, 39] it is explained that an increase in current density about the electrode perimeter often occurs as a result of low electrode impedance coupled with large tissue resistance. This chapter neglects such edge effects. The remaining system parameter values used to solve Eqs. (3.2), (3.3), (3.14) and conditions (3.5)-(3.7) are given in Table 3.3.

Table 3.3 Parameter values

ρ_e	electrode density	$7850 \text{ kg}/\text{m}^3$
c_e	electrode specific heat	$450 \text{ J}/\text{kgK}$
k_e	electrode thermal conductivity	$16 \text{ W}/\text{mK}$
T_a	arterial blood temperature	37°C
T_∞	ambient temperature	20°C
V_{app}	applied voltage	400 V

3.3.4 Damage evaluation

Thermal damage occurs when tissue temperature is elevated over a period of time. In [40] a thermal dose estimate is proposed to quantify thermal damage in part so that damage may be compared between different time-at-temperature situations. In this model, the level of damage is described in a time-temperature relation as in [40] in which thermal damage is represented in minutes of exposure at 43°C by:

$$t > t_p \quad EM_{43} = \int R^{(43-T)} dt \quad (3.24)$$

where $R = \begin{cases} 0.25 & T < 43^\circ \text{C} \\ 0.5 & T > 43^\circ \text{C} \end{cases}$ and T is the temperature. In this model the thermal dose is

calculated beginning with the onset of the first applied electric pulse. The sensitivity of biological tissue to thermal dose varies significantly between animal species and specific tissue type [41]. While it is reported for pig muscle, fat and skin tissues that significant

chronic histological damage and gross appearance thermal dose thresholds begin at values $EM_{43} > 80 \text{ min}$ [41], values of EM_{43} in the range 240-340 minutes have been used to denote permanent thermal tissue damage for muscle, liver and kidney tissues[29, 40-42].

3.4 Discretization and solution procedure

The computational domain is discretized using a variable rectangular grid consisting of 112,760 cells for cases without a blood vessel and 158,720 cells for the two cases with a blood vessel. A fully implicit finite volume approach is used to solve Eqs. (3.2-3.6), (3.19), and (3.22). Temperature, T , and Joule heat, Q_j , are evaluated at the cell centers while the electric potential, ϕ , is evaluated at the cell boundaries. A global 5 ms time step is used for transient Eqs. (3.2-3.4). Interface conductivity is represented by a harmonic mean similar to that described in [43], while a resistor representation is used between the cell center and the convection boundary conditions (3.12) and (3.13). For the two cases in which a blood vessel is modeled, an upwind scheme is adopted to represent advection in the blood vessel domain in Eq. (3.3). For the transient cases, the convection coefficient, h , used in boundary conditions (3.12) and (3.13) is updated at each time step and the fluid properties of air used to solve Eqs. (3.16) and (3.17) are evaluated at the film temperature T_f of the previous time step. For the steady state solution these properties are evaluated at the film temperature of the previous iteration. Data given in [36] is curve fit for temperature dependence of each air property. Variables T , ϕ , and Q_j are solved using an implicit point iterative method. Since it is expected that between time steps dramatic temperature changes occur not over the entire solution domain, but locally within and about the target region, a localized convergence

measure is used between iterations. The convergence criterion is defined as $\varepsilon = |T_{i,j,k}^{p+1} - T_{i,j,k}^p|$ where p is iteration and convergence is established when $\varepsilon < 10^{-5}$. The value of the convergence parameter, ε , was decreased until the maximum local percent difference (between solutions of decreasing ε value) was less than 1%. Equation (3.24) is integrated numerically. Computations were performed on a 3.0 GHz Intel Xeon processor. Typical CPU times were 8-12 hours.

3.5 Results and discussion

To validate the numerical solution, comparisons with analytic solutions were made. The temperature solution of the one-dimensional steady form of Eq. (3.1) was obtained in the vertical y -direction at a location away from the target region with a prescribed boundary conditions (3.8) and (3.12) using $h = 700 \text{ W/m}^2\text{K}$, $T_\infty = 10^\circ\text{C}$, and $T_a = 37^\circ\text{C}$. Thermal comparisons in Figure 3.3a show excellent agreement. The analytic and numerical one-dimensional (along the x -axis) solutions of the electric potential and Joule heat Eqs. (3.20) and (3.23) are obtained using SC post-electric breakdown conductivity value of $\sigma_{SC} = 0.01 \text{ S/m}$ is used and the remaining composite tissue conductivity values are the same as in Table 3.2. A Figure 3.3b shows good agreement between computed and analytic solutions.

3.5.1 Electrical solution

The distribution of Joule heat is presented in Figure 3.4. The area shown is a close-up of the target region in the x - y plane at the axial center of the target region ($z = 5 \text{ mm}$). In this case

the SC is represented using a full electroporation conductivity breakdown value, $\sigma_{SC} = 0.01 S/m$. The dashed line represents the target region boundary and the dotted lines represent the composite tissue interfaces. Since the electrical properties of the composite region shown are symmetric about the center, it is not surprising to see that the Joule heating is similarly symmetric. The two-dimensional contours in the top of the target region and local jumps (sudden increases) of the Joule heat value about the corners of the fat - tissue interface imply increased vertical current densities, reflecting the strong two-dimensional electric potential drops that result from the non-uniformity of electrical properties at these locations. In the lower section of the target region (where the composite properties do not change vertically) the contours are predominately one-dimensional (in the x -direction) with levels of highest heating occurring at locations of lowest tissue electrical conductivity. A closer look at this section is made to gain further insight into the effects of the composite variation in conductivity and specifically of the role that the SC plays in Joule heating. Figure 3.5 shows the spatial dependence of the electrical solution along the x -axis at the center of the target region ($y = 0, z = 10 \text{ mm}$) for three values of electrical conductivities of the SC ($\sigma_{SC} = 10^{-5}, 10^{-3}, \text{ and } 10^{-2} S/m$). For a very resistive SC the heating is greatest within the SC and negligible Joule heating occurring throughout the interior layers. This is explained by the obvious fact that the primary voltage drop occurs across this thin $15 \mu m$ layer. Increasing the SC conductivity allows for a larger current, noticeably increasing the potential drops across the composite layers and subsequently increasing the local Joule heating value. The local magnitudes of Joule heat added are in decreasing order SC , epidermis, dermis, fat and subcutaneous muscle tissue, which corresponds to the order of increasing composite conductivity value. It is important to note that potential drops across the composite layers of

Figure 3.5 are nearly linear mirroring those of the one-dimensional analytic profile presented in Figure 3.3.

To compare these composite findings with an electrical homogenous tissue case, consider the homogenous solutions to Eqs. (3.20) and (3.23) which are one-dimensional in the x -direction:

$$\phi(x) = V_{app} \left(\frac{1}{2} + \frac{x}{W} \right) \quad (3.25)$$

$$Q_J = \sigma \left(\frac{V_{app}}{W} \right)^2 \quad (3.26)$$

In the homogenous case both the potential drop and the Joule heat are constant throughout the target region. Assigning a homogenous conductivity value of $\sigma = 0.05 \text{ S/m}$, the resulting

constant values of potential drop and Joule heat added are $\frac{d\phi}{dx} = 400 \text{ V/cm}$ and

$Q_J = 8 \times 10^7 \text{ W/m}^3$, respectively. The spatial variations of the composite solutions contrast strongly with the solution of a homogenous domain and great care should be taken when assuming homogenous tissue properties in external plate electroporation studies.

The results of the composite electrical analysis show that the local heating is dependant not only on local composite conductivity, but also to a great extent on the conductivity of the SC .

To bridge dependence of local composite temperature rise to local property values and SC electrical conductivity, the following one-dimensional case is presented. Neglecting the vertical changes in composite property values at the top of the target region results in the electrical profile presented in Figure 3.3. Substituting Eq. (3.23) for Joule heat into the one-dimensional form of the modified Pennes' bioheat equation, Eq. (3.1), and assuming that contributions of conduction, metabolic heat generation, blood perfusion, and convection at

the boundaries are negligible over a short pulse duration, the temperature rise of each of the composite layers is:

$$\Delta T_m = \text{pulse duration} \times \frac{\sigma_m \left(\frac{d\phi_m(x)}{dx} \right)^2}{\rho_m c_m} \quad (3.27)$$

The potential drop across the composite layer, $\frac{d\phi_m(x)}{dx}$, has the analytic solution:

$$\frac{d\phi_m}{dx} = \frac{V_{app}}{\sigma_m B} \quad (3.28)$$

where B is the sum of the n composite layers' resistances: $B = \sum_{i=1}^n \left(\frac{\delta_i}{\sigma_i} \right)$ and δ is the

composite layer thickness. Substituting Eq. (3.28) into Eq. (3.27) the temperature rise is evaluated for its dependence on the value of SC conductivity using composite properties and dimensions presented in Table 3.2 and a corresponding muscle tissue thickness of 5.3 *mm*. Composite temperature rises over a range of SC conductivities presented in Figure 3.6 are representative of a single 400 *V/cm* pulse 150 *ms* in duration. As the conductivity of the SC is increased, the increase in current is reflected in temperature rises of the inner composite regions. Previous calculations show that the dermis receives more heat than the fat layer, however, due to its low heat capacity, the temperature rise of the fat layer noticeably exceed that of the dermis.

3.5.2 Thermal solution

The transient thermal solutions of the electroporation model are now addressed. The electroporation process modeled consists of five 150 *ms* long square wave pulses administered at 1 second intervals with an applied voltage of 400 *V*. Because it is anticipated

that the maximum thermal damage occurs near the axial center of the electroporated region, the x - y plane at the axial target region midpoint ($z = 5 \text{ mm}$) is chosen to depict the transient temperature and thermal damage profiles in Figures 3.7 and 3.8. Symmetry of the electrical and physical model is used so that results are shown only for the positive x half of the model. Times shown begin at the end of the last pulse cycle. To model the electrical breakdown of the SC , the conductivity of the SC is assigned a value of $\sigma_{SC} = 10^{-5} \text{ S/m}$ for the first 5 ms of the first pulse and $\sigma_{SC} = 10^{-2} \text{ S/m}$ thereafter. Transient thermal contours for the composite case are shown in Figure 3.7. Here the dashed line represents the target region boundary and the dotted line separates the composite layers. The steady solution shows that the target region (which is exposed to free convection on vertical as well as horizontal surfaces) is cooler than the rest of the tissue domain. The maximum temperature contours are presented at $t = 0 \text{ s}$ (immediately after the last pulse cycle of the electroporation process). Recalling the results of the electrical solution it is not surprising to find negligible temperature rises in the electrically conductive muscle tissue layer. However, maximum temperature rises occur along the vertical fat-dermal interface while it is expected from the results depicted in Figure 3.6 that the maximum temperature rises occur in the SC and epidermal layers. To explain this discrepancy it is noted that for each of the 1 Hz pulse cycles, Joule heat is added during the first 150 ms pulse with an 850 ms period remaining during which heat is conducted from the thin $50 \text{ }\mu\text{m}$ dermal layer to the electrode. As time passes, heat is convected out of the upper surface of the target region and conducted into the electrode and lower tissue domain. At times less than 30 seconds the location of highest temperature remains in the vertical center of the target region and about the fat composite layer. At five minutes after the last pulse cycle the temperatures of the target region have reached a level low enough to produce

negligible damage, nearly returning to steady state values. For this reason the thermal damage of Eq. (3.24) is evaluated from the beginning of the first pulse until 300 s after the end of the last pulse cycle.

The transient solution of the homogenous tissue case is presented in Figure 3.8. The thermophysical and biological parameter values used in the calculation of the homogenous transient thermal solution were evaluated as volumetric averages of those in the composite target region. The averaged values used are: $k = 0.35 \text{ W/mK}$, $\rho = 1013 \text{ kg/m}^3$, $c = 3383 \text{ J/kgK}$, $q''' = 386 \text{ W/m}^3$, and $\omega = 1.14 \text{ kg/m}^3\text{s}$. The steady temperature solution of the homogenous case is similar, but slightly lower in magnitude than that of composite solution reflecting the importance of the more insulative and perfusive properties of the dermis and fat layers of the composite case. The pulse parameters used are the same as in the composite case, however, the homogenous conductivity value of $\sigma = 0.05 \text{ S/m}$ is used which corresponds to the previously mentioned uniform target region heating value of $Q_J = 8 \times 10^7 \text{ W/m}^3$. This electrical uniformity results in a nearly uniform temperature rise (seen at $t = 0 \text{ s}$) contrasting with the local temperature rises of the composite case. At this time, evidence of heat conducted to the electrodes is noticeable by the temperature drop along the tissue – electrode boundary, while the slight vertical temperature variation at the top of the target region shows the much slower rate at which free convection contributes to the cooling. Keeping this in mind and recalling the similar cooling of the epidermis by the electrodes of the composite case, it is evident that conduction to the electrodes plays a major role in the nearby temperature distribution. As time progresses the highest temperatures remain near the center. The heated tissue cools by conducting heat to the electrodes and to

the tissue below the target region seen by the slow temperature rise there. At the end of the five minute cooling process the target region is again near its initial temperature state.

3.5.3 Thermal damage

Thermal damage of Eq. (3.24) is evaluated until five minutes after the end of the last pulse cycle and the results are presented in Figure 3.9 for two composite cases with SC conductivities $\sigma_{sc} = 10^{-3}$ and $10^{-2} S/m$ and for the homogenous case. As expected from the previous thermal analysis, damage contours of the composite cases focus locally in the fat and dermis composite layers, while maximum damage of the homogenous case is in the central target region. EM_{43} comparisons between the two composite tissues show much greater damage for the case with the higher electrical conductivity reflecting a higher rise in initial temperature. It should be noted that Eq. (3.24) does not capture damages associated with thermal removal of tissue.

3.5.4 Blood vessel

The advective effects of a $0.6 mm$ blood vessel with an averaged blood velocity of $6 mm/s$ are presented in Figure 3.10. The homogenous damage profile in the $x-y$ plane at $z = 5 mm$ show that the blood vessel significantly cools the center of the target region minimizing thermal damage near the blood vessel and reduces maximum damage levels to half of those in the case without a blood vessel (Figure 3.9). Figures 3.10a and 3.10b show the data superimposed along the $x-z$ plane at the blood vessel center ($y = 0 mm$). In the homogenous tissue comparison the blood vessel skews the damage profile in the downwind direction reflecting significant cooling of the tissue near the vessel and at the inlet of the target region. However, portions of damage profiles further away from the vessel are relatively unaffected. Since the heating of the composite model occurs primarily in the dermis and fat layers, the

low level damage profiles of the composite model show negligible effects of the presence of the blood vessel positioned several mm from the fat-dermis interface. The solid contour representing composite thermal damage in the presence of a blood vessel covers a slightly larger area in Figure 3.9b. The phenomenon of increased maximum thermal damage risk with small blood vessel presence is explored in detail in [44].

3.6 Conclusions

A transient three-dimensional model of *in vivo* skin electroporation has been developed in which the importance of representing the separate composite layers of the skin is established. The electrical solution of the skin composite model is strongly dependant on the electrical resistance of the *SC* and shows a strong one-dimensional tendency parallel to the induced electric field. The highly electrically resistive *SC* and epidermis layers receive the largest Joule heating, while in the more conductive subcutaneous muscle tissue heating is negligible. This chapter makes the assumption that the permeabilization of the *SC* is and occurs within the first 5 *ms* of pulsing. The decrease in *SC* electrical resistance associated with skin electroporation significantly increases heating of the dermis and subcutaneous fat layers. The thermal effects of *in vivo* electroporation are characterized by a sudden jump in temperature followed by a much more gradual cooling process. The low heat capacity of fat promotes temperature rises associated with Joule heating. The dermis and subcutaneous fat layers are at great risk of thermal damage with the reduction of *SC* electrical conductivity.

3.7 References

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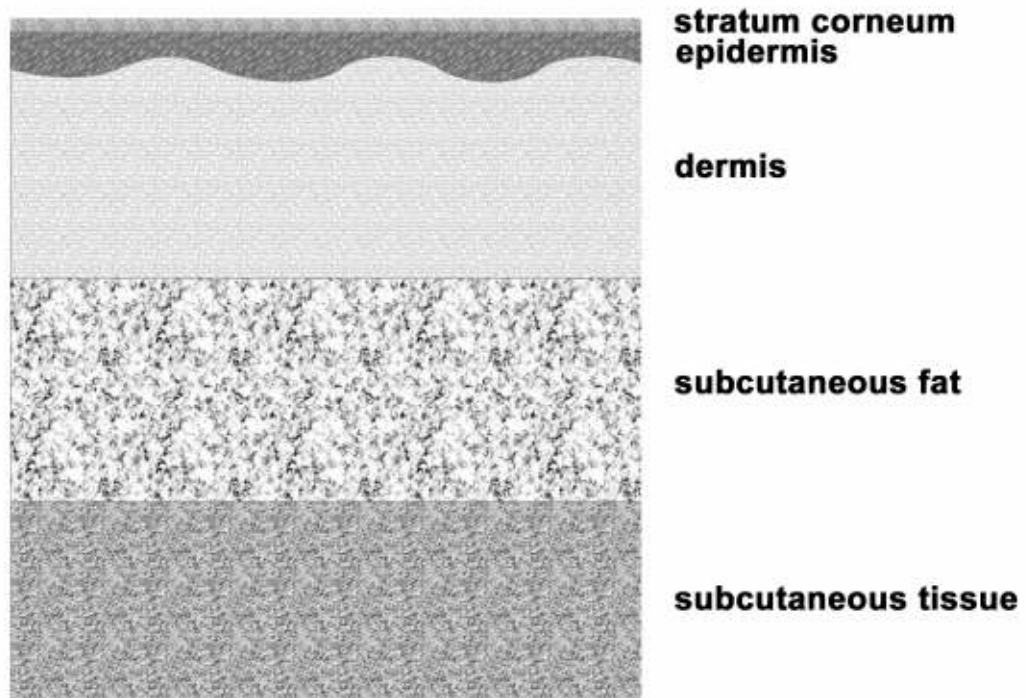


Figure 3.2 Composite skin model

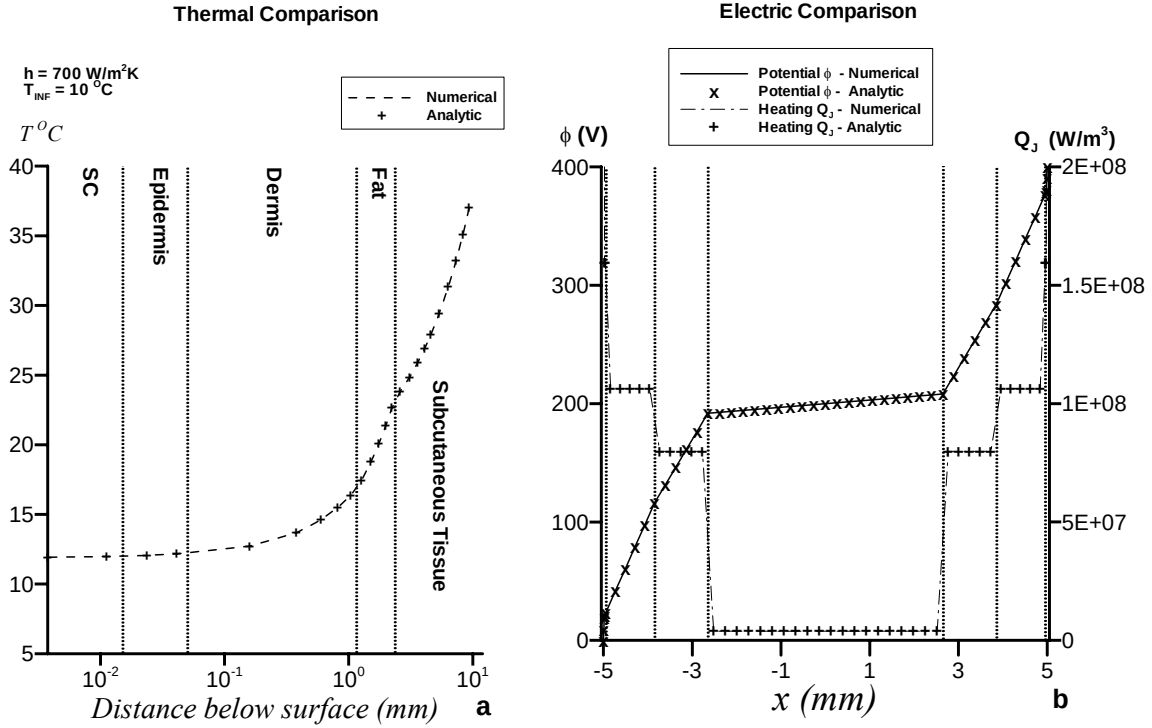


Figure 3.3 Comparisons between numerical code and analytic solution (one-dimensional): (a) Steady state temperature (°C), in which the domain represents vertical depth below the skin's surface. (b) Electric (potential and heating) in which the domain represents a horizontal line between the electrodes. Dotted lines separate composite layers.

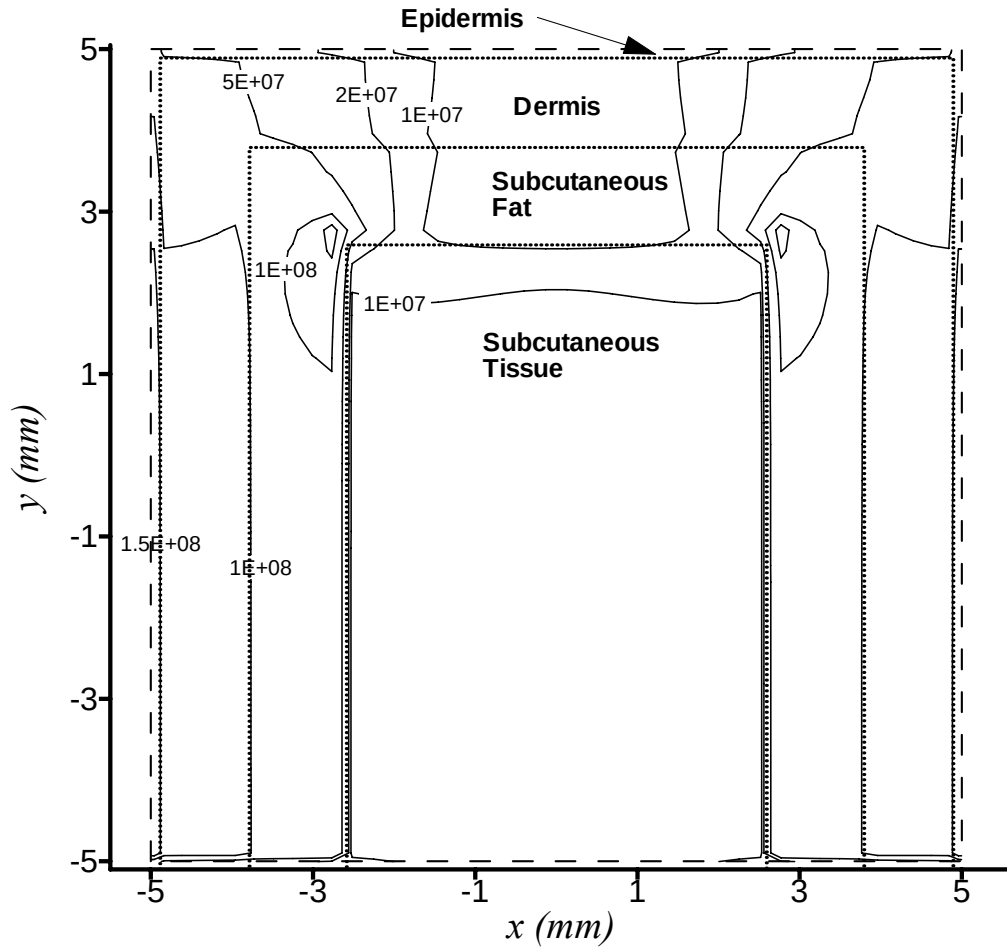


Figure 3.4 Joule heat solution (Q_J W/m^3) in the x - y plane at the axial midpoint of the target region ($z = 5$ mm) during an electroporation pulse with an applied potential of $400V$ using SC conductivity value ($\sigma_{SC} = 0.01S/m$). Dotted lines separate composite layers. Dashed line denotes target region boundary

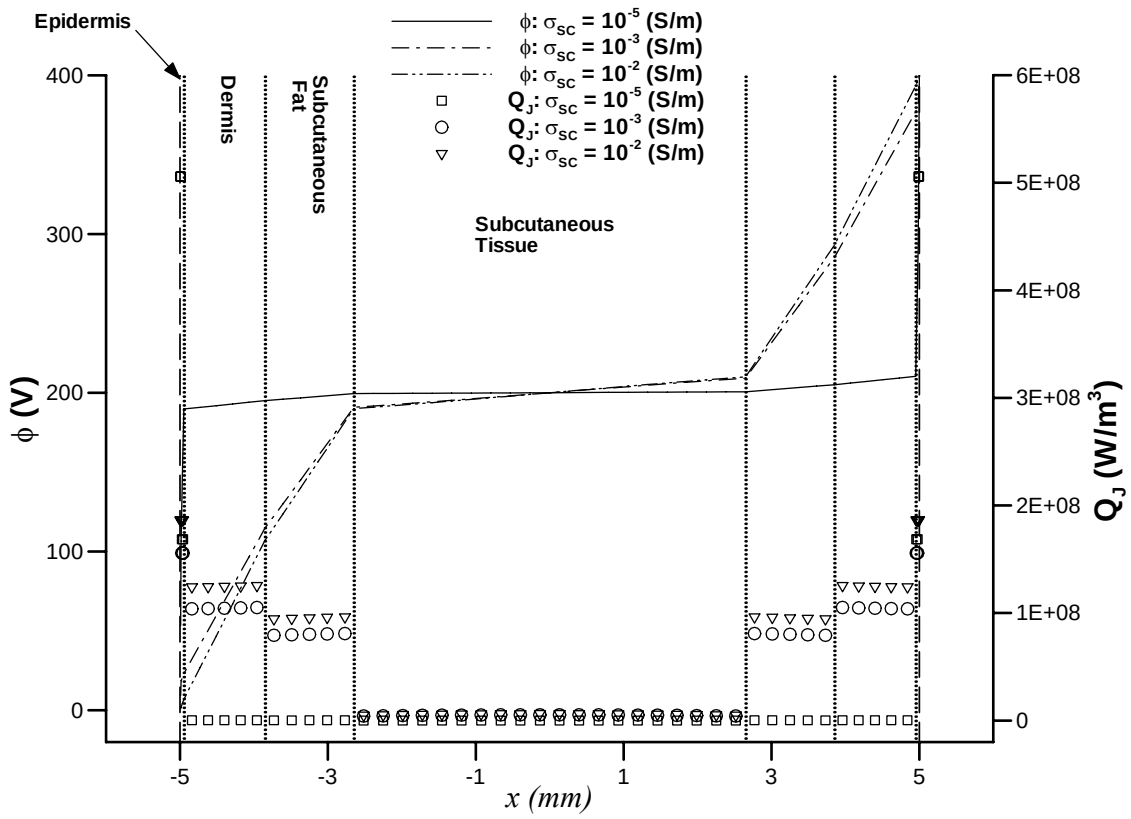


Figure 3.5 Composite tissue property effects on electrical solution during electroporation pulse: comparison effects between three representative SC conductivities. Solution shown lies between the electrodes at target region vertical and axial midpoints ($y = 0mm$, $z = 5mm$). Dotted lines denote interfaces between composite regions, long dashed lines denote skin-electrode interfaces.

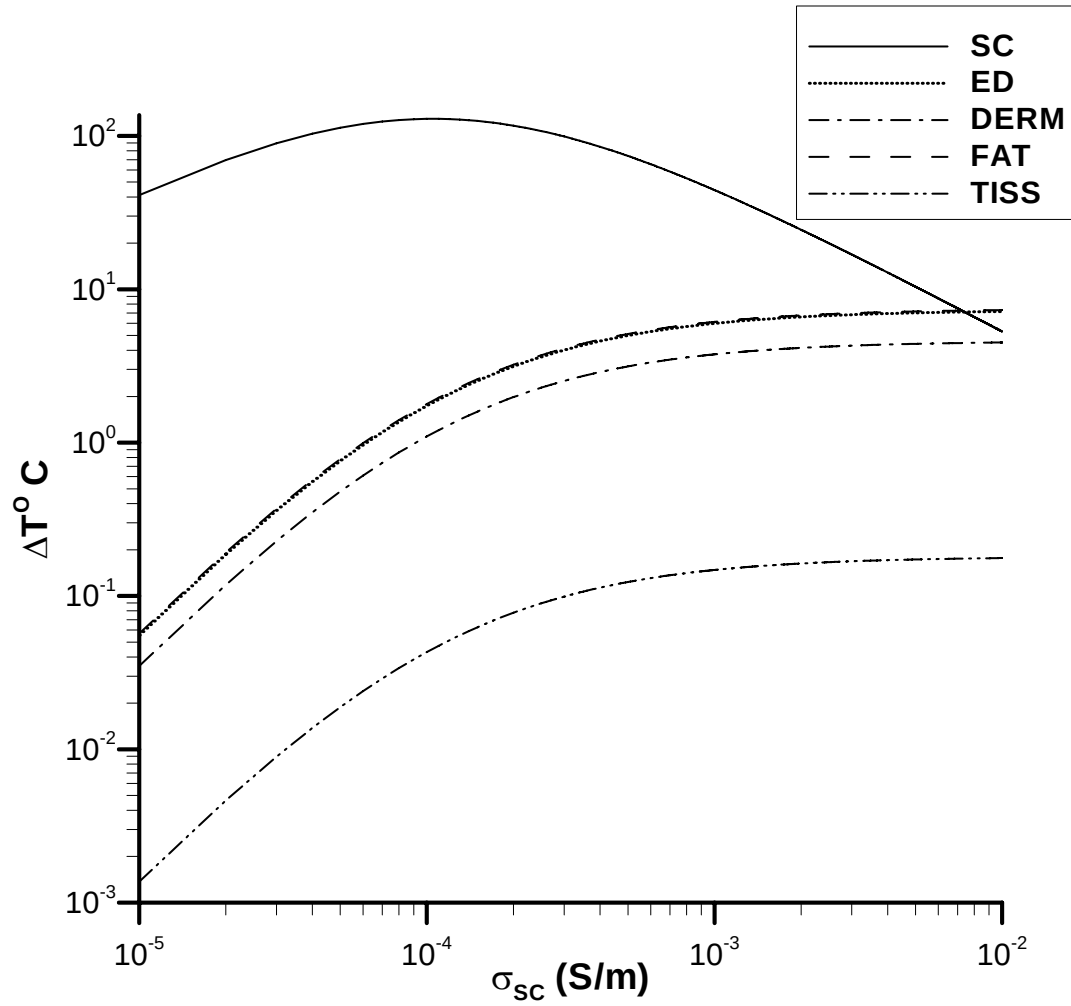


Figure 3.6 Electroporation pulse associated composite temperature rise vs. *SC* electrical conductivity. Representative electroporation pulse is of 150ms duration with an applied voltage of 400V.

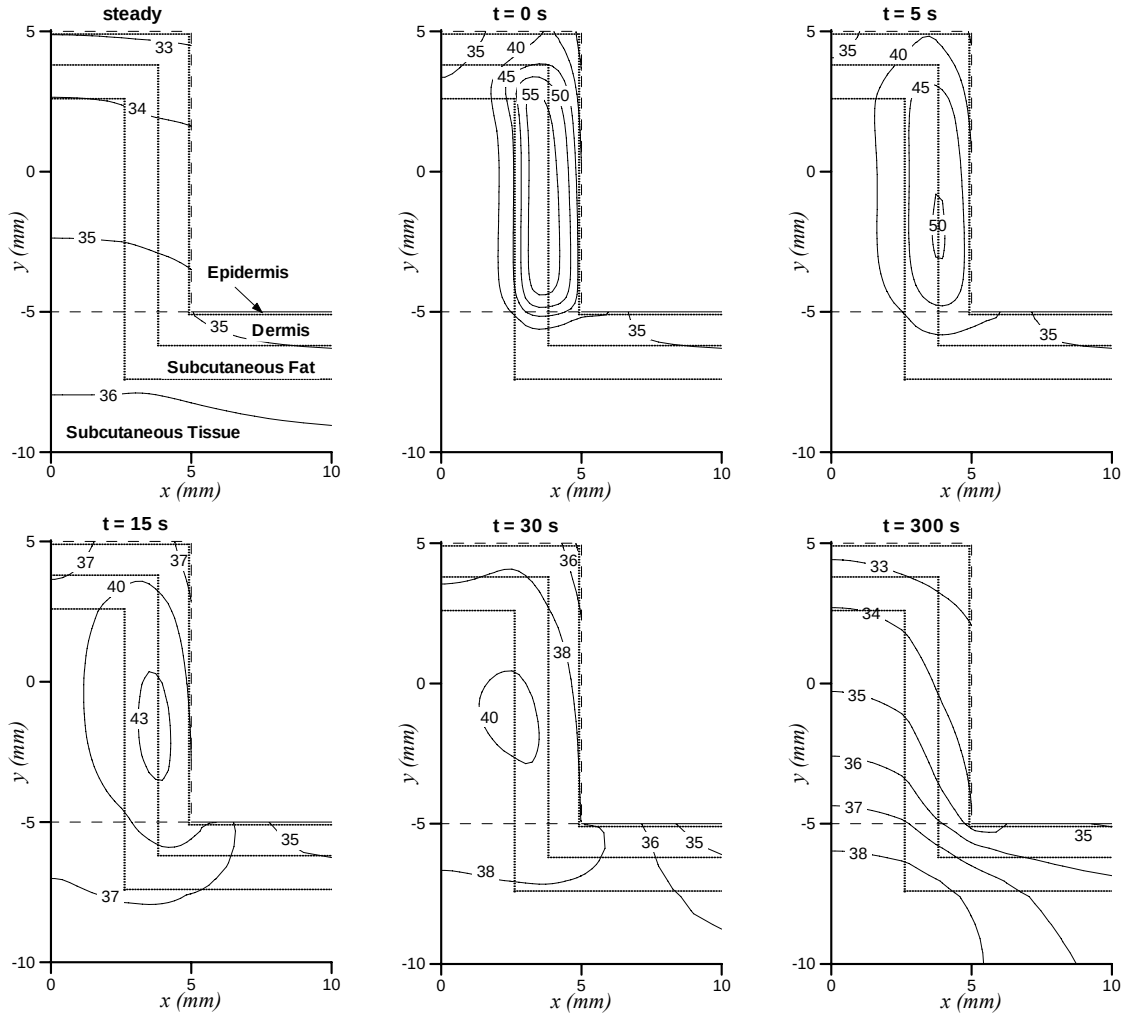


Figure 3.7 Composite model post-electroporation transient thermal solution ($^{\circ}\text{C}$) at the target region axial midpoint ($z = 5\text{ mm}$). Electroporation consisted of 5 pulses of 150 ms duration spaced 1 s apart with an applied voltage of 400 V . Dotted lines denote interfaces between composite regions, dashed lines denote the target region boundary, and times shown begin after application of the last pulse cycle.

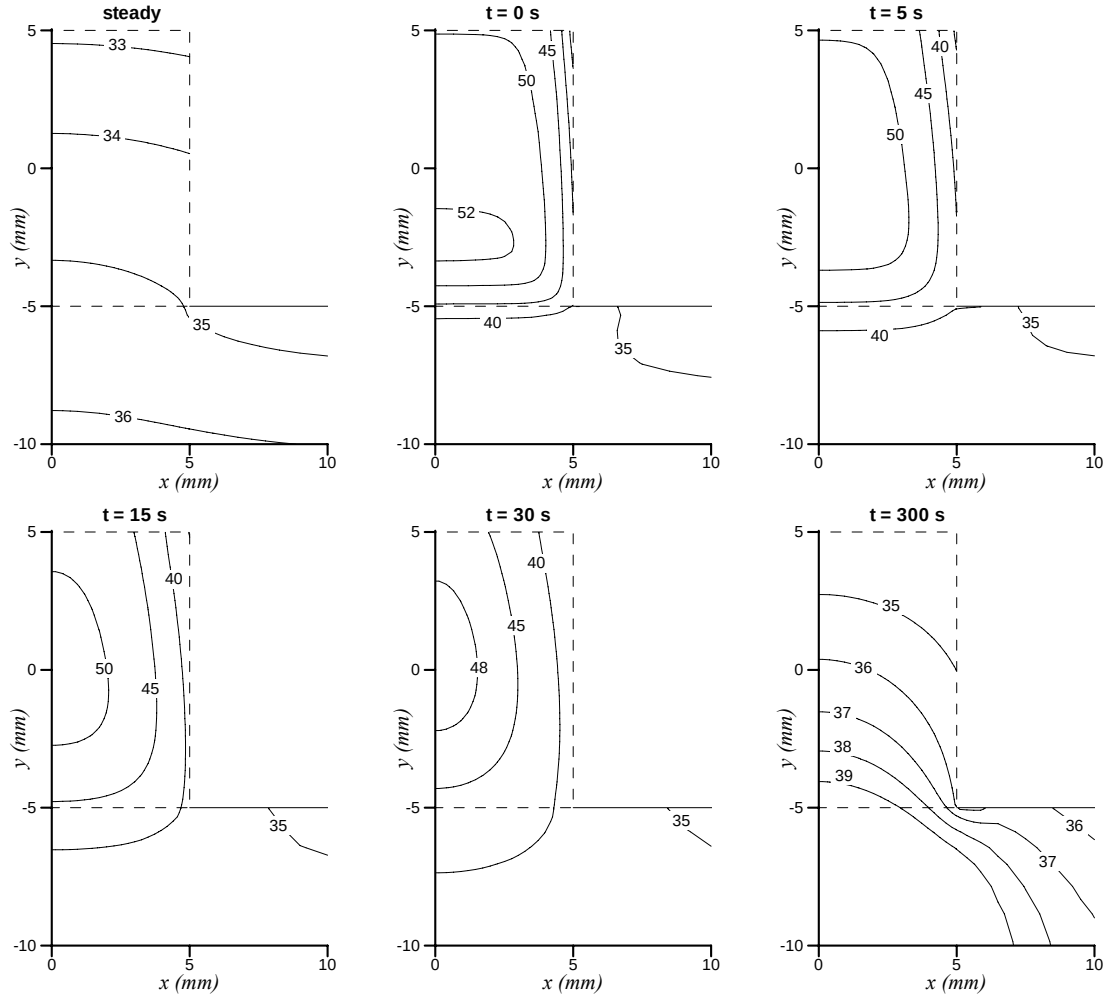


Figure 3.8 Homogenous tissue model post-electroporation transient thermal solution ($^{\circ}\text{C}$) at the target region axial midpoint ($z = 5\text{mm}$). Electroporation consisted of 5 pulses of 150ms duration spaced 1s apart with an applied voltage of 400V .

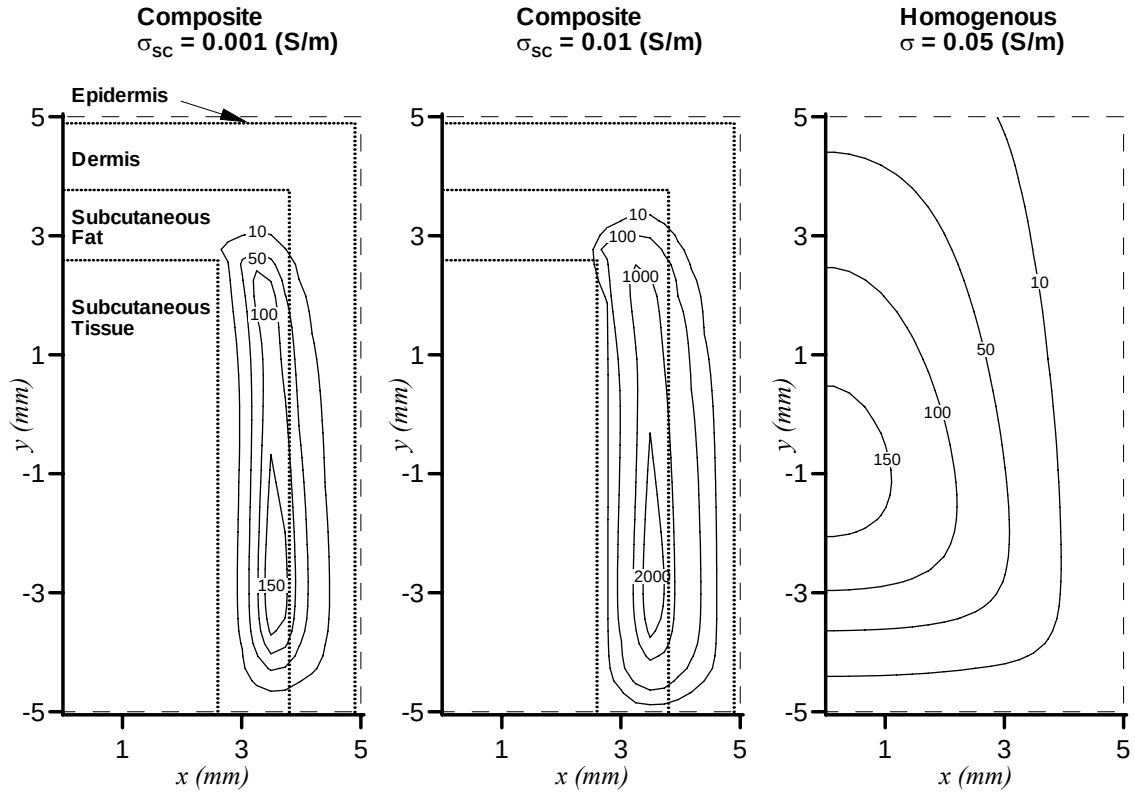


Figure 3.9 Thermal Damage comparisons: EM_{43} (minutes) evaluated at $t = 300s$ (after last pulse cycle) along the x - y plane at the axial midpoint of the target region ($z = 5mm$). Electroporation consisted of 5 pulses 150ms in duration spaced 1s apart with an applied voltage of 400V. Dashed line represents target region boundary, dotted lines represent composite tissue interfaces.

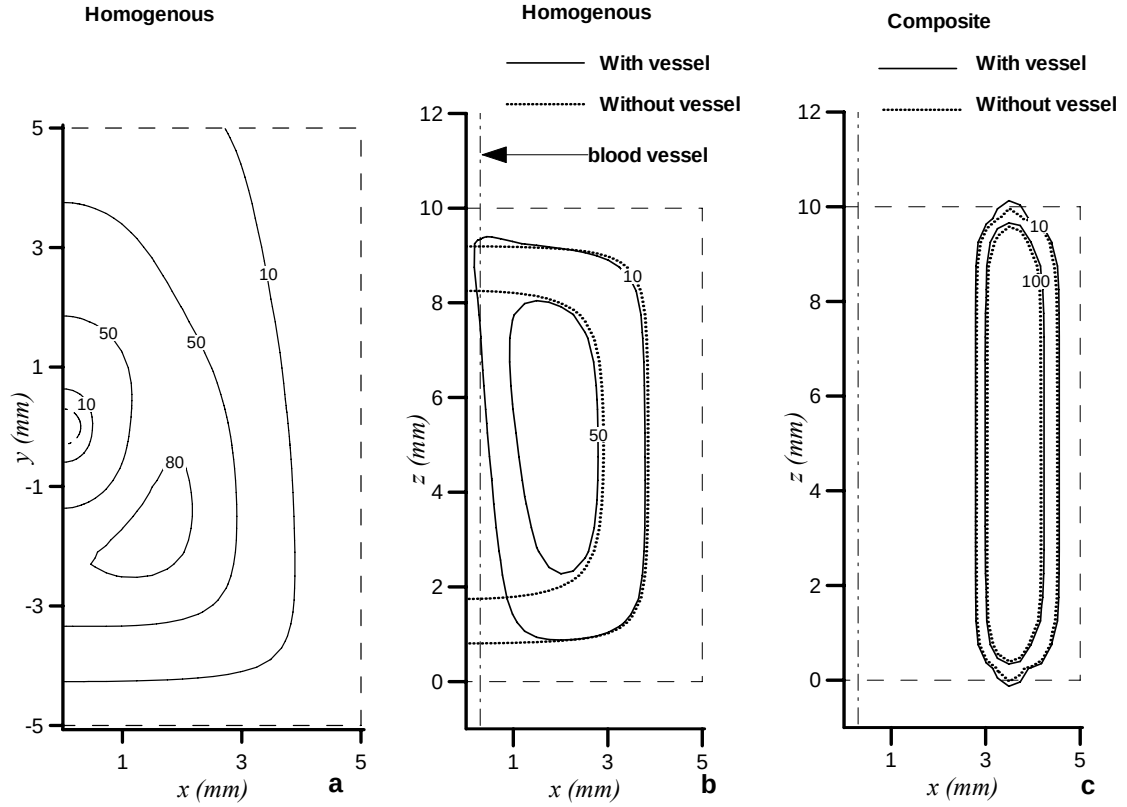


Figure 3.10 Blood vessel effects on thermal damage EM_{43} (minutes) evaluated at $t = 300s$ (after last pulse cycle): (a) Homogenous tissue model: the x - y plane at the axial midpoint of the target region ($z = 5mm$), (b) Homogenous tissue model, (c) Composite tissue model: the x - z plane at the vertical midpoint of the target region ($y = 0mm$). Electroporation consisted of 5 pulses 150ms in duration spaced 1s apart with an applied voltage of 400V. Dashed line represents target region boundary, dotted lines represent composite tissue interfaces, and dash-dot-dotted line represents position of blood vessel outer radius.

4 Thermal damage reduction associated with *in vivo* skin electroporation: a numerical investigation justifying aggressive pre - cooling

Electroporation is an approach used to enhance transdermal transport of large molecules in which the skin is exposed to a series of electric pulses. Electroporation temporarily destabilizes the structure of the outer skin layer, the stratum corneum, by creating microscopic pores through which agents, which ordinarily are unable to pass into the skin, are able to pass through this outer barrier. Of possible concern when exposing biological tissue to an electric field is thermal tissue damage associated with Joule heating. In order to find the electrical and transient thermal solutions associated with this process, this study develops a three-dimensional transient finite-volume composite model of *in vivo* skin electroporation. The electroporation process modeled consists of five 150 *ms* long DC square wave pulses administered at 1 second intervals with an applied voltage of 400 *V*. This chapter finds that minor thermal influence of the electrode plate and the of a small presence blood vessel have a large impact on thermal damage. An aggressive pre-cooling technique is presented which is shown to dramatically reduce the risk of thermal damage.

4.1 Introduction

Electroporation is a technique in which the cell membrane is altered in order to increase permeability into the cell cytosol by inducing an electric field in which the transmembrane potential exceeds a critical threshold [1-5]. The lipid bilayer matrix of the thin (10 – 20 μm) outermost layer of the skin, the stratum corneum (SC), acts as a barrier inhibiting transdermal drug delivery. It has been shown that electroporation changes the structure of the SC creating

aqueous pores [6-8]. The breakdown of the SC barrier allows for increased molecular transport and radically reduces the electrical resistance of the skin [6, 9-12].

In dealing with *in vivo* biological tissues exposed to an electric field it is important to consider the temperature rise associated with Joule heating. The severity of this temperature rise is proportional to pulse length and magnitude and is strongly tied to tissue electrical properties. Experimental results show that transdermal molecular flux rates increase with increased pulse duration and magnitude [13-16]. Experiments involving homogenous tissue electroporation have shown that the success of large molecule gene transfer is strongly tied to the amount of heat generated [17, 18]. Extensive and novel numerical studies have been conducted for homogenous tissue cases in which factors influencing thermal responses of electroporation are explored. An innovative application of electroporation is proposed in [19] in which irreversible electroporation is used to induce non-thermal killing of cancerous tissue. In [20] one and two dimensional finite element models are implemented in order to gain useful insight of the temperature related effects of physical influences such as vasculature, convection, metabolic heat generation, and electrode geometry and spacing. A homogenous tissue model of *in vivo* plate electroporation is developed in [21] in which thermal response to electroporation pulse parameters and vasculature is explored. In [22] the importance of the composite layer representation of skin electroporation is stressed. The purpose of this chapter is to not only address factors affecting thermal damage of *in vivo* skin electroporation but also to show that pre-cooling the tissue reduces the risk of thermal damage, allowing the experimentalist to use pulse parameters which increase the likelihood of successful drug delivery.

4.2 Model geometry

This chapter considers external parallel plate electroporation in which the electrodes are in direct contact with the skin. Figure 4.1 shows the geometric model representation of the system in which a small section of tissue is raised and two thin electrodes are positioned on either side of the target region (at the axial center of the system). A small blood vessel is modeled running axially through the center of the raised tissue. Table 4.1 lists the model dimensions used. The coordinate system origin lies at the target region center (the blood vessel center) for the x and y axes, and for the z axis, along the axial upwind side of the target region entrance. The blood vessel's circular circumference is modeled by a polygon of 16 equal sides. The electrode plates are represented by rectangular bars that act as cooling fins to the electroporated tissue.

A representative composite model is used to depict the skin and underlying tissue layers of the human volar forearm. The outer skin layer, the epidermis, is without vasculature. The previously mentioned outermost layer of the epidermis, the SC, is highly electrically insulative and acts as a protective barrier to the skin preventing molecule transport. The inner skin layer is the highly vascular dermis. The dermal skin blood flow exceeds metabolic requirements and the excess blood flow primarily regulates body temperature [23]. Below the dermis is a thermally insulative fat layer that acts to conserve body heat. The innermost layer is the subcutaneous tissue layer which in this study is modeled as muscle tissue. Figure 4.2 shows this composite layer model used to represent the skin and underlying tissue.

Table 4.1 Model dimensions

Symbol	Description	Value (mm)
R	Blood vessel radius	0, 0.3
W	Electrode spacing	10
H	Electrode face dimension, Target region height	10
TH	Electrode thickness	1
LF	Electrode length above Target region	100
LZM	Upwind axial length (pre-target region)	15
LZP	Downwind axial length (post-target region)	15
LYM	Vertical depth below Target region	9.805
LXM	Horizontal width past Target region	8.5

4.3 Mathematical model

4.3.1 Thermal energy

In finding the thermal solution, the system is divided into three sub-domains: vessel, composite tissue, and electrode plate.

To represent the composite tissue domain, the Pennes' bioheat equation [24] is used along with an additional source term:

$$\rho_m c_m \frac{\partial T_m}{\partial t} = \nabla \cdot (k_m \nabla T) - \omega_m c_b (T - T_a) + q_m''' + Q_J \quad (4.1)$$

where ρ is the density, c is the specific heat, k is the thermal conductivity, T is the temperature, T_a is the arterial temperature, Q_J is the Joule heat generated from the induced electric field, q_m''' is the metabolic volumetric heat generation, ω_m is the non-directional blood flow associated with perfusion, t is the time, and x , y , and z are the Cartesian coordinate

directions. The subscript m refers to one of the four composite tissue layers: stratum corneum (SC), epidermis (ED), dermis (D), subcutaneous fat (F), and subcutaneous muscle tissue (T).

Table 4.2 Composite parameter values

			SC	ED	D	F	T	b
thickness	mm	δ	0.015 [23]	0.035 [23]	1.1 [31]	1.2 [31]	-	-
thermal conductivity	W/mK	k	0.2 [27]	0.209 [30]	0.293 [30]	0.23 [28]	0.5 [28]	0.572 [28]
density	kg/m^3	ρ	1500 [28]	1110 [28]	1116 [28]	850 [29]	1040 [28]	1060 [28]
heat capacity (specific)	J/kgK	c	3600 [29]	3600 [29]	3800 [29]	2300 [29]	3800 [29]	3840 [28]
perfusion	kg/m^3s	ω	0	0	2.33 [32]	0.545 [32]	0.695 [32]	-
metabolic heat generation	W/m^3	q'''	0	0	200 [29]	5 [29]	800 [29]	-
Electrical conductivity	S/m	σ	10^{-5} [28] Pre-breakdown	0.01	0.015	0.02 [28]	0.41 [28]	0.62 [28]

Vascular lock is a phenomenon characterized and termed in [25] in which blood perfusion is obstructed after the application of an electric pulse: after the pulse is administered, blood perfusion is temporarily (depending on pulse severity) stopped. Following these results, in this model the blood perfusion of Eq. (4.1) is neglected in the target region after the first pulse application:

$$\rho_m c_m \frac{\partial T_m}{\partial t} = \nabla \cdot (k_m \nabla T_m) - A [\omega_m c_b (T_m - T_a)] + q_m''' + Q_J \quad (4.2)$$

where $A = \begin{cases} 1 & \text{outside target region} \\ 0 & \text{inside target region} \end{cases}$

The cases addressed in this study involve the effects associated with the presence of a small blood vessel in the center of the composite tissue domain. To represent the blood vessel domain, the following energy equation is used:

$$\rho_b c_b \left(\frac{\partial T_b}{\partial t} + w \frac{\partial T_b}{\partial z} \right) = \nabla \cdot (k_b \nabla T_b) + Q_J \quad (4.3)$$

where the subscript b refers to blood parameters, and w is the axial blood velocity. The effects of pulsation resulting from heartbeat are neglected and the blood is treated as a Newtonian fluid. The axial blood flow is assumed to have a parabolic profile and since the coordinate system origin lies in the axial center of the vessel, the blood velocity distribution is defined as:

$$w = 2w_{AV} \left(1 - \frac{(x^2 + y^2)}{R^2} \right) \quad (4.4)$$

where w_{AV} is the averaged blood velocity.

A representative blood vessel radius of 0.3 mm is used with the corresponding average blood velocity, w_{AV} , of 6 mm/s based on data given in [26]. The vessel wall is assumed to have the same property values as the surrounding tissue. The biological composite tissue and blood properties are presented in Table 4.2.

The electrode plate section is represented by the transient conduction equation:

$$\rho_e c_e \frac{\partial T_e}{\partial t} = \nabla \cdot (k_e \nabla T_e) \quad (4.5)$$

where the subscript e refers to electrode plate parameters. Electrode property values are given in Table 4.3. Parameter temperature dependencies are neglected in this study.

Commonly in parallel plate electroporation a conductive gel is used between the electrodes and the target region, thus in this model electrical and thermal contact resistance at the electrode-tissue interface are neglected.

Table 4.3 Parameter values

ρ_e	electrode density	7850 kg/m^3
c_e	electrode specific heat	450 J/kgK
k_e	electrode thermal conductivity	16 W/mK
T_a	arterial blood temperature	37°C
T_∞	ambient temperature	20°C
V_{app}	applied voltage	400 V

4.3.2 Boundary and initial conditions

Initial temperature distributions of the tissue and blood region domains are calculated from the steady state forms of Eqs. (4.1) and (4.3) without the induced electric field:

$$0 = \nabla \cdot (k_m \nabla T_m) - \omega_m c_b (T_m - T_a) + q_m''' \quad (4.6)$$

$$\rho_b c_b w \frac{\partial T_b}{\partial z} = \nabla \cdot (k_b \nabla T_b) \quad (4.7)$$

Blood perfusion within the target region is not neglected in Eq. (4.6) and the electrodes are not included in the model for the steady state solution. The electrodes are added to the model at the onset of the first electroporation pulse, and unless otherwise specified are left in contact with the target region for all remaining computations. Initially electrodes are assumed to have the same temperature as the surroundings, T_∞ :

$$T_e(x, y, z, 0) = T_\infty \quad (4.8)$$

The temperature of the lower face of the composite tissue domain is assumed to be equal to the arterial blood temperature, T_a :

$$T_T(x, y_{\min}, z, t) = T_a \quad (4.9)$$

The remaining boundary faces of the composite tissue domain not exposed to the ambient environment are assumed thermally insulated:

$$\begin{aligned} \frac{\partial}{\partial z} T_m(x, y, z_{\min}, t) &= \frac{\partial}{\partial z} T_m(x, y, z_{\max}, t) = 0 \\ \frac{\partial}{\partial x} T_m(x_{\min}, y, z, t) &= \frac{\partial}{\partial x} T_m(x_{\max}, y, z, t) = 0 \end{aligned} \quad (4.10)$$

The blood vessel inlet temperature is assumed to be equal to T_a while axial thermal conduction at the outlet is neglected:

$$T_b(x, y, z_{\min}, t) = T_a \quad (4.11)$$

$$\frac{\partial}{\partial z} T_b(x, y, z_{\max}, t) = 0 \quad (4.12)$$

Along the outer surface of the composite domain (the SC), a convective flux boundary condition is imposed onto all sides exposed to the ambient air:

$$q'' = h(T_{SC} - T_\infty) = -k \left. \frac{\partial T_{SC}}{\partial n} \right|_{\text{surface}} \quad (4.13)$$

where q'' is the heat flux convected from the exposed face, h is the convection coefficient, T_∞ is the ambient air temperature, and n is the normal direction to the surface.

A convective flux boundary condition is imposed onto all electrode sides exposed to the ambient air:

$$q'' = h(T_e - T_\infty) = -k_e \left. \frac{\partial T_e}{\partial n} \right|_{surface} \quad (4.14)$$

For the electrode plate, the face of the end away from the tissue domain is treated as an insulated fin tip:

$$q'' = 0 = -k_e \left. \frac{\partial T_e}{\partial y} \right|_{tip} \quad (4.15)$$

The convection coefficient, h , of boundary conditions (4.13) and (4.14) is modeled to reepresent the natural convection of air, and it is solved from:

$$h = \frac{Nu \cdot k_f}{L} \quad (4.16)$$

where Nu is the average Nusselt number over the face, k_f is the fluid film thermal

conductivity, and L is a characteristic length scale defined as : $L = \begin{cases} \text{Vertical} : H_s \\ \text{Horizontal} : \frac{A_s}{P_s} \end{cases}$ where

H_s is the vertical surface height, A_s is the horizontal surface area, and P_s is the horizontal surface periphery.

Correlations described in [33] are used to evaluate the averaged Nusselt number, Nu , as follows:

For vertical surfaces:

$$Nu = \left\{ 0.825 + \frac{0.387 \cdot Ra^{1/6}}{\left[1 + (0.492/Pr)^{9/16} \right]^{8/27}} \right\}^2 \quad (4.17)$$

For horizontal surfaces cooling:

$$Nu = 0.54 Ra^{1/4} \quad (4.18)$$

For the horizontal surfaces heating:

$$Nu = 0.27 Ra^{1/4} \quad (4.19)$$

Pr is the Prandtl number and Ra is the Rayleigh number which is defined as:

$$Ra = \frac{g\beta(T_s - T_\infty)L^3}{\alpha\nu} \quad (4.20)$$

where g is gravity, β is the coefficient of volumetric thermal expansion, α is the thermal diffusivity, ν is the kinematic viscosity, and T_s is the surface temperature. The surface temperature value used in Eq. (4.20) is obtained by averaging the temperature over the surface. The values of the fluid properties k_f , Pr, β , α , and ν are evaluated at the air film temperature, T_f , which is defined as:

$$T_f = \frac{(T_s + T_\infty)}{2} \quad (4.21)$$

4.3.3 Electric field power generation

The electrical conductivity of the SC is several orders of magnitude lower than the underlying composite layers. As the barrier function of the SC is destabilized by electroporation induced pore formation, transdermal electric currents are given less resistive routes by which to pass this barrier, thus lowering the effective SC resistivity.

Experimentally it has been shown *in vitro* that when exposed to an intense electric field the electrical resistance of the skin temporarily drops two to three orders of magnitude in a time period of micro to milliseconds depending on pulse intensity and length [6, 9, 10]. In [14] *in vitro* experiments show that for intense, short pulses the SC resistance drops from $50\text{ k}\Omega$ to $60\text{ }\Omega$ and with some SC resistance recovery occurring between pulses separated by time periods from 5 s to 5 min. To model the passive breakdown of the SC electrical resistivity, the conductivity of the SC is prescribed a published pre-breakdown value of $\sigma_{SC} = 10^{-5}\text{ S/m}$

[28] during the first 5 *ms* of the first electroporation pulse, and thereafter is increased to a value of $\sigma_{SC} = 10^{-2} \text{ S/m}$. It is assumed that the electrical properties of the SC do not recover to the pre-pulse values during the 5 *s* time interval during which the 5 pulses of 150 *ms* duration are applied. These homogenous effective SC conductivities are used in calculations as it is beyond the scope and purpose of this chapter to model localized current passage through the SC. Current passing through normal skin is believed to follow two parallel pathways: one is through the cell matrix composing the SC and the other through skin appendages such as hair follicles and sweat glands [35]. It is beyond the scope of this study to model the local pathways through the skin, so here uniform conductivities are used within composite layers. In [35] it is shown that even at moderate voltages the capacitive charging time associated with non-Ohmic behavior of the skin is very short and may be neglected at times greater than 1 *ms*. This behavior is neglected in this study.

The lipid bilayer matrix configuration of the SC is without question the greatest contributor to electrical resistance in the skin [23], and it is this layer that undergoes the greatest change in electrical properties during skin electroporation, subsequently making the SC the greatest contributor to overall increase in current density [22]. Furthermore the authors are unaware of any experimental data describing passive electrical property changes in electroporated skin tissue other than that of the SC. For these reasons only the electroporation associated changes in electrical properties of the SC are considered in this study. Epidermal and dermal electrical conductivities given in Table 4.2 have been chosen to reflect electrical resistance drop with increased depth into the skin [23]. It has been shown that effective electrical resistance of biological tissue decreases with frequency [34], and this chapter uses the electrical properties at the low frequency range.

During electroporation the induced electric field causes a temperature rise due to Joule heating. The power supplied is found as follows. The electric potential is solved from the Laplace equation:

$$\nabla \cdot (\sigma_m \nabla \phi) = 0 \quad (4.22)$$

where ϕ is the electric potential and σ_m is the composite electrical conductivity. This equation is solved using the following boundary conditions: at one electrode the electric potential is zero, while at the other electrode the potential is the same as the applied voltage,

V_{app} :

$$\phi\left(-\frac{W}{2}, y, z\right) = 0, \quad \phi\left(\frac{W}{2}, y, z\right) = V_{app} \quad (4.23)$$

where W is the distance between the electrodes. In this study a Direct current pulse is modeled with an applied voltage value of $V_{app} = 400 \text{ V}$ and a plate spacing of $W = 1 \text{ cm}$.

All other boundaries of the target area are assumed electrically insulated:

$$\frac{\partial \phi}{\partial n} = 0 \quad (4.24)$$

where n represents the normal direction to the insulated face.

The power due to Joule heating is then defined as:

$$Q_J = \sigma_m |\nabla \phi|^2 \quad (4.25)$$

where σ_m is the tissue electrical conductivity. Obviously there is no Joule heating outside the target region or at times during which the electric pulses are not applied.

In [36, 37] it is explained that an increase in current density about the electrode perimeter often occurs as a result of low electrode impedance coupled with large tissue resistance. This

chapter neglects such edge effects. The remaining system parameter values are given in Table 4.3.

4.3.4 Damage evaluation

Thermal damage occurs when tissue temperature is elevated over a period of time. In [38] a thermal dose estimate is proposed to quantify thermal damage in part so that damage may be compared between different time-at-temperature situations. In this model, the level of damage is described in a time-temperature relation as in [38] in which thermal damage is represented in minutes of exposure at 43°C by:

$$EM_{43} = \int R^{(43-T)} dt \quad (4.26)$$

where $R = \begin{cases} 0.25 & T < 43^\circ C \\ 0.5 & T > 43^\circ C \end{cases}$ and T is the temperature. In this model the thermal dose is

calculated beginning with the onset of the first applied electric pulse.

The sensitivity of biological tissue to thermal dose varies significantly between animal species and specific tissue type [39]. While it is reported for pig muscle, fat and skin tissues that significant chronic histological damage and gross appearance thermal dose thresholds begin at values $EM_{43} > 80 \text{ min}$ [39], values of EM_{43} in the range 240-340 minutes have been used to denote permanent thermal tissue damage for muscle, liver and kidney tissues[26, 38-40].

4.4 Discretization and solution procedure

The computational domain is discretized using a variable rectangular grid consisting of 112,760 cells for cases without a blood vessel and 158,720 cells for the case with a blood vessel. A fully implicit finite volume approach is used to solve Eqs. (4.1-4.3), (4.5-4.7),

(4.22), and (4.25). Temperature, T , and Joule heat, Q_j , are evaluated at the cell centers while the electric potential, ϕ , is evaluated at the cell boundaries. A global 5 ms time step is used for transient Eqs. (4.1-4.3), (4.5). Interface conductivity is represented by a harmonic mean similar to that described in [41], while a resistor representation is used between the cell center and the convection boundary conditions (4.13) and (4.14). An upwind scheme is adopted to represent advection in the blood vessel domain of Eq. (4.3). For the transient cases, the convection coefficient, h , used in boundary conditions (4.13, 4.14) is updated at each time step and the fluid properties used to solve Eqs. (4.17-4.19) are evaluated at the film temperature T_f of the previous time step. For the steady state solution these properties are evaluated at the film temperature of the previous iteration. Data given in [33] is curve fit for temperature dependence of each fluid property. Variables T , ϕ , and Q_j are solved using an implicit point iterative method. Since it is expected that between time steps dramatic temperature changes occur not over the entire solution domain, but locally within and about the target region, a localized convergence measure is used between iterations. The convergence criterion is defined as $\varepsilon = |T_{i,j,k}^{p+1} - T_{i,j,k}^p|$ where p is iteration and convergence is established when $\varepsilon < 10^{-5}$. The value of the convergence parameter, ε , was decreased until the maximum local percent difference (between solutions of decreasing ε value) was less than 1%. Equation (4.26) is integrated numerically. Computations were performed on a 3.0 GHz Intel Xeon processor. Typical CPU times were 8-12 hours.

4.5 Results and Discussion

To validate the numerical solution, comparisons with analytic solutions were made. The temperature solution of the one-dimensional steady form of Eq. (4.1) was obtained in the

vertical y -direction at a location away from the target region with a prescribed boundary conditions (4.9) and (4.13) using $h = 700 W/m^2 K$, $T_\infty = 10^\circ C$, and $T_a = 37^\circ C$. The one-dimensional solutions of the electric potential and Joule heat Eqs. (4.22) and (4.25) are obtained in the horizontal x -direction at the center of the target region ($y = 0$, $z = 5 \text{ mm}$) without the blood vessel. A SC post-electric breakdown conductivity value of $\sigma_{SC} = 0.01 S/m$ is used and the remaining composite tissue conductivity values are the same as in Table 4.2. Figure 4.3 shows good agreement between computed and analytic solutions.

4.5.1 Electrical solution

The electrical solutions of Eqs. (4.22) and (4.25) are found for a DC electroporation pulse with an applied voltage $V_{app} = 400 \text{ V}$. The distribution of Joule heat is presented in Figure 4.4. The area shown is a close-up of the target region in the x - y plane at the axial center of the target region ($z = 5 \text{ mm}$). In this case the SC is represented at full electroporation breakdown with an electrical conductivity value of $\sigma_{SC} = 0.01 S/m$. The dashed line represents the target region boundary and the dotted lines represent the composite tissue interfaces. The blood vessel is represented by the circle in the center. The symmetry of the Joule heat solution reflects the symmetry of the composite model electrical property values. Large vertical current densities resulting from the non-uniformity of electrical properties of the composite system about the corners of the composite interfaces are implied by the two-dimensional increases in the Joule heat contour values especially about the corners of the fat-dermis interface. In the lower section of the target region (where the composite properties do not change vertically) the contours are predominately one-dimensional in the x -direction with

levels of highest heating occurring at locations of lowest tissue electrical conductivity. To gain further insight into the electrical solution variations with respect to composite layer, Figure 4.5 shows the electrical solution along the x -axis at the center of the target region ($y = 0, z = 5 \text{ mm}$) for a SC electrical conductivity value of $\sigma_{sc} = 10^{-2} \text{ S/m}$. At the blood vessel tissue interface the slight increase in current density is reflected by the increase in Joule heat. The local magnitudes of Joule heat added are in decreasing order SC, epidermis, dermis, fat and subcutaneous muscle tissue, which corresponds to the order of increasing composite conductivity value. The strong one-dimensional tendencies of the potential drops across the composite layers of Fig. 5 are nearly linear, mirroring those of the one-dimensional analytic profile presented in Figure 4.3.

4.5.2 Thermal solution

The transient thermal solutions of the electroporation model are now addressed. The electroporation process modeled consists of five 150 ms long DC square wave pulses administered at 1 second intervals with an applied voltage of 400 V . Two representative planes are chosen to depict the thermal and damage solutions: the x - y plane at the axial target region midpoint $z = 5 \text{ mm}$ (Figures 4.6-4.9, and 4.11-4.12) and the x - z plane at the blood vessel center $y = 0 \text{ mm}$ (Figures 4.8 and 4.12). Symmetry of the electrical and physical model is utilized so that results are shown only for the positive x half of the model. To model the electrical breakdown of the SC, the conductivity of the SC is assigned a value of $\sigma_{sc} = 10^{-5} \text{ S/m}$ for the first 5 ms of the first pulse and $\sigma_{sc} = 10^{-2} \text{ S/m}$ thereafter. Transient thermal contours are shown in Figure 4.6 where times shown begin at the end of the last pulse cycle. The maximum temperature contours are presented at $t = 0 \text{ s}$ (immediately after

the last pulse cycle of the electroporation process). Recalling the results of the electrical solution it is not surprising to find negligible temperature rise in the least electrically resistive layer, the subcutaneous muscle tissue. From the results depicted in Figures 4.4 and 4.5 it is expected that the maximum temperature rises occur in the SC and epidermal layers, and clearly this is not the case in the thermal solution. To explain this discrepancy it is noted that for each of the 1 Hz pulse cycles, Joule heat is added during the first 150 *ms* pulse with an 850 *ms* period remaining during which heat is conducted from the thin 50 μm dermal layer to the thermally conductive electrode. The maximum temperature rises occur along the vertical fat-dermis interface reflects the low specific heat of the fat layer. As time passes, evidence of heat conducted to the electrodes is noticeable by the temperature drop along the tissue-electrode boundary, while the slight vertical temperature variation at the top of the target region shows the much slower rate at which free convection contributes to the cooling. At times less than 45 seconds the location of highest temperature remains in the vertical center of the target region and about the fat composite layer. At five minutes after the last pulse cycle the temperatures of the target region have reached a level low enough to produce negligible damage. For this reason the thermal damage of Eq. (4.26) is evaluated from the beginning of the first pulse until 300 *s* after the end of the last pulse cycle.

To get a closer look at the electrode influence, thermal damage comparisons are conducted between the scenario in which the electrodes are removed immediately after the last pulse cycle and one in which the electrodes remain in place during the entire 5 minutes after electrical pulse. In Figure 4.7 the thermal damage solution of both cases yields nearly identical high damage contours about the vertical fat-dermis interface. Recalling the higher temperature contours of Figure 4.6, this result is anticipated. However the electrodes'

cooling effects are evident in the lower level damage profiles. Post electroporation electrode removal results in slower cooling of the outer dermis and epidermis composite layer as evidenced by the tendency of lower level damage profiles to extend to the edge of the target region.

Vascular effects are investigated in Figure 4.8 for which EM_{43} profiles are shown comparing the cases with and without the presence of a small blood vessel. The low and moderate level contours are primarily very similar, which suggests no direct interaction between the heated fat-dermis interface and the blood vessel. In this case the two are separated by a distance of nearly 3 mm . However an unanticipated result is seen in both the $x-z$ and $x-y$ planes which clearly shows that the case with the blood vessel reaches a greater maximum thermal damage value; the blood vessel acts to add heat to the target region. A comparison between steady state solutions of Figure 4.9 shows that the blood vessel advects heat into the target region slightly raising the steady state temperature uniformly. Recalling that the steady state solutions are used as initial conditions, it follows that the maximum temperature achieved during electroporation is slightly higher for the blood vessel case. Because the vessel adds heat to the muscle tissue, the conduction of heat to from the high temperature fat layer into to the muscle tissue is inhibited. This results in a slower cooling of the high temperature region which explains the associated higher maximum damage values.

4.5.3 Aggressive cooling technique

The sensitivity of thermal damage found from the results of Figures 4.8 and 4.9 prompted the authors to conduct a theoretical exercise. If the small elevated temperature associated with the vessel greatly contributes to total thermal damage, should not the reverse hold true as well? An aggressive cooling technique is proposed in which the temperature of the target

region is reduced by exposing the model forearm to a cold water bath at $T_{\infty} = 10^{\circ}C$ for one minute (water properties are used in Eqs. (4.13) and (4.17-4.19)). Then a recovery time period of one minute is allowed in which the tissue is exposed to the ambient air at $T_{\infty} = 10^{\circ}C$ (air properties are used in Eqs. (4.13) and (4.17-4.19)). This recovery time is added primarily to model the time period during which an experimentalist would prepare the target region site prior to the application of the electrodes. Immediately following the recovery period, the electrodes are modeled in place and the electroporation pulse cycles are administered. A representative flow diagram of the procedure is given in Figure 4.10. Figure 4.11 shows the temperature profiles during aggressive cooling and subsequent recovery heating as well as the maximum temperature profile of the system following electroporation. Noting the difference in temperature profiles just prior to electroporation (60 s recovery for the pre-cooled case and steady state for the un-cooled case) it is not surprising that after electroporation, high temperature profiles associated with the cooling technique (seen at $t = 0$ s of Figure 4.11) are nearly $5^{\circ}C$ lower than those of the un-cooled case depicted in Figure 4.6. Comparing the pre-cooled thermal damage profiles of Figure 4.12 to those of the un-cooled profiles of Figure 4.8, it is obvious that using an aggressive cooling technique results in much lower thermal damage. Cooling the target region prior to electroporation results in a lower maximum temperature and greatly reduces the risk of thermal damage. It should be noted that reduced perfusion associated with decreased skin surface temperature resulting in slower cooling is not modeled in this study, which means that in practice greater aggressive cooling is required to lower target region temperature. Furthermore in this study during the cooling the physical model represented the raised configuration of Figure 4.1. In practice the target region may not be raised during cooling which results in a lower convection affected.

Again to achieve similar target region temperatures, in practice greater cooling requirements are expected.

4.6 Conclusions

A transient three-dimensional model of *in vivo* skin electroporation representing the composite layers of the skin has been developed in which electrical and transient thermal solutions are found. The electrical solution of the skin composite model shows a strong one-dimensional tendency parallel to the induced electric field. The highly electrically resistive SC and epidermis layers receive the largest Joule heating, while in the more conductive subcutaneous muscle tissue heating is negligible. The dermis and subcutaneous fat layers are at great risk of thermal damage. A blood vessel running deep in the subcutaneous muscle tissue indirectly increases the maximum value of thermal damage by adding heat to the target region. Leaving the electrodes attached to the skin after electroporation promotes cooling, thereby reducing thermal damage of the outer composite layers. This promotes research directed towards increasing the cooling capacity of electroporation calipers. Effectively cooling tissue prior to electroporation reduces the risk of thermal damage, allowing the experimentalist to use pulse parameters which increase the likelihood of successful drug delivery.

4.7 References

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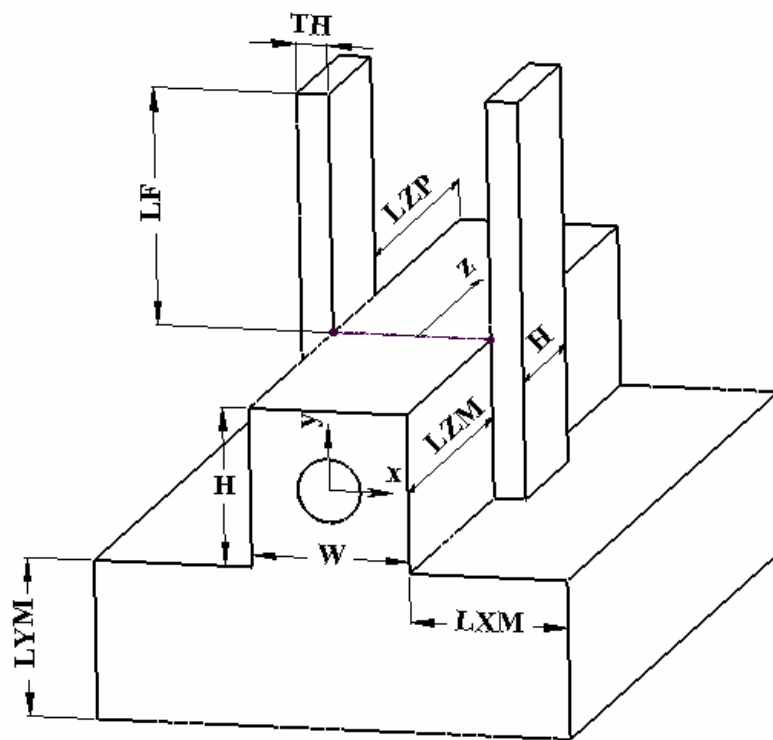


Figure 4.1 Model geometry

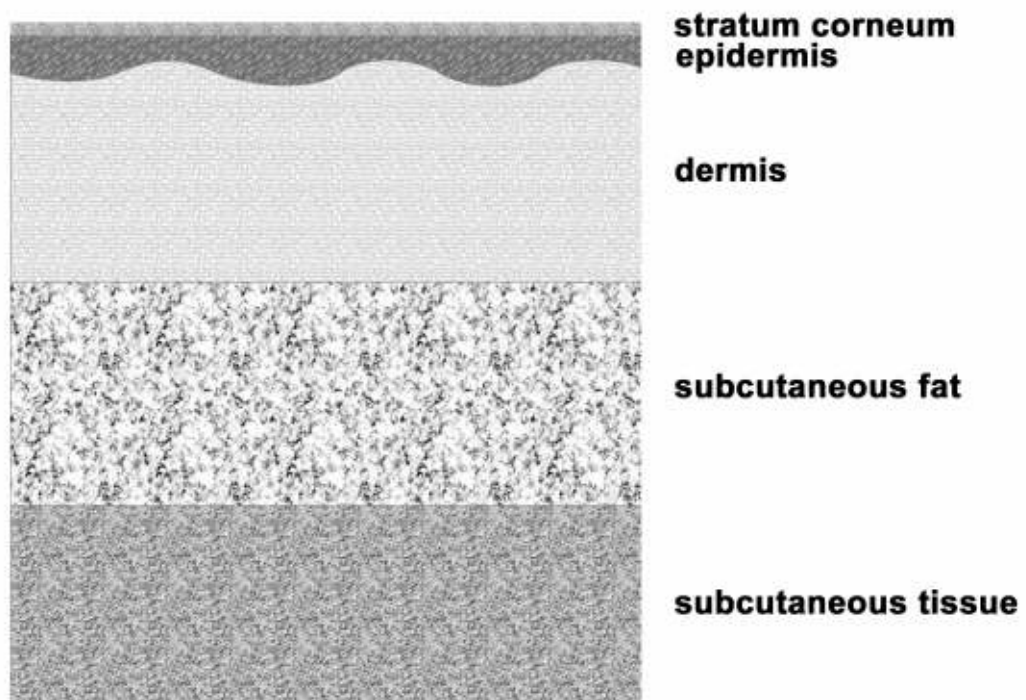


Figure 4.2 Composite skin model

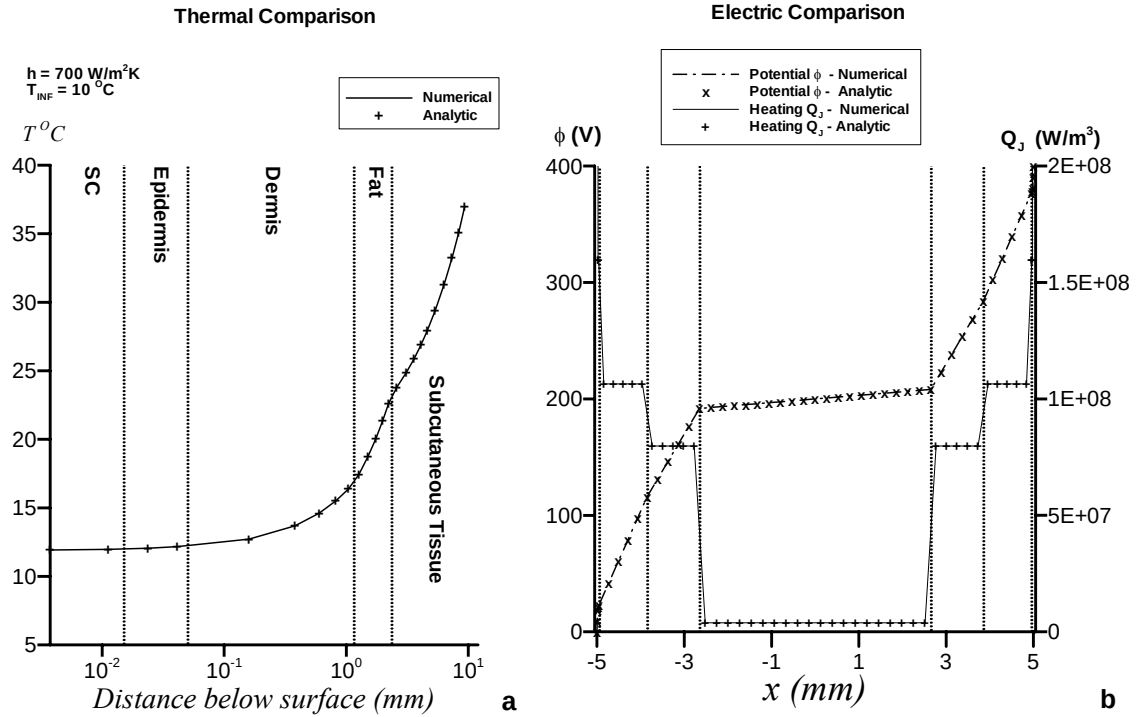


Figure 4.3 Comparisons between numerical code and analytic solution (one-dimensional). (a) Steady state temperature ($^{\circ}\text{C}$), in which the domain represents vertical depth below the skin's surface. (b) Electric (potential and heating) in which the domain represents a horizontal line between the electrodes. Dotted lines separate composite layers.

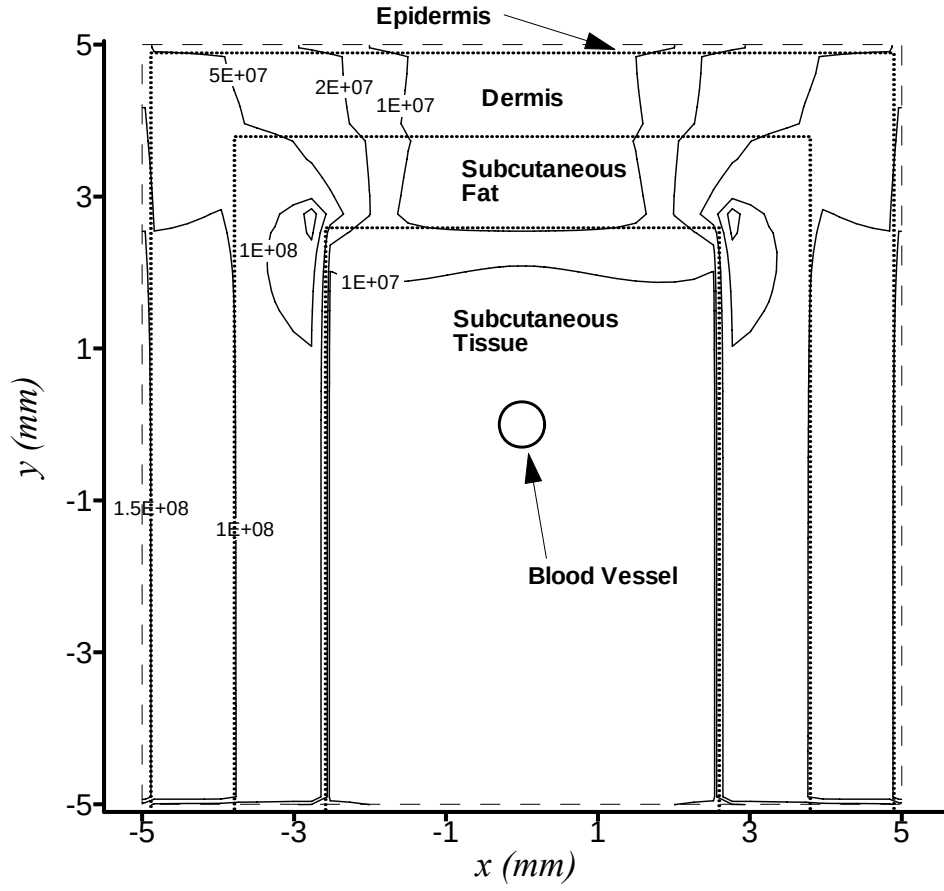


Figure 4.4 Joule heat solution ($Q_J W/m^3$) in the x - y plane at the axial midpoint of the target region ($z = 5mm$) during an electroporation pulse with an applied potential of $400V$ using SC conductivity value ($\sigma_{SC} = 0.01S/m$). Dotted lines separate composite layers. Dashed line denotes target region boundary.

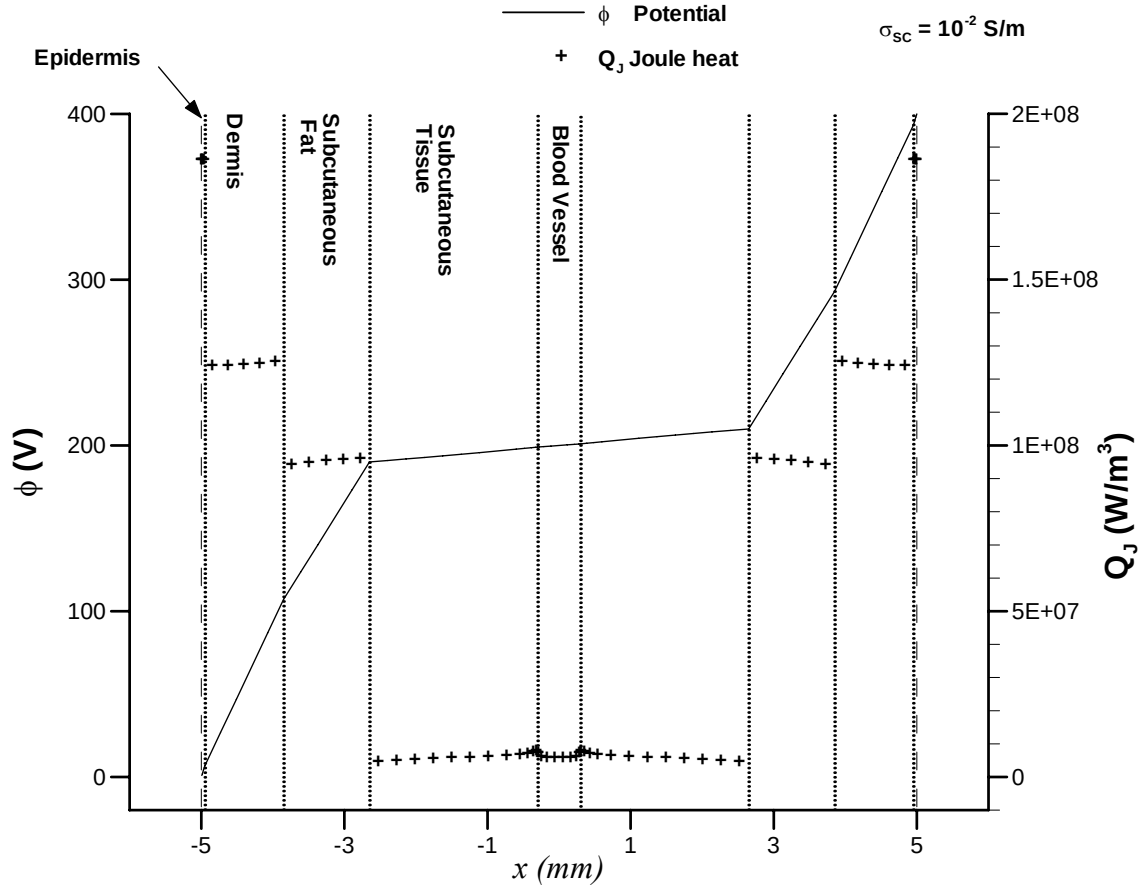


Figure 4.5 Composite tissue property effects on electrical solution during electroporation pulse. Solution shown lies between the electrodes at target region vertical and axial midpoints ($y = 0\text{mm}$, $z = 5\text{mm}$). Dotted lines denote interfaces between composite regions, dashed lines denote skin-electrode interfaces, and SC conductivity used denotes post-EP breakdown value.

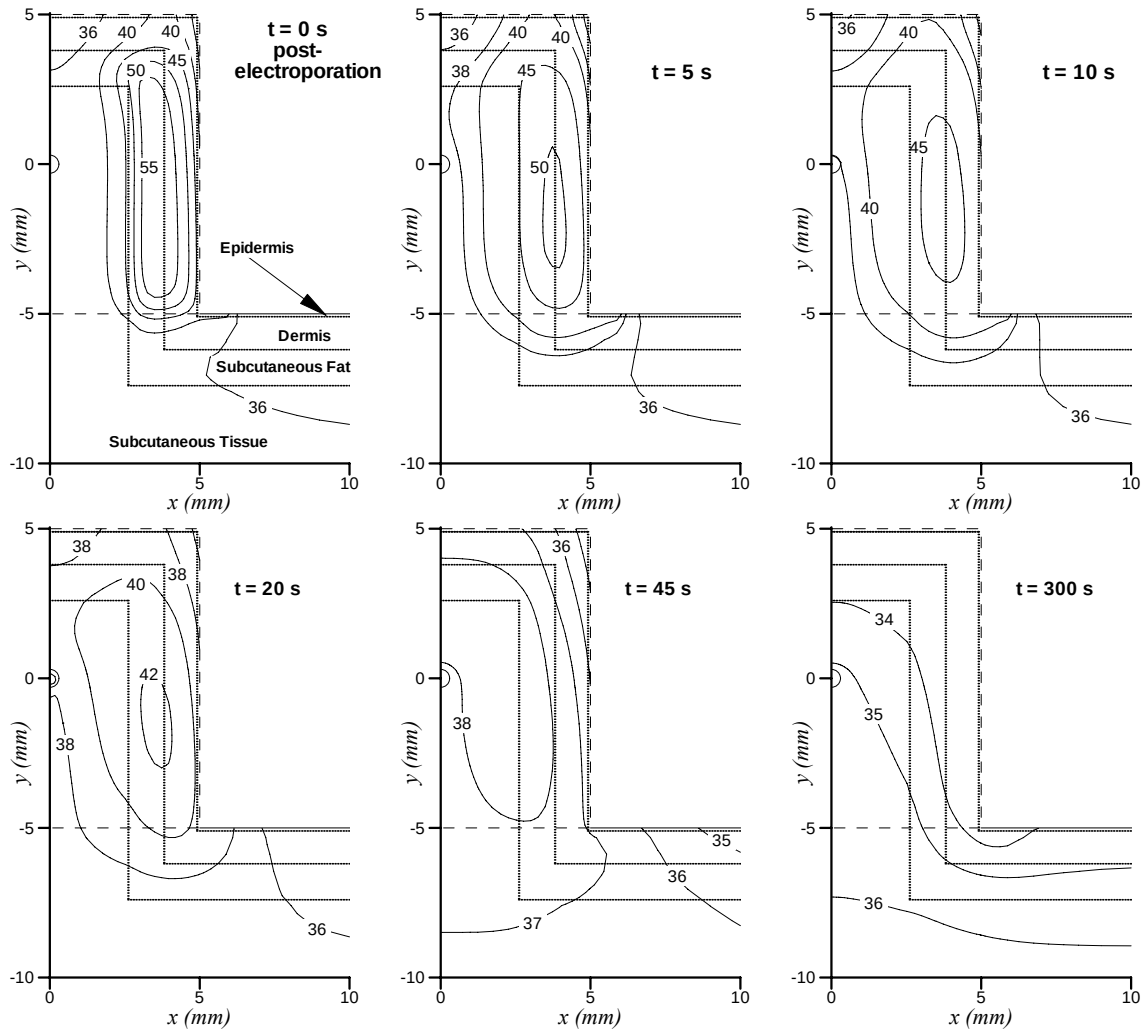


Figure 4.6 Post electroporation transient thermal solution ($^{\circ}\text{C}$) at the target region axial midpoint ($z = 5\text{mm}$).

Electroporation consisted of 5 pulses of 150ms duration spaced 1s apart with an applied voltage of 400V.

Dotted lines denote interfaces between composite regions, dashed lines denote the target region boundary, and times shown begin after application of the last pulse cycle.

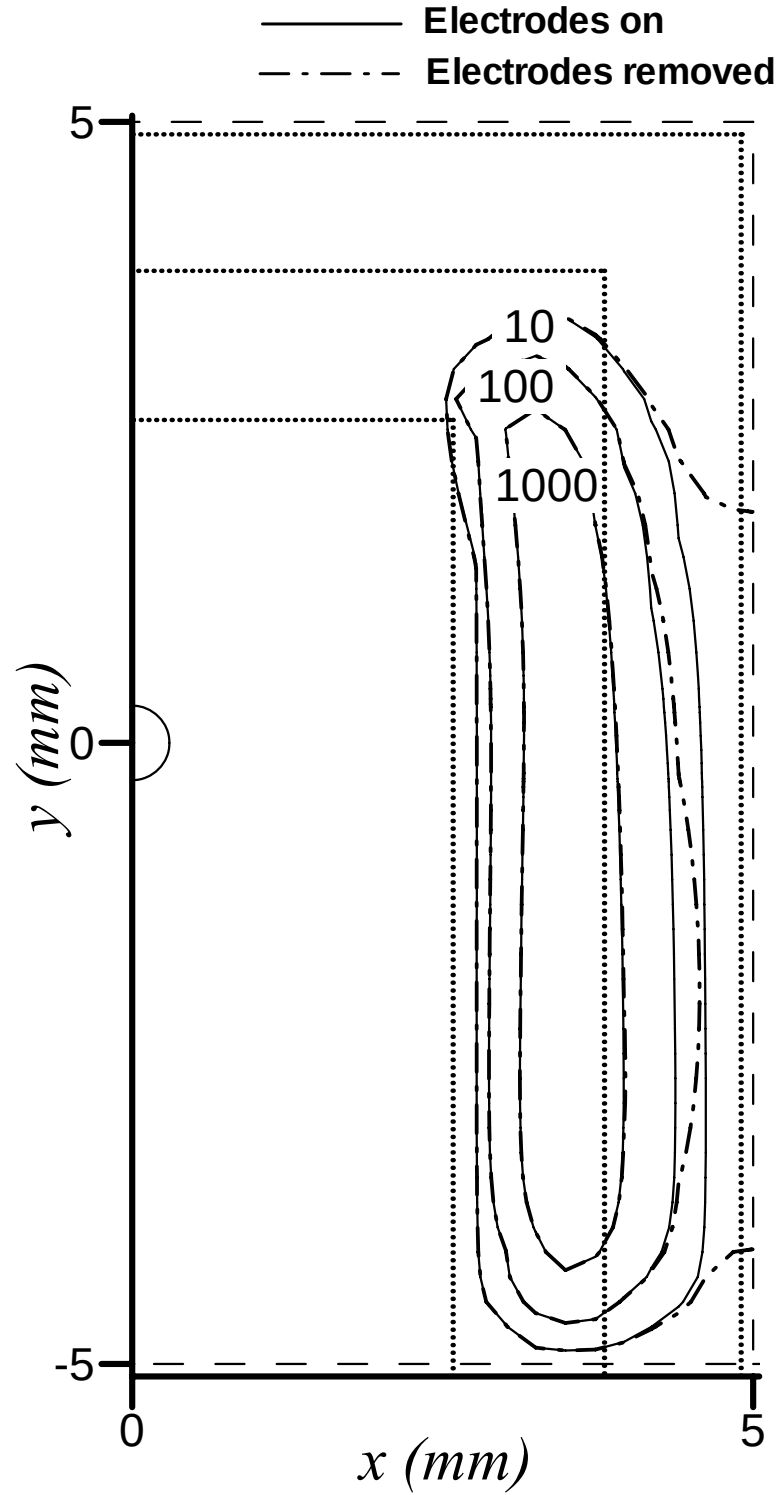


Figure 4.7 Electrode effects on thermal damage EM_{43} (minutes) evaluated at $t = 300s$ (after last pulse cycle) at the axial midpoint of the target region ($z = 5mm$). Electroporation consisted of 5 pulses $150ms$ in duration spaced $1s$ apart with an applied voltage of $400V$. Dotted lines denote interfaces between composite regions, dashed lines denote the target region boundary.

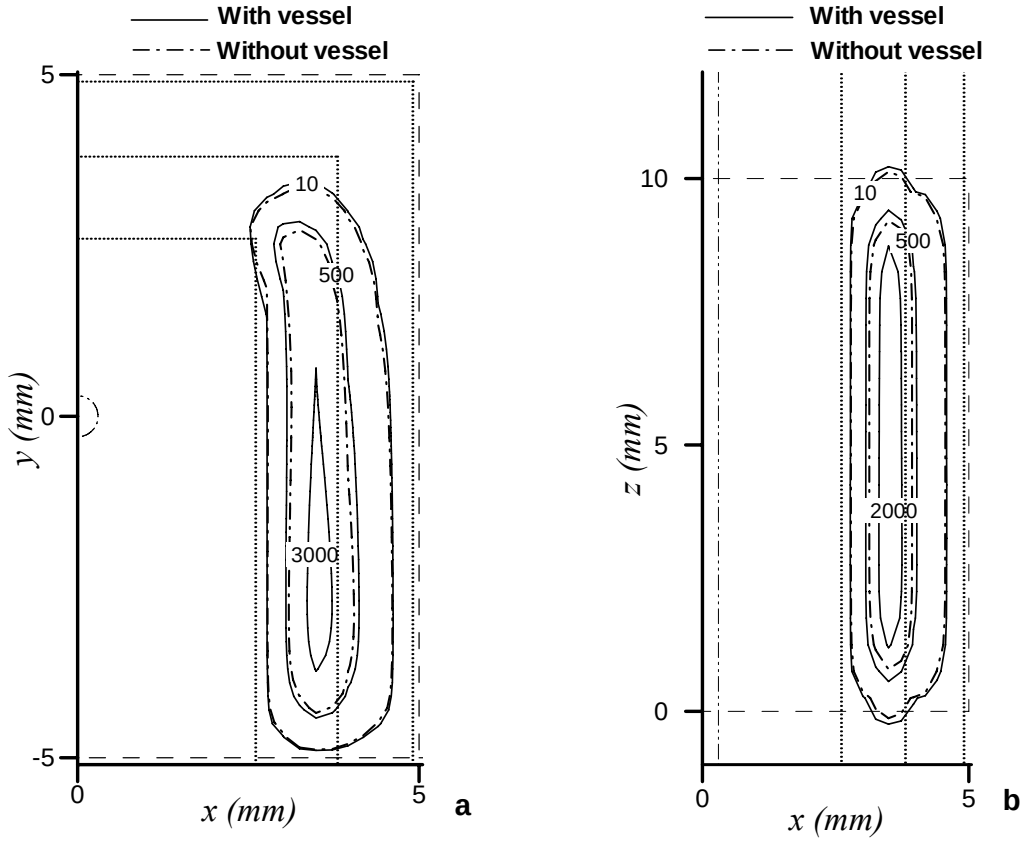


Figure 4.8 Blood vessel effects on Thermal damage EM_{43} (minutes) evaluated at $t = 300\text{ s}$ (after last pulse cycle): (a) the x - y plane at the axial midpoint of the target region ($z = 5\text{ mm}$), (b) the x - z plane at the vertical midpoint of the target region ($y = 0\text{ mm}$). Electroporation consisted of 5 pulses 150 ms in duration spaced 1 s apart with an applied voltage of 400 V . Dashed line represents target region boundary, dotted lines represent composite tissue interfaces, and dash-dot-dotted line represents position of blood vessel outer radius.

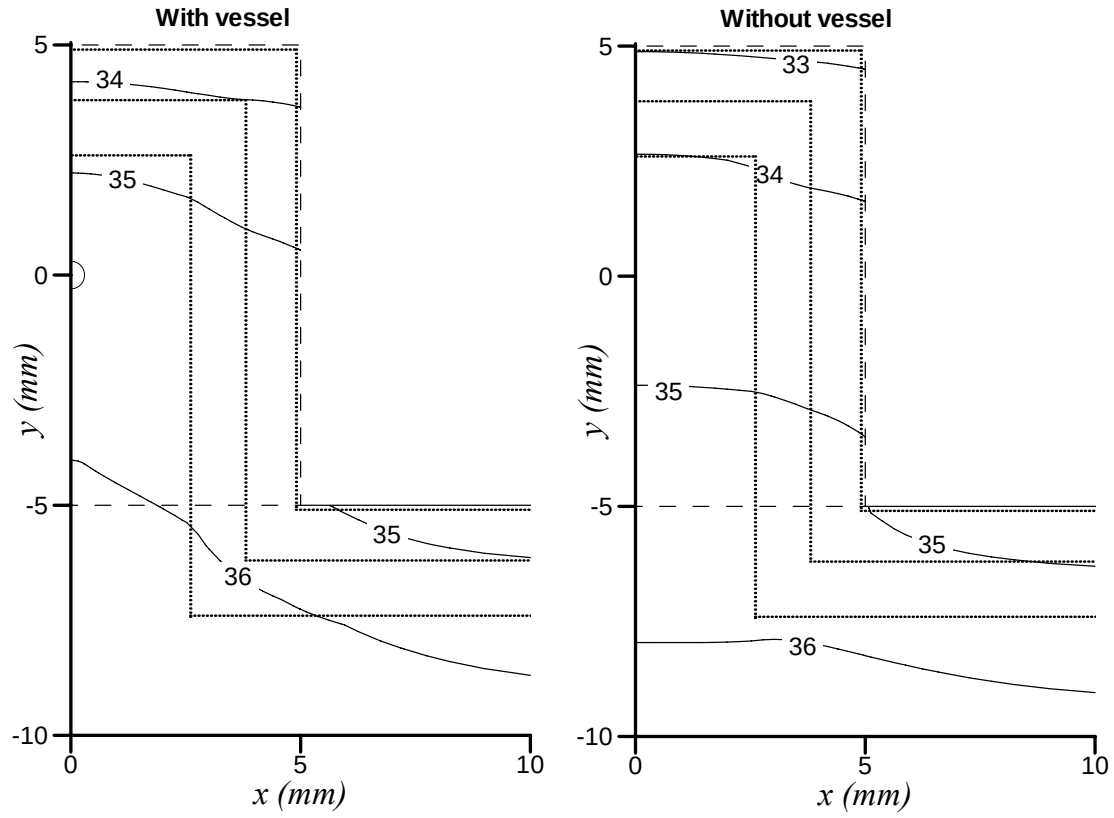


Figure 4.9 Blood vessel effects on steady state solution ($^{\circ}\text{C}$) at the axial midpoint of the target region ($z = 5\text{mm}$). Dashed line represents target region boundary, dotted lines represent composite tissue interfaces.

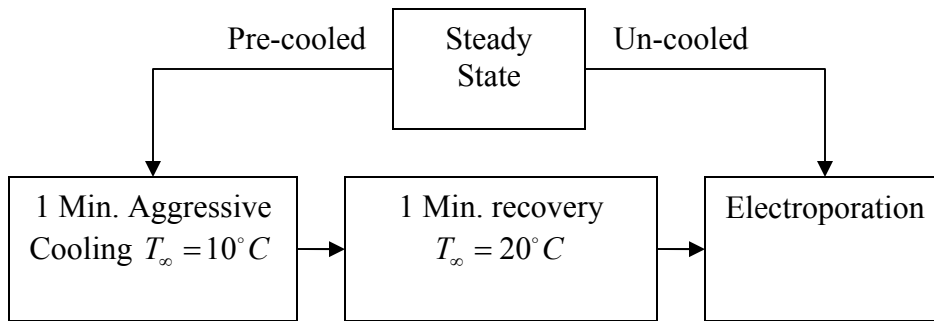


Figure 4.10 Cooling technique algorithm

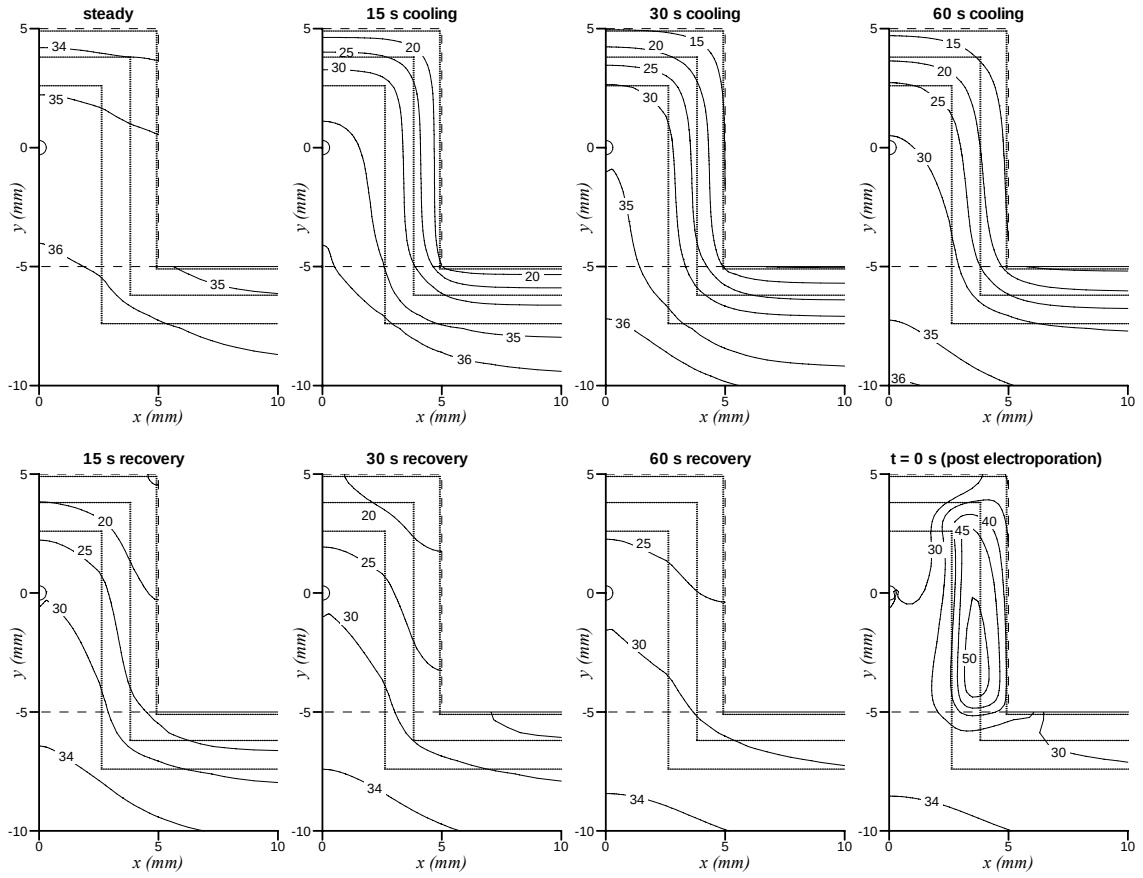


Figure 4.10 Transient aggressive cooling and recovery thermal solutions ($^{\circ}\text{C}$): at the axial midpoint of the target region ($z = 5\text{mm}$), dashed line represents target region boundary, dotted lines represent composite tissue interfaces. Data represented in post-electroporation thermal solution is from an electroporation model consisting of 5 pulses 150ms in duration spaced 1s apart with an applied voltage of 400V .

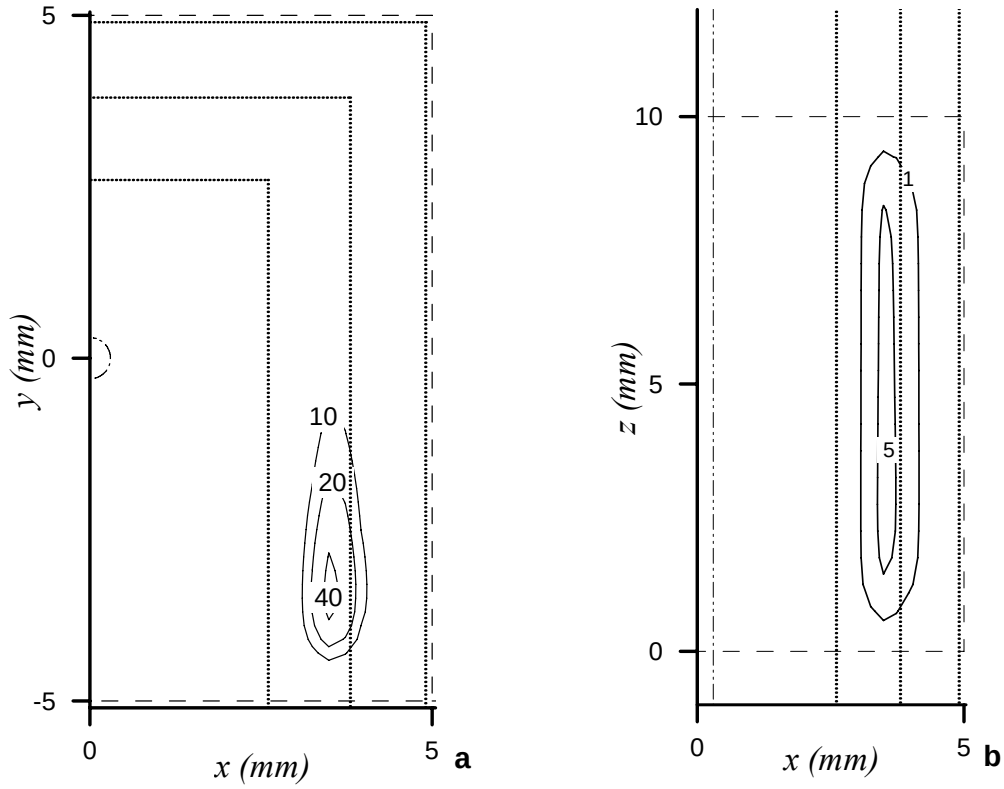


Figure 4.11 Aggressive cooling results showing reduced thermal damage EM_{43} (minutes) at $t = 300s$ (after last pulse cycle): (a) the x - y plane at the axial midpoint of the target region ($z = 5mm$), (b) the x - z plane at the vertical midpoint of the target region ($y = 0mm$); Dashed line represents target region boundary, dotted lines represent composite tissue interfaces, and dash-dot-dotted line represents position of blood vessel. Electroporation consisted of 5 pulses 150ms in duration spaced 1s apart with an applied voltage of 400V.

5 Local temperature rises influence *in vivo* electroporation pore development: a numerical stratum corneum lipid phase transition model

Electroporation is an approach used to enhance transdermal transport of large molecules in which the skin is exposed to a series of electric pulses. Electroporation temporarily destabilizes the structure of the outer skin layer, the stratum corneum, by creating microscopic pores through which agents, which ordinarily are unable to pass into the skin, are able to pass through this outer barrier. Long duration electroporation pulses can cause localized temperature rises which result in thermotropic phase transitions within the lipid bilayer matrix of the stratum corneum. This chapter focuses on electroporation pore development resulting from localized Joule heating. This study presents a theoretical model of electroporation which incorporates stratum corneum lipid melting with electrical and thermal energy equations. A transient finite volume model is developed representing electroporation of *in vivo* human skin in which stratum corneum lipid phase transitions are modeled as a series of melting processes. The results confirm that applied voltage to the skin results in high current densities within the less resistive regions of the stratum corneum. The model captures highly localized Joule heating within the stratum corneum and subsequent temperature rises which propagate radially outward. Electroporation pore development resulting from the decrease in resistance associated with lipid melting is captured by the lipid phase transition model. As the effective pore radius grows current density and subsequent Joule heating values decrease.

5.1 Introduction

The thin ($10 - 20 \mu m$) outermost layer of the skin, the stratum corneum (SC), acts as a barrier inhibiting transdermal drug delivery. The SC is composed of flat dead cell shells, called corneocytes, which are interconnected by a lipid lamellar bilayer structure in a crystalline-gel phase. The barrier function of the SC is primarily associated with this lamellar lipid structure in the extracellular space of the SC [1,2]. During skin electroporation, the skin is exposed to a series of intense electric pulses in order to change the structure of the SC by creating aqueous pores [3-5]. The breakdown of the SC barrier allows for increased molecular transport and radically reduces the electrical resistance of the skin [3, 6-9]. At temperatures around $70^{\circ}C$ the lamellar structure of SC is compromised [10-14] and subsequently the barrier function of the SC is dramatically reduced [11, 15]. It is recognized that localized Joule heating associated with electroporation is likely to contribute to increased permeability of the SC by lipid chain melting [9, 16-20]. In [16, 17] *in vitro* studies show that for moderate voltage, long duration pulses, electroporation pores originate within existing trans- SC appendageal pathways (sweat glands or hair follicles) and it is postulated that large current densities within the pore result in localized Joule heating and temperature rises sufficient to cause lipid phase transitions. Further *in vitro* experiments show that the effective pore radius of the localized transport region (LTR) is proportional to pulse duration which is associated with Joule heating [18]. Evidence of lipid phase change brought on by localized Joule heating is also presented in [19] where correlations between mass permeability of the SC and local temperature rise above lipid phase transition are established. In [21] a theoretical model is developed in which a high voltage electroporation pulse about an existing small diameter pore causes temperature rises which are sufficient to thermally alter the SC's lipid structure.

The focus of the current study is to model the time evolution of an electroporation pore with emphasis on the coupling of the thermal and electrical solutions with the *SC* lipid phase transition. As the lipid configuration changes from lamellar to disordered due to local temperature rises, the model incorporates dramatic drops in electrical resistance.

5.2 Physical model

This chapter considers the effects of skin electroporation in the direct vicinity of an electroporation pore. Typically in external skin electroporation a section of skin coated with an applicator gel is pinched between two electrodes (see Figure 1a). Computations performed in this study rely on symmetry at the axial midplane ($z = W/2$), therefore only the half domain is modeled. In this study a small section of tissue near the center of the electrode is used to represent the target region. Figure 5.1b shows the composite representation of this configuration consisting of electrodes (*e*), gel (*g*), and skin. The skin model consists of four sections: *SC*, epidermis (*ED*), dermis (*D*), and subcutaneous fat (*FAT*). This model uses a cylindrical domain with an outer radius $R_o = 1.5mm$. Figure 5.1c shows a representation of the *SC* prior to electroporation in which a pre-existing pore of radius $R_p = 15\mu m$ at the axial center passes through the *SC*. Prior to electroporation, the lipid structure connecting the corneocytes of the *SC* is the lamellar and uniform. During electroporation a large current density through the pore causes local temperature rises within the *SC*. Figure 5.1d shows the lamellar structure in phase transition and that the effective pore radius, R_{eff} , extends to the region at which *SC* lipids are unaffected by the temperature rises. The axial axis origin begins at the *SC* – Gel interface and is oriented positive into the skin, while the radial axis origin is at the center. Table 5.1 lists the composite thicknesses used in this model which are representative of the human volar forearm.

Table 5.1 Composite material dimensions and properties

			<i>SC</i>	<i>ED</i>	<i>D</i>	<i>FAT</i>	<i>e</i>	<i>g</i>
thickness	<i>mm</i>	δ	0.015 [22]	0.035 [22]	1.1 [23]	1.2 [23]	1	0.1
thermal conductivity	<i>W/mK</i>	<i>k</i>	0.2 [26]	0.209 [27]	0.293 [27]	0.23 [25]	16	0.6
density	<i>kg/m³</i>	ρ	1500 [25]	1110 [25]	1116 [25]	850 [28]	7850	1000
heat capacity (specific)	<i>J/kgK</i>	<i>c</i>	3600 [28]	3600 [28]	3800 [28]	2300 [28]	450	4180
perfusion	<i>kg/m³s</i>	ω	0	0	2.33 [29]	0.545 [29]	-	-
metabolic heat generation	<i>W/m³</i>	q'''	0	0	200 [28]	5 [28]	-	-
Electrical conductivity	<i>S/m</i>	σ	10^{-5} [25] $\sigma_{SC,MELT} = 0.0075$	0.01	0.015	0.02 [25]	-	1.5

5.3 Mathematical model

5.3.1 Thermal energy

In formulating the thermal model, the system is divided into three sub-domains: composite tissue, applicator gel, and electrode plate.

To represent the composite tissue domain, the Pennes' bioheat equation [24] is used along with an additional source term:

$$\rho_m c_m \frac{\partial T_m}{\partial t} = \nabla \cdot (k_m \nabla T) - \omega_m c_b (T - T_a) + q'''_m + Q_J \quad (5.1)$$

where ρ is the density, c is the specific heat, k is the thermal conductivity, T is the temperature, T_a is the arterial temperature, Q_J is the Joule heat generated from the induced electric field, q''' is the metabolic volumetric heat generation, ω_m is the non-directional blood flow associated with perfusion, t is the time, and x , y , and z are the Cartesian coordinate directions. The parameter c_b in the perfusion term is the specific heat of blood, which is assigned a value of $c_b = 3800 J/kgK$ [25]. The subscript m refers to one of the three composite skin layers: (*SC*), (*ED*), (*D*), and (*FAT*). Tissue property values used are listed in Table 5.1.

The gel layer (*g*), situated between the electrode and the *SC* is represented by the transient conduction equation with an added source term representing Joule heating:

$$\rho_g c_g \frac{\partial T_g}{\partial t} = \nabla \cdot (k_g \nabla T_g) + Q_J \quad (5.2)$$

This domain also includes the pre-existing trans-*SC* pore which is contained in the central $15\mu m$ radius section of the *SC*. The applicator gel is modeled to have similar property values as those of water; these are listed in Table 5.1.

The electrode plate section (*e*) is represented by the transient conduction equation:

$$\rho_e c_e \frac{\partial T_e}{\partial t} = \nabla \cdot (k_e \nabla T_e) \quad (5.3)$$

where the subscript *e* refers to electrode plate parameters. In this study the electrode is modeled as stainless steel, and thus electrode property values shown in Table 5.1 are representative of stainless steel.

5.3.2 Boundary and initial conditions

Initially the temperatures of the tissue, and gel domains are assumed to be equal to the arterial blood temperature, T_a :

$$T_m(r, z, 0) = T_a \quad (5.4)$$

$$T_g(r, z, 0) = T_a \quad (5.5)$$

The electrodes are added to the model at the onset of the first electroporation pulse, and unless otherwise specified are left in contact with the target region for all remaining computations. Initially electrodes are assumed to have the same temperature as the surroundings, T_∞ :

$$T_e(r, z, 0) = T_\infty \quad (5.6)$$

The outer radial faces of the tissue, gel and electrode domains are assumed thermally insulated:

$$\frac{\partial}{\partial r} T_m(R_o, z, t) = 0 \quad (5.7)$$

A convective flux boundary condition is imposed onto the electrode face exposed to the ambient air:

$$q'' = h(T_e - T_\infty) = -k_e \frac{\partial T_e}{\partial z} \Big|_{\text{surface}} \quad (5.8)$$

where q'' is the heat flux convected from the exposed face, h is the convection coefficient, T_∞ is the ambient air temperature, and n is the normal direction to the surface.

Symmetry is applied at the skin fold axial midpoint:

$$\frac{\partial}{\partial z} T_{FAT}(r, W/2, t) = 0 \quad (5.9)$$

where W is the plate spacing. Table 5.2 lists further parameter values.

Table 5.2 Additional parameter values

T_a	arterial blood temperature	$37^\circ C$
T_∞	ambient temperature	$20^\circ C$
h	convection coefficient	$15 W/m^2 K$
V_{app}	applied voltage	$300 V$

5.3.3 Electric field power generation

During electroporation the induced electric field causes a temperature rise due to Joule heating. The power supplied is found as follows. The electric potential is solved from the Laplace equation:

$$\nabla \cdot (\sigma_m \nabla \phi) = 0 \quad (5.10)$$

where ϕ is the electric potential and σ_m is the composite electrical conductivity, and the subscript m refers to one of the composite layers (*SC*), (*ED*), (*D*), (*FAT*), or (*g*). This equation is solved using the boundary condition at the upper electrode-gel interface, in which the potential is the same as the applied voltage, V_{app} :

$$\phi(r, -\delta_g) = V_{app} \quad (5.11)$$

where δ_g is the thickness of the gel section.

The second boundary condition uses symmetry of the system imposing a potential value equal to half of the applied voltage at the target region axial middle:

$$\phi(r, W/2) = V_{app}/2 \quad (5.12)$$

where W is the spacing between electrodes.

In this study a Direct current pulse is modeled with an applied voltage value of $V_{app} = 300V$.

The outer radial surface of the target region is assumed electrically insulated:

$$\frac{\partial}{\partial r}\phi(R_o, z) = 0 \quad (5.13)$$

The power due to Joule heating is then defined as:

$$Q_J = \sigma_m |\nabla \phi|^2 \quad (5.14)$$

Obviously there is no Joule heating outside the target region or at times during which the electric pulses are not applied. In [30] it is shown that even at moderate voltages the capacitive charging time associated with non-Ohmic behavior of the skin is very short and may be neglected at times greater than 1 ms . This behavior is neglected in this study.

Epidermal and dermal electrical conductivities given in Table 1 have been estimated to reflect electrical resistance drop with increased depth into the skin [22].

5.3.4 Lipid phase change

The lipid structure is known to become destabilized at elevated temperatures. It is generally accepted that there exist four main endothermic transitions in the *SC* in the temperature range $40\text{-}130^\circ\text{C}$ which have been independently confirmed by differential scanning calorimetry [10-14], although evidence exists of additional thermal transitions [31, 32]. This chapter focuses on three high temperature transitions of the *SC*, adopting the convention used in [31, 32], phase changes E, F, and G. The enthalpic peaks of phase change G occur at temperatures above 90°C and have been associated with protein denaturation [12, 13, 31]. Secondary lipid melting has been associated with phase change F at temperatures around 80°C and has been linked to lipids covalently bonded to corneocytes [12,13]. Of great interest in this chapter is phase change E which has primarily been attributed to the disordering of the lamellar lipid phase [13] and has been documented in numerous

differential scanning calorimetry studies at endothermic peak temperatures from 65 - 72°C [32]. Using X-ray diffraction microscopy it has been shown that the lipid lamellar structure in the *SC* is evident up to temperatures about 60°C and then disappears within 10°C [2,33]. Polarized light thermal microscopy of lipids extracted from the *SC* directly shows an overall fluidization of lipid structures at 60°C [31]. Further evidence of lipid chain melting and increased permeabilization at this phase change is given in [11,15] where it is shown that the *SC* becomes more permeable to water flux abruptly at 70°C.

To capture *SC* lipid phase transition this study borrows from enthalpy methods which were originally designed to model melting and solidification processes occurring over a temperature range [34, 35]. A rectangular shaped specific heat vs. temperature curve is used to model phase transitions as in [36, 37]. The heat of transition is modeled using apparent specific heats as follows:

$$c_{SC,APP} = c_{SC} + c_{SC,L} \quad (5.15)$$

where $c_{SC,APP}$ is the apparent *SC* specific heat, and $c_{SC,L}$ is the latent specific heat which is represented as:

$$c_{SC,L} = \frac{\Delta H_{PC}}{T_{PC2} - T_{PC1}} \quad (5.16)$$

where ΔH is the latent heat, T_2 and T_1 are the representative phase change end and beginning temperatures, and the subscript *PC* refers to one of the three phase transitions: E, F, or G.

Table 5.3 lists the values used in computations for the three *SC* phase change regions.

Table 5.3 *SC* enthalpic phase transition data

Phase change	Description	T_1 ($^{\circ}\text{C}$)	T_2 ($^{\circ}\text{C}$)	ΔH (J/kg)
E	Liquidization of lamellar lipids	65	75	5300 [13]
F	Liquidization of protein associated lipids	75	90	4100 [13]
G	Denaturation of proteins	90	110	5100 [13]

In light of the previously discussed *SC* lipid thermal behavior and permeability studied, this model uses phase transition E to model *SC* lipid melting during which the fraction of lipid melting is defined as:

$$\varphi = \frac{(H - c_{SC}T)}{\Delta H_E} \quad (5.17)$$

Where H is total enthalpy and is defined:

$$H = \int_{T_{E1}}^{T_{E2}} c_{SC,APP} dT \quad (5.18)$$

which given the assumed linear endothermic absorption over the lipid melting region E, the fraction of lipid melt may be represented:

$$\varphi = \begin{cases} 0 & : T < T_{1,E} \\ \frac{T - T_{E,1}}{T_{E,2} - T_{E,1}} & : T_{E,1} < T < T_{E,2} \\ 1 & : T_{E,2} < T \end{cases} \quad (5.19)$$

The electrical conductivity of the *SC* is several orders of magnitude lower than the underlying composite layers. The lamellar structure of the bi-layer matrix greatly reduces ionic permeability. With the fluidization the lamellar lipid extracellular sheets in phase transition E comes increased *SC* ionic permeability. Electrical resistance has been shown to

drop by two orders of magnitude in temperature range of this phase change [38] which also suggests the barrier function of the *SC* is compromised in transition E.

In this chapter the local *SC* effective electrical conductivity increase is related to lipid melt fraction by the relation:

$$\sigma_{SC}^* = \sigma_{SC} + \varphi(\sigma_{SC,MELT} - \sigma_{SC}) \quad (5.20)$$

Where σ_{SC} is the normal electrical conductivity, and $\sigma_{SC,MELT}$ is the electrical conductivity associated with the *SC* after full lipid melting. In this study a value of

$\sigma_{SC,MELT} = 0.0075 \text{ S/m}$ is used.

In [19, 39] is noted that long pulse duration is linked to increased post-pulse electrical resistance recovery times ($> 5 \text{ min.}$). In [40] the electric and mass transport barrier properties of *SC* regions experiencing lipid thermal phase transition by electroporation show very long recovery times ($> 30 \text{ min.}$). *SC* lipid structural changes associated with localized Joule heating during electroporation remain for some time after cooling of the *SC* [19]. Polarized light thermal microscopy of *SC* and extracted lipid samples heated to 130°C show that as the *SC* samples return to room temperature, the restructuring of the lipids shows evidence of aggregate variation [31]. Because of these findings and in light of the relatively short time periods that transience is modeled, upon cooling this study does not consider lamellar restructuring of the lipid bilayers and subsequent electrical recovery. Thus Eq. (5.19) is recast as:

$$\varphi^{t+1} = \begin{cases} \max(\varphi^t, 0) & : T < T_{1,E} \\ \max\left(\varphi^t, \frac{T - T_{E,1}}{T_{E,2} - T_{E,1}}\right) & : T_{E,1} < T < T_{E,2} \\ 1 & : T_{E,2} < T \end{cases} \quad (5.21)$$

where the superscript t and $t + 1$ refers to the previous and current time steps, respectively.

5.4 Discretization and solution procedure

The computational domain is discretized using a variable cylindrical grid consisting of 11,880 cells. A fully implicit finite volume approach is used to solve Eqs. (5.1-5.3), (5.10), and (5.14). Temperature, T , and Joule heat, Q_J , are evaluated at the cell centers while the electric potential, ϕ , is evaluated at the cell edges. A global $1ms$ time step is used for transient Eqs. (5.1-5.3). Interface conductivity is represented by a harmonic mean similar to that described in [41], while a resistor representation is used between the cell center and the convection boundary condition (5.8). The SC specific heats and electrical properties and lipid melt fractions of Eqs. (5.15) -(5.21) are updated at each time step using the local temperature solution of the previous time step. Variables T and ϕ are solved using an implicit point iterative method. Since it is expected that between time steps dramatic temperature changes occur not over the entire solution domain, but locally within and about the target region, a localized convergence measure is used between iterations. The convergence criterion is defined as $\varepsilon = |T_{i,j}^{p+1} - T_{i,j}^p|$ where p is iteration and convergence is established when $\varepsilon < 10^{-5}$.

5.5 Results and discussion

To validate the numerical solution, comparisons with analytic solutions were made. The temperature solution of the one-dimensional steady form of Eq. (5.1) was obtained in the axial z -direction using parameter values $h = 500 W/m^2 K$ and $T_\infty = 10^\circ C$, and setting a prescribed temperature at the target region half width $T(W/2) = T_a$. The one-dimensional solutions of the electric potential and Joule heat Eqs. (5.22) and (5.25) are obtained along the axial direction. A SC conductivity value of $\sigma_{SC} = 10^{-5} S/m$ is used and the remaining

composite tissue conductivity values are the same as in Table 5.2. Figures 5.2a-b show good agreement between computed and analytic solutions.

5.5.1 Single long pulse

In order to show the phenomenon associated with the thermal and electrical solutions during pore formation a single pulse case is presented first. Pulsation influences will be presented in later sections. The case is presented in which a single $300V$ pulse is applied for $750ms$.

Figure 5.3 shows the potential solution in the half domain at the three representative times.

Early in pulse application at $t = 10ms$ (Figure 5.3a) nearly the entire potential drop of the domain occurs across the SC , with minor two – dimensional diffusive effects visible about the pre-existing appendageal pore. The small insert shows the lipid melt fraction contour $\phi = 0$, representing the innermost radial boundary at which lipids have not yet been thermally altered. As time progresses evidence of pore growth is clearly visible in the potential solution. At $250ms$ pulsing (Figure 5.3c), the potential drop across the SC drops significantly with two-dimensional effects visible in the potential solution along the outer pore radius. The radius of the border at which the SC is unaltered grows to nearly 0.6 mm as pulsing time is increased to $750ms$ (Figure 5.3d). At the end of the $750ms$ pulse the potential solution shows a drastically reduced potential drop across the SC .

Figure 5.4 shows the results of the Joule heating as well as a detailed look at the SC electrical conductivity. Joule heat and SC conductivity solutions are presented at $10ms$ pulsing in Figure 5.4a. At this time heating is highly localized near the existing $15\mu\text{m}$ pore. Increased SC conductivity by lipid melting is restricted near the existing pore radius reflecting that high temperature effects have not yet propagated far into the SC . At $100ms$ pulsing the heating and

increased *SC* conductivity have propagated to larger radial positions (Figure 5.4b). At this time large value Joule heating contours have split into two localized regions. One remains at the conductive gel filled pore- epidermis interface reflecting large localized current spreading at the pore outlet. The other is within the *SC* near the interface between high and low electrical conductivities. As the pulsing time increases to 250 and 750ms (Figures 5.4c-b) maximum Joule heat value contours occur locally within the *SC* along the border between low and high electrical conductivities (effectively the outer radius at which the *SC* lipids experience phase transition E). As time progresses and the *SC* increased conductivity radius grows, lower level Joule heating matches this radius and reaches epidermal depths. The maximum values of Joule heat decrease with increasing pore radius (decreasing current density).

Transient thermal solutions of the single 750ms 300V pulse are presented in Figure 5.5. A close-up of the *SC* is presented in Figure 5.5a which depicts the thermal solution at 10ms pulsing. At this early stage of pore development the temperature rise occurs locally in the vicinity of the pre-existing pore. The very large current density through the pore results in temperature contours exceeding 120°C. As the effective pore radius grows, the system no longer achieves these intense maximum temperatures, although the contours propagate outward and down into the skin. At 100ms (Figure 5.5b) the phase transition temperatures have propagated from the pre-existing pore into the *SC*. At pulse times 250 and 750ms (Figures 5.5c-d) significant temperature rises are seen in the deeper dermal regions and at greater outward radial locations although the intense temperatures associated are no longer evident. The tissue regions below the effective pore radius continue to grow as the pulse continues. By 750ms pulsing phase transition temperatures have propagated radially to

distances of 0.5mm . The horizontal contour lines within the *SC* and gel layers reflect heat conducted to the electrode, while slower cooling due to conduction and perfusion within the dermis is evident in the dispersed thermal contours below the *SC*.

5.5.2 Multiple pulses

In practice multiple shorter pulses are used instead of long single pulses. This is done predominantly in order to avoid heating sections of viable tissue to high temperatures as seen in the $70\text{-}80^\circ\text{C}$ contours of Figures 5.5c-d that extend into the dermis. A series of 4 electroporation pulses is modeled in which 300V pulses are applied for 250ms at 1s intervals. The thermal solutions at the end of each 250ms electroporation pulse are presented in Figure 5.6. Inserts in Figure 5.6 depict a close-up of the *SC* (dashed rectangle) in which the lipid melt fraction is represented by the solid contour lines and Joule heating value contour $Q_j = 10^{10} \text{ W/m}^3$ is represented by the dash-dot line. At the end of the first pulse the region of lipids experiencing phase transition E has reached 3 mm . As pulsing continues (Figures 5.6b-d) this region continues to grow, although at a decreased rate. This decreased growth rate indicates the lower current density associated with increased pore radius. The high Joule heating contour is consistently distributed within in the *SC* region of phase transition indicating that localized Joule heating is greatest within the *SC*. This results in the lipid melt pattern seen in the high melt fraction contour, $\varphi = 0.9$, which extends radially outward and from lower levels to the surface. The multiple pulse thermal solution differs from the single pulse primarily due to cooling between pulses. By breaking the pulse up into multiple pulses, a cooling period has been introduced. The results are evident in the lower temperatures attained specifically in the center of the domain (Figure 5.6b-d). As the number of pulses increase, the thermal contours broaden indicating the increase in effective pore

radius. Although the center of the domain cools between pulses, localized Joule heating is evident in the pockets of higher temperature contour lines near the lipid melt border. Ideally the Joule heating and subsequent temperature rise should be isolated as closely to the *SC* as possible. Noting the differences between thermal solutions of the single long pulse case in Figure 5.5d and the multiple pulse case in Figures 5.6b-d it is obvious that allowing conductive, perfusive, and convective influences act between pulse applications tends cool the lower tissue domain.

In order to exaggerate the cooling effects between the 4 pulses, the time between the 250ms, 300V pulse applications is increased to two minutes. By increasing the time between pulses, thermal energy within the target region is allowed to dissipate prior to the onset of the next pulse: effectively what is done is that the target region is allowed to cool to 35-36°C prior to application of pulses 2-4 (data not shown). The thermal results are presented in Figure 5.7 at the end of each 250ms pulse, and again the inserts depict lipid melt fractions and Joule heating contour values of $Q_J = 10^{10} \text{ W/m}^3$. Cooling the target region between pulses brings out the localized heating influences on the thermal solution seen in the higher temperature contours which emanate from the region directly below the *SC* effective pore radius.

Similarities between the solutions at the ends of pulses 2-4 (Figures 5.7b-d) show that little change occurs in the system with increased pulse number for long cooling times between pulses. This is especially evident in the lack of growth of lipid phase transition within the *SC* with respect to the closer pulse spacing results of Figure 5.6. Pore growth of 1s and 120s pulse spacing is compared in Figure 5.8 which shows the effective pore radius at the end of each pulse (Figure 5.8a) as well as the growth of the pore during pulsing (Figures 5.8b-e).

The effective pore radius is defined by the minimum radius at which lipids are unaffected by

thermal phases change. Regardless of pulse spacing, the first pulse produces the greatest pore growth followed by much slower rate of growth in later pulses. Subsequent pulses result in reduced pore growth of the 120s spacing case due to the increased dissipation between pulses. The 1s spaced pulse reaches lipid phase transition earlier because the tissue temperature is higher at the onset of the second pulse. During pulse 4 there is no increase in effective pore radius for the widely spaced pulse which is an indication that at this time localized Joule heating alone is not sufficient to reach phase transition temperatures. This agrees with the previous results which relate decreased current density and subsequent Joule heating with increased pore size. Experimentally this has also been shown to be the case in [18] for which tissue samples allowed to cool for long times between pulse applications show a limit in the pore diameter regardless of number of pulses delivered.

5.6 Conclusions

A transient axi-symmetric model of in vivo skin electroporation incorporating *SC* lipid melting has been developed in which electrical and transient thermal solutions are found. The electrical solution of the skin composite model shows potential drop across the *SC* decreases as the electroporation pore grows. This is a result of increased electrical conductivity with lipid phase transition. Heating and temperature rises are highly localized occurring not over the entire target region, but within the vicinity of the electroporation pore. The very electrically resistive *SC* receives the largest Joule heating values which focus within the region of lipid phase transition. As a result of large Joule heating, lipid phase transition temperatures are attained during pulsing. The results show that *SC* lipid phase transition occurs in a pattern from the skin interior to the surface and radially outward. The rate of pore growth decreases with increased pore radius and pulsing time as a consequence of decreased

current density. Longer pulse spacing allows for greater diffusion of heat between pulses thus lowering the maximum temperatures at the beginning of subsequent pulses.

Electroporation pore growth is then inhibited by the resulting lower local maximum temperatures.

5.7 References

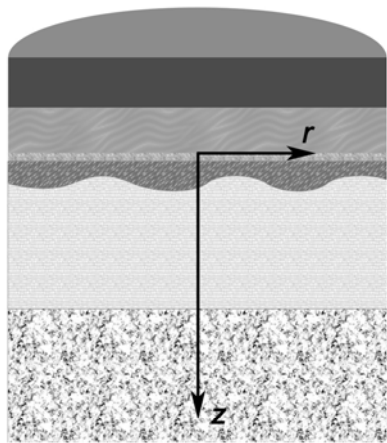
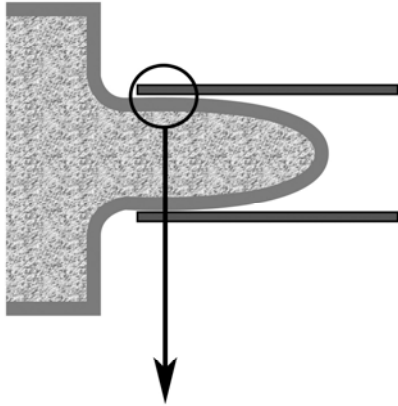
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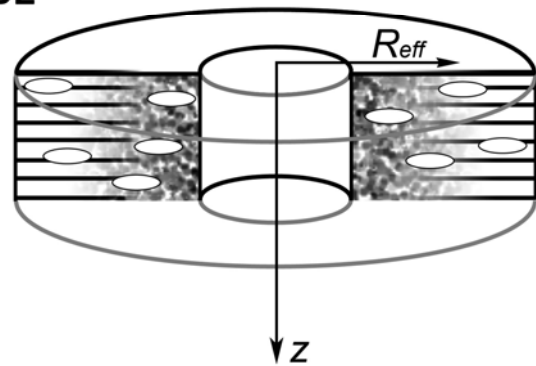
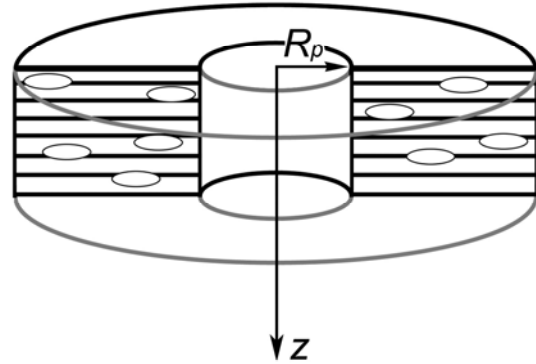
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a SKIN FOLD



b COMPOSITE REPRESENTATION

**c CLOSE-UP:
SC - PREEXISTING PORE**



d SC - LIPID MELTING

Figure 5.1 Physical model description: (a) electroporation skin fold, (b) composite skin layers (cylindrical), (c) close-up of trans- SC pre-existing pore with unperturbed lipid configuration, (d) close-up of electroporated SC: lipids undergoing structural phase transition.

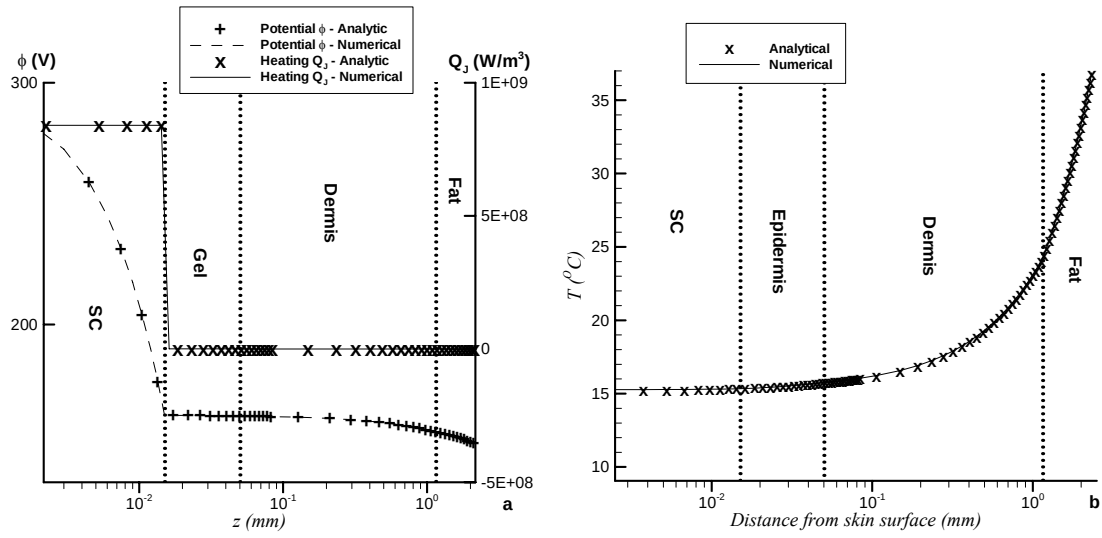


Figure 5.2 Comparisons between numerical code and analytic solution (one-dimensional): (a) Electric (potential and heating) in which the domain represents a vertical line between the electrodes using $V_{app} = 300V$. (b) Steady state temperature (°C), in which the domain represents vertical depth below the skin's surface using convection coefficient $h = 500W/m^3$. Dotted lines separate composite sections

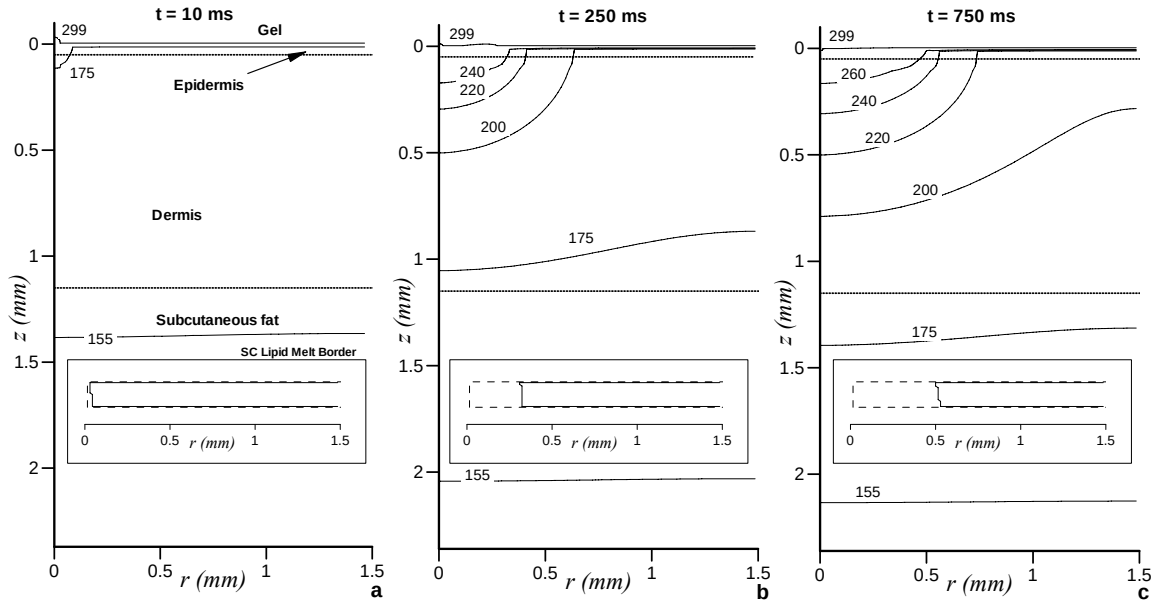


Figure 5.3 Potential solution at representative pulsing times of a single 300V pulse. Dotted lines represent interfaces between composite layers. Inserts show boundary of zero lipid melt fraction contour ($\phi = 0$) in a close-up of the SC (dashed rectangle) using aspect ratio ($x:y = 1:10$)

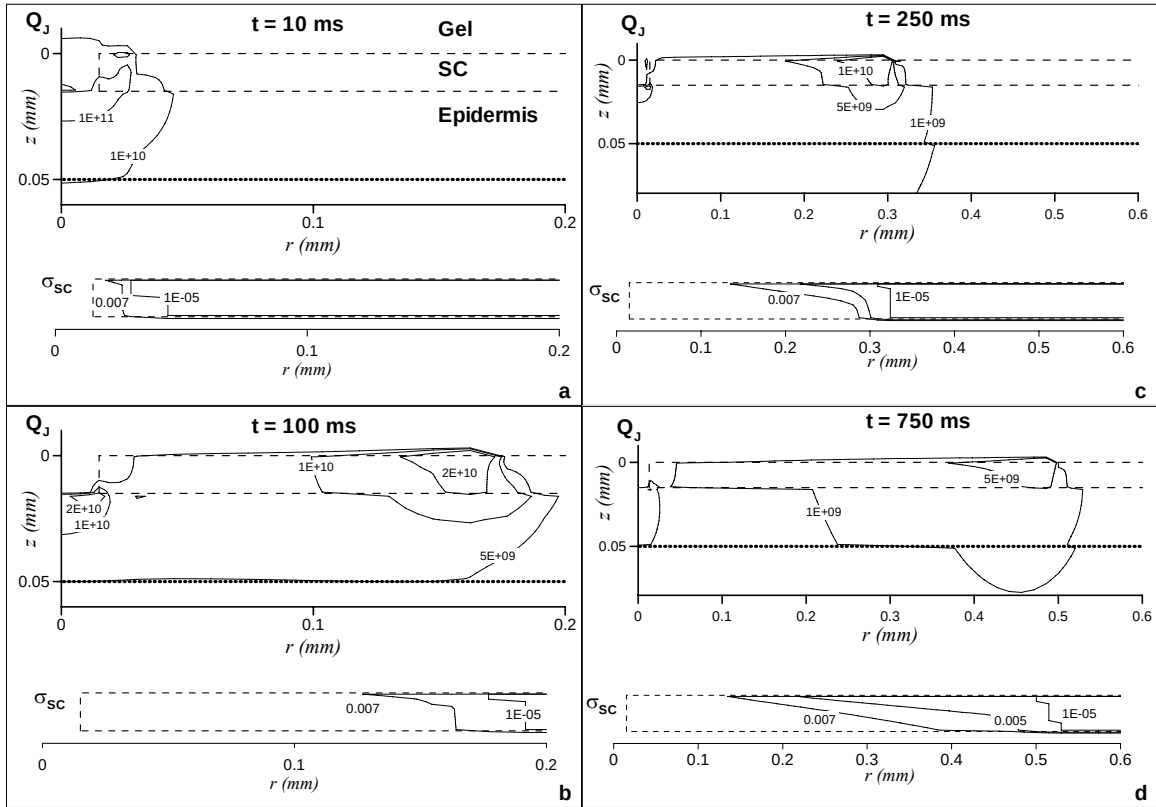


Figure 5.4 Upper panel sections: Joule heat solution (Q_J W/m^3) in the vicinity of the electroporation pore during an electroporation pulse with an applied potential of $300V$. In upper panels (c) and (d) $x:y = 1:2$ aspect ratios are used. Dotted line denotes dermal-epidermal junction. Dashed rectangle denotes SC boundary. Lower panel sections: SC electrical conductivity (S/m). In lower panels (c) and (d) $x:y = 0.35:1$ aspect ratios are used. Dashed rectangle denotes SC boundary.

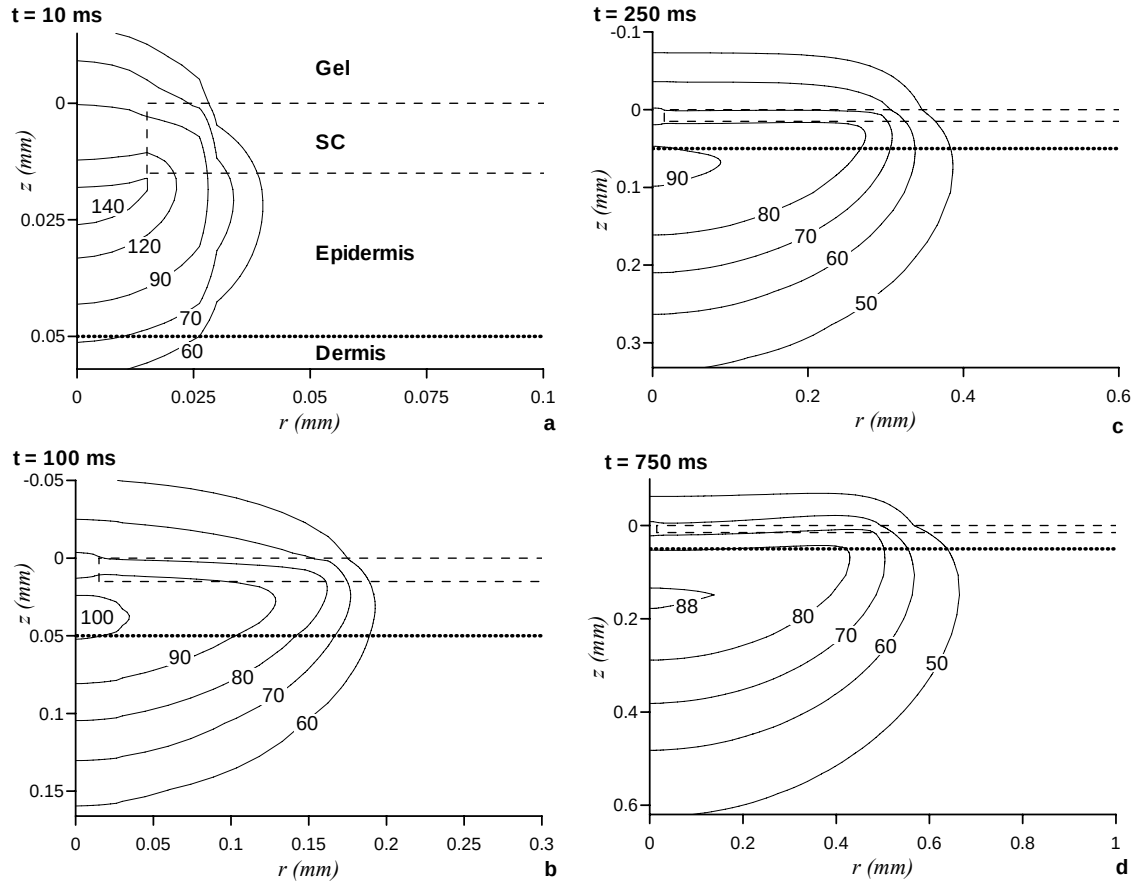


Figure 5.5 Transient thermal solution ($^{\circ}\text{C}$) at representative pulsing times of a single pulse with an applied voltage of 300V. Dotted line denotes dermal-epidermal junction. Dashed rectangle denotes SC boundary.

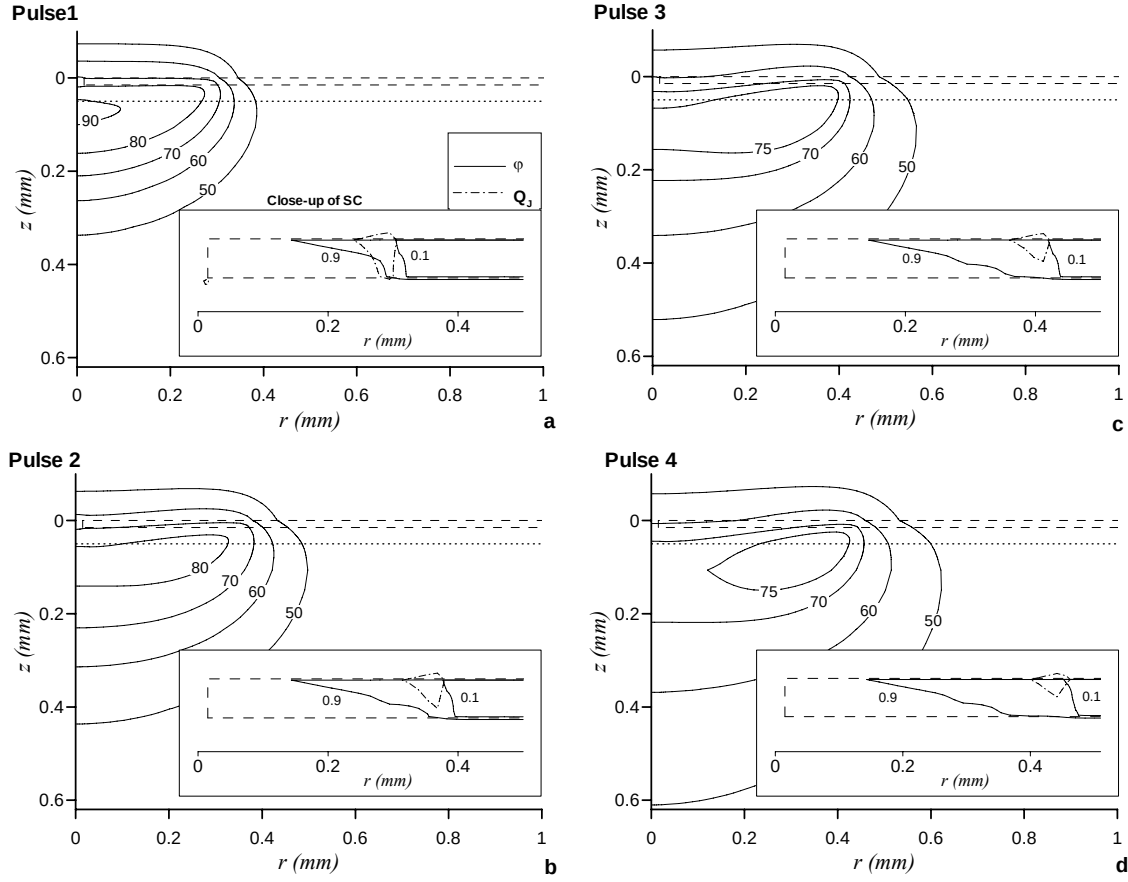


Figure 5.6 Multiple short pulse spacing thermal solutions ($^{\circ}\text{C}$) at the end of each of four 250ms 300V pulses applied at 1s intervals. Dotted line denotes dermal-epidermal junction. Dashed line denotes SC boundary. Inserts show lipid melt fraction contours (solid line) and contour of large Joule heat value $Q_J = 10^{10} \text{ W/m}^3$ (dashed dotted line) in a close-up of the SC (dashed rectangle) using aspect ratios ($x:y = 1:4$)

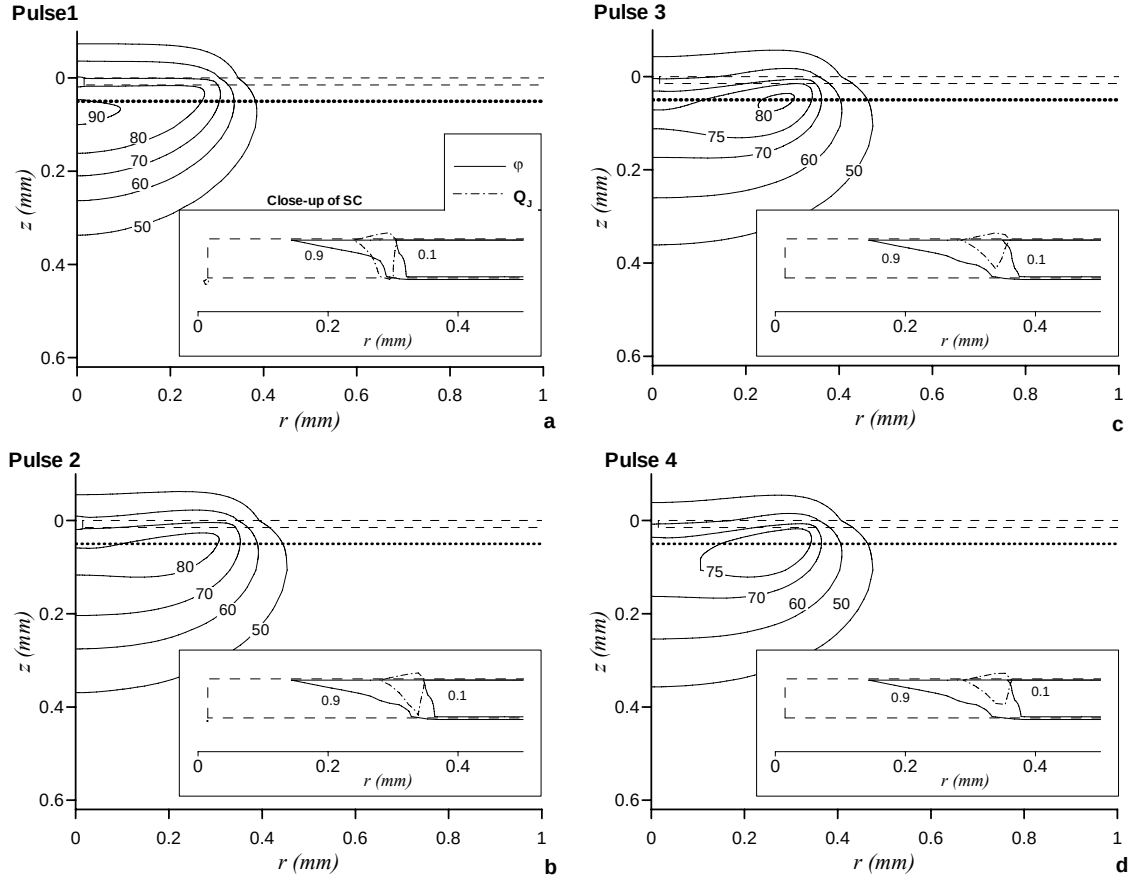


Figure 5.7 Multiple long pulse spacing thermal solutions ($^{\circ}\text{C}$) at the end of each of four 250ms 300V pulses applied at 120s intervals. Dotted line denotes dermal-epidermal junction. Dashed line denotes SC boundary. Inserts show lipid melt fraction contours (solid line) and contour of large Joule heat value $Q_J = 10^{10} \text{ W/m}^3$ (dashed dotted line) in a close-up of the SC (dashed rectangle) using aspect ratios ($x:y = 1:4$)

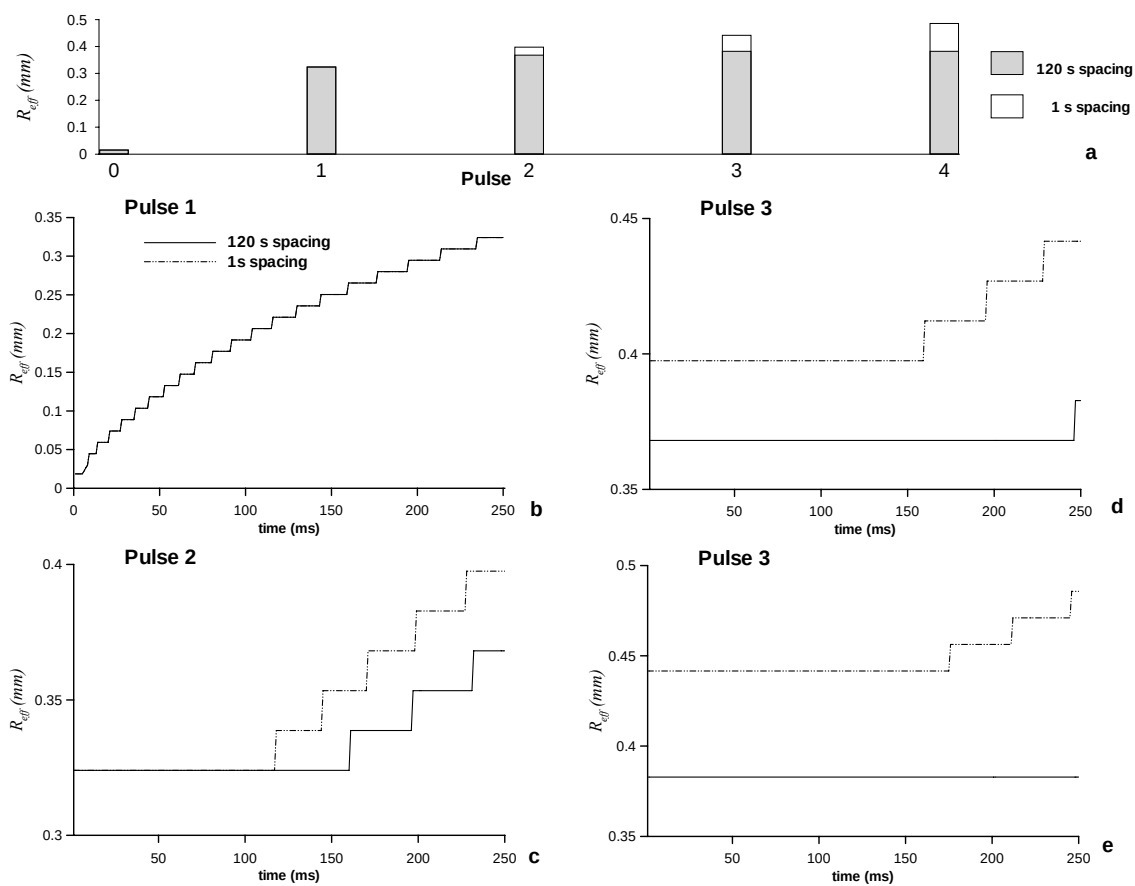


Figure 5.8 Effective pore radius growth comparisons between 250ms 300V pulses spaced at 1s intervals and at 120s intervals. Effective pore radius is defined by the minimum radius at which lipids are unaffected by thermal phases change.

6 Thermal *in-vivo* skin electroporation pore development and charged macromolecule trans-dermal delivery: a numerical study of the influence of chemically enhanced lower lipid phase transition temperatures

Electroporation is an approach used to enhance transdermal transport of large molecules in which the skin is exposed to a series of electric pulses. Electroporation temporarily destabilizes the structure of the outer skin layer, the stratum corneum, by creating microscopic pores through which agents, which ordinarily are unable to pass into the skin, are able to pass through this outer barrier. Long duration electroporation pulses can cause localized temperature rises which result in thermotropic phase transitions within the lipid bilayer matrix of the stratum corneum. Chemical agents applied to the skin can reduce the lipid phase transition temperatures. This chapter studies the benefits of the combination of the chemical enhancer, terpene d-limonene with low voltage electroporation pulses in order to further aid in electroporation pore development resulting from fluidization of the lipid structures within the stratum corneum. A transient finite volume model is developed in which the thermal and electrical behavior associated with electroporation of *in vivo* human skin is analyzed and lipid phase transition represented by a melting process. The Nernst-Planck model is used to represent the electrophoretic assisted transport of large charged molecules through the skin. The results show that the lower lipid phase transition temperatures associated with the topical application of chemical enhancers to the skin allow for solute penetration of the skin reaching radial locations much further than in the untreated case. Solute transport solutions of both cases exhibit local accumulation of concentrations below

the stratum corneum - epidermis interface which exceed concentration values initially contained within the applicator gel.

6.1 Introduction

The thin ($10 - 20 \mu m$) outermost layer of the skin, the stratum corneum (SC), acts as a barrier inhibiting transdermal drug delivery. The SC is composed of flat dead cell shells, called corneocytes, which are interconnected by a lipid lamellar bilayer structure in a crystalline-gel phase. The barrier function of the SC is primarily associated with this lamellar lipid structure in the extracellular space of the SC [1, 2]. Electroporation has been shown to greatly increase the success of transdermal delivery of large molecules [3-5]. During skin electroporation, the skin is exposed to a series of intense electric pulses in order to change the structure of the SC by creating microscopic aqueous pores [6-8]. The breakdown of the SC barrier allows for increased molecular transport and radically reduces the electrical resistance of the skin [6, 9-12]. Although the primary effects of electroporation are these microscopic transient pores, under certain pulse parameters secondary effects occur resulting from Joule heating and the associated highly localized regions of elevated temperatures. At temperatures around $70^{\circ}C$ the SC lipid lamellar structure is compromised [13-17] and subsequently the barrier function of the SC is dramatically reduced [14, 18]. It is recognized that localized Joule heating associated with electroporation is likely to contribute to increased permeability of the SC by lipid chain melting [4, 19-22]. In [4, 19] *in vitro* studies show that for moderate voltage, long duration pulses, electroporation pores originate within existing trans- SC appendageal pathways (sweat glands or hair follicles) and it is postulated that large current densities within the pore result in localized Joule heating and temperature rises sufficient to cause lipid

phase transitions within the surrounding *SC*. Further *in vitro* experiments show that the effective pore radius of the localized transport region (LTR) is proportional to pulse duration which is associated with Joule heating [20]. Evidence of lipid phase change brought on by localized Joule heating is also presented in [21] where correlations between mass permeability of the *SC* and local temperature rise above lipid phase transition are established. The skin barrier property has also been shown to be overcome by chemical penetration enhancers which act to destabilize the transport hindrance by interacting with the *SC* bilayer structure resulting in increased passive diffusion fluxes [23]. One class of chemical enhancers, terpenes, which are found in some plant oils, have been shown to decrease the temperatures at which *SC* lipids experience phase transition [16] and increase transdermal mass flux in iontophoresis (a very low voltage electro-assisted transport method) [24]. Chemical enhancers have been used in conjunction with electroporation experimentally, however only to prolong the recovery of the electroporated *SC* bilayers [4].

Previous models of transdermal electrically assisted molecular transport focusing on iontophoresis are predominantly one dimensional, and treat the skin as a homogenous membrane [25-28]. By treating the *SC* as a homogenous membrane any local variations of the electric field within the *SC* are neglected, and the radial gradients in mass flux cannot be represented. By modeling the skin as a single membrane, variations in axial concentration of solute deposition are lost. In [29] a theoretical model is developed in which a high voltage electroporation pulse about an existing small diameter pore causes temperature rises which are sufficient to thermally alter the *SC*'s lipid structure.

The current study's purpose is first to develop a two-dimensional transient model of skin electroporation which captures thermal pore development and local solute transport. The

model emphasizes the coupling of the thermal and electrical solutions with the *SC* lipid phase transition. As the lipid configuration changes from lamellar to disordered due to local temperature rises, the model incorporates dramatic drops in electrical and transport resistance. Using this model, the study shows that combining terpene enhancer with skin electroporation greatly increases the success of transdermal mass transport.

6.2 *Physical model*

This chapter considers the effects of skin electroporation in the direct vicinity of an electroporation pore. Typically in external skin electroporation a section of skin coated with an applicator gel is pinched between two electrodes (see Figure 6.1a). Computations performed in this study rely on symmetry at the axial mid-plane ($z = W/2$), therefore only the half domain is modeled. In this study a small section of tissue near the center of the electrode is used to represent the target region. Figure 6.1b shows the composite representation of this configuration consisting of electrodes (*e*), gel (*g*), and skin. The skin model consists of four sections: *SC*, epidermis (*ED*), dermis (*DERM*), and subcutaneous fat (*FAT*). This model uses a cylindrical domain with an outer radius $R_o = 1mm$. Figure 6.1c shows a representation of the *SC* prior to electroporation in which a pre-existing pore of radius $R_p = 5\mu m$ at the axial center passes through the *SC*. Prior to electroporation, the lipid structure connecting the corneocytes of the *SC* is the lamellar and uniform. During electroporation a large current density through the pore causes local temperature rises within the *SC*. Figure 6.1d shows the lamellar structure in phase transition and that the effective pore radius, R_{eff} , extends to the region at which *SC* lipids are unaffected by the temperature rises. The axial axis origin begins at the *SC* – Gel interface and is oriented positive into the

skin, while the radial axis origin is at the center. Table 6.1 lists the composite thicknesses used in this model.

Table 6.1 Composite material dimensions and properties

			<i>SC</i>	<i>ED</i>	<i>DERM</i>	<i>FAT</i>	<i>g</i>	<i>e</i>
Thickness	<i>mm</i>	δ	0.015 [30]	0.035 [30]	1.1 [31]	1.2 [31]	1	1
Thermal conductivity	<i>W/mK</i>	<i>k</i>	0.2 [34]	0.209 [35]	0.293 [35]	0.23 [33]	0.6	16
Density	<i>kg/m³</i>	ρ	1500 [33]	1110 [33]	1116 [33]	850 [36]	1000	7850
Heat capacity (specific)	<i>J/kgK</i>	<i>c</i>	3600 [36]	3600 [36]	3800 [36]	2300 [36]	4180	450
Perfusion	<i>kg/m³s</i>	ω	0	0	2.33 [37]	0.545 [37]	-	-
Metabolic heat generation	<i>W/m³</i>	q'''	0	0	200 [36]	5 [36]	-	-
Electrical conductivity	<i>S/m</i>	σ	10^{-5} [33] ($\sigma_{SC,MELT} = 0.001$)	0.01	0.015	0.02 [33]	1.5	-
Electrophoretic mobility	<i>m² / Vs</i>	<i>m</i>	10^{-17} ($m_{SC,MELT} = 5 \times 10^{-11}$)	10^{-10}	2×10^{-10}	10^{-10}	10^{-8}	-
Diffusivity	<i>m² / s</i>	<i>D</i>	10^{-17} ($D_{SC,MELT} = 10^{-13}$)	10^{-12}	10^{-12}	10^{-12}	10^{-9}	-

6.3 Mathematical model

6.3.1 Thermal energy

In formulating the thermal model, the system is divided into three sub-domains: composite tissue, applicator gel, and electrode plate.

To represent the composite tissue domain, the Pennes' bioheat equation [32] is used along with an additional source term:

$$\rho_i c_i \frac{\partial T}{\partial t} = \nabla \cdot (k_i \nabla T) - \omega_i c_b (T - T_a) + q_i''' + Q_J \quad (6.1)$$

where ρ is the density, c is the specific heat, k is the thermal conductivity, T is the temperature, T_a is the arterial temperature, Q_J is the Joule heat generated from the induced electric field, q_i''' is the metabolic volumetric heat generation, ω_m is the non-directional blood flow associated with perfusion, t is the time. The parameter c_b in the perfusion term is the specific heat of blood, which is assigned a value of $c_b = 3800 J/kgK$ [33]. The subscript i refers to one of the three composite skin layers: *SC*, *ED*, *DERM*, or *FAT*.

The gel layer (*g*), situated between the electrode and the *SC* is represented by the transient conduction equation with an added source term representing Joule heating:

$$\rho_g c_g \frac{\partial T_g}{\partial t} = \nabla \cdot (k_g \nabla T_g) + Q_J \quad (6.2)$$

This domain also includes the pre-existing trans-*SC* pore which is contained in the central $5\mu m$ radius section of the *SC*.

The electrode plate section (*e*) is represented by the transient conduction equation:

$$\rho_e c_e \frac{\partial T_e}{\partial t} = \nabla \cdot (k_e \nabla T_e) \quad (6.3)$$

where the subscript e refers to electrode plate parameters. Tissue, electrode and gel property values used are listed in Table 6.1.

6.3.2 Boundary and initial conditions

Initially the temperatures of the composite tissue, and gel domains are assumed to be equal to the arterial blood temperature, T_a :

$$T_i(r, z, 0) = T_a \quad (6.4)$$

$$T_g(r, z, 0) = T_a \quad (6.5)$$

The electrodes are added to the model at the onset of the first electroporation pulse, and unless otherwise specified are left in contact with the target region for all remaining computations. Initially electrodes are assumed to have the same temperature as the surroundings, T_∞ :

$$T_e(r, z, 0) = T_\infty \quad (6.6)$$

The outer radial faces of the tissue, gel and electrode domains are assumed thermally insulated:

$$\frac{\partial}{\partial r} T(R_o, z, t) = 0 \quad (6.7)$$

A convective flux boundary condition is imposed onto the electrode face exposed to the ambient air:

$$q'' = h(T_e - T_\infty) = -k_e \left. \frac{\partial T_e}{\partial z} \right|_{surface} \quad (6.8)$$

where q'' is the heat flux convected from the exposed face, h is the convection coefficient, and T_∞ is the ambient air temperature.

Symmetry is applied at the skin fold axial midpoint:

$$\frac{\partial}{\partial z} T_{FAT}(r, W/2, t) = 0 \quad (6.9)$$

where W is the plate spacing. Table 6.2 lists further parameter values.

Table 6.2 Operational parameter values

Computational domain radius	R_o	1 mm
Pre-existing pore radius	R_p	$5\mu\text{m}$
Arterial blood temperature	T_a	37°C
Ambient temperature	T_∞	20°C
Convection coefficient	h	$15\text{ W/m}^2\text{K}$
Applied voltage	V_{app}	275V
Pulse duration	τ_p	200ms
Pulse interval	τ_s	1s
Number of pulses delivered	N_p	4

6.3.3 Electric field power generation

During electroporation the induced electric field causes a temperature rise due to Joule heating. The power supplied is found as follows. The electric potential is solved from the Laplace equation:

$$\nabla \cdot (\sigma_i \nabla \phi) = 0 \quad (6.10)$$

where ϕ is the electric potential and σ_i is the composite electrical conductivity, and the subscript i refers to one of the composite layers: g , SC , ED , $DERM$, or FAT . This equation is solved using the boundary condition at the upper electrode-gel interface, in which the potential is the same as the applied voltage, V_{app} :

$$\phi(r, -\delta_g) = V_{app} \quad (6.11)$$

where δ_g is the thickness of the gel section. The second boundary condition uses symmetry of the system imposing a potential value equal to half of the applied voltage at the target region axial middle:

$$\phi(r, W/2) = V_{app}/2 \quad (6.12)$$

where W is the spacing between electrodes.

In this study a direct current pulse is modeled with an applied voltage value of $V_{app} = 275V$.

The outer radial surface of the target region is assumed electrically insulated:

$$\frac{\partial}{\partial r} \phi(R_o, z) = 0 \quad (6.13)$$

The power due to Joule heating is then defined as:

$$Q_J = \sigma_i |\nabla \phi|^2 \quad (6.14)$$

Obviously there is no Joule heating outside the target region or at times during which the electric pulses are not applied. In [38] it is shown that even at moderate voltages the capacitive charging time associated with non-Ohmic behavior of the skin is very short and may be neglected at times greater than 1 ms . This behavior is neglected in this study.

Equation (6.10) neglects the influence of the charged molecules of the solute on the electrical distribution of the large magnitude pulse.

6.3.4 Mass transport

Electrically driven transdermal delivery is negotiated by the three modes of transport: electrophoresis, electroosmosis, and diffusion. Studies focusing on electrically driven transport of large charged molecules show that electro-osmotic effects are negligible

compared to electrophoresis forces [39]. Homogenous tissue *in vivo* electroporation studies show that electrophoretic forces dominate in the transport of large molecules [40], [41]. In skin electroporation the pulse parameters are short (compared to iontophoresis), thus electro-osmosis plays a negligible role, while electrophoresis effects are the primary effects in transdermal delivery of charged molecules [4, 5].

The transport of solute through the *SC* and into the underlying domain is formulated from the Nernst-Planck equation [42] as follows:

$$\frac{\partial C^*}{\partial t} = -\nabla \cdot (m_i C^* \nabla \phi) - \nabla \cdot (D_i \nabla C^*) \quad (6.15)$$

where C^* is the concentration, D is the diffusivity, m is the electromobility, and the subscript i refers to one of the composite layers: *g*, *SC*, *ED*, *DERM*, or *FAT*. Solute concentration is represented in dimensionless form by the relation:

$$C = \frac{C^*}{C_o} \quad (6.16)$$

where C_o is the initial concentration within the gel layer.

Using relation (6.16), Eq. (6.15) is represented as:

$$\frac{\partial C}{\partial t} = -\nabla \cdot (m_i C \nabla \phi) - \nabla \cdot (D_i \nabla C) \quad (6.17)$$

Equation (6.17) is solved using a zero flux conditions:

$$\left. \frac{\partial C}{\partial r} \right|_{r=R_o} = \left. \frac{\partial C}{\partial r} \right|_{r=0} = \left. \frac{\partial C}{\partial z} \right|_{z=-\delta_g} = 0 \quad (6.18)$$

The last boundary condition used is justified by the assumption that, given the short time periods of evaluation and small magnitude transport parameter values, the solute is not expected to be transported to the lower computational boundary:

$$C(r, W/2, t) = 0 \quad (6.19)$$

Initially all the solute is contained within the gel layer:

$$C(r, z, 0) = \begin{cases} 1: & z < 0 \\ 0: & z > 0 \end{cases} \quad (6.20)$$

Exact correlations between electromobility and molecular weight and charge are not available although in vitro experimental measurements of DNA movement in tumor tissues show that electrophoresis increases transport over passive diffusion by several orders of magnitude, and that transport increases with pulse intensity [39]. In vitro studies also show that collagen content and structure acts to reduce electromobility of large molecules in tumor tissue [39, 43]. Electrophoresis studies in polyacrylamide and agarose gels have been conducted to estimate mobility of DNA within the interstitial spaces of living tissue [44,[45]. These studies show that mobility varies inversely with gel concentration in the order of magnitude range $10^{-9} - 10^{-8} \text{ m}^2/\text{Vs}$. Diffusion coefficients of DNA have been obtained experimentally in agarose gels and result in a range of values $10^{-12} - 10^{-14} \text{ m}^2/\text{s}$ depending on gel concentration [44]. Diffusion coefficients and electromobility values used in the current study have been chosen with the findings in mind and are given in Table 6.1.

6.3.5 Lipid phase change

The lipid structure is known to become destabilized at elevated temperatures. It is generally accepted that there exist four main endothermic transitions in the *SC* in the temperature range 40-130°C which have been independently confirmed by differential scanning calorimetry [13-17], although evidence exists of additional thermal transitions [46, 47]. This chapter focuses on three high temperature transitions of the *SC*, adopting the convention used in [46, 47], phase changes E, F, and G. The enthalpic peaks of phase change G occur at

temperatures above 90°C and have been associated with protein denaturation [15-46].

Secondary lipid melting has been associated with phase change F at higher temperatures around 80 °C and has been linked to lipid covalently bonded to corneocytes [15, 16]. Of great interest in this chapter is phase change E which has primarily been attributed to the disordering of the lamellar lipid phase [16] and has been documented in numerous differential scanning calorimetry studies at endothermic peak temperatures from 65 - 72°C [47]. Using X-ray diffraction microscopy it has been shown that the lipid lamellar structure in the *SC* is evident up to temperatures about 60°C and then disappears within 10°C [2, 48]. Polarized light thermal microscopy of lipids extracted from the *SC* directly shows an overall fluidization of lipid structures at 60°C [46]. Further evidence of lipid chain melting and increased permeabilization at this phase change is given in [14, 18] where it is shown that the *SC* becomes more permeable to water flux abruptly at 70°C. Table 6.3 lists the temperature ranges over which each of the three phase transitions are represented.

Skin penetration enhancers have been shown to increase the permeability of the *SC* by increasing the diffusivity, probably by disrupting the lipid bilayer of the *SC* [23]. In [16] the terpene d-limonene is shown to reduce both E and F phase transition temperatures by 20°C. Enthalpic curves generated by differential scanning calorimetry also show that using this enhancer broadens the endotherm associated with phase change G. These findings are reflected in the enthalpy and phase change temperatures used in this study and provided in Table 6.3.

Table 6.3 *SC* lipid enthalpic phase transition data reflecting findings of [16]

Phase change	Association	Without enhancer $[T_1, T_2](^{\circ}C)$	Terpene d-limonene $[T_1, T_2](^{\circ}C)$	Without enhancer $\Delta H (J/kg)$	Terpene d-limonene $\Delta H (J/kg)$
E	Fluidization of lamellar lipids	[65, 75]	[45, 55]	5500	6000
F	Fluidization of protein associated lipids	[75, 90]	[55, 70]	4000	3900
G	Denaturation of proteins	[90, 110]	[70, 110]	4700	3100

To capture *SC* lipid phase transition this study borrows from enthalpy methods which were originally designed to model melting and solidification processes occurring over a temperature range [49, 50]. A rectangular shaped specific heat vs. temperature curve is used to model phase transitions as in [51, 52]. The heat of transition is modeled using apparent specific heats as follows:

$$c_{SC,APP} = c_{SC} + c_{SC,L} \quad (6.21)$$

where $c_{SC,APP}$ is the apparent *SC* specific heat, and $c_{SC,L}$ is the latent specific heat which is represented as:

$$c_{SC,L} = \frac{\Delta H_{PC}}{T_{PC2} - T_{PC1}} \quad (6.22)$$

where ΔH is the latent heat, T_2 and T_1 are the representative phase change end and beginning temperatures, and the subscript *PC* refers to one of the three phase transitions: *E*, *F*, or *G*.

Table 6.3 lists the values used in computations for the three *SC* phase change regions.

In light of the previously discussed *SC* lipid thermal behavior and permeability studied, this model uses phase transition E to model *SC* lipid melting during which the fraction of lipid melting is defined as:

$$\varphi = \frac{(H - c_{SC}T)}{\Delta H_E} \quad (6.23)$$

where H is the total enthalpy and is defined:

$$H = \int_{T_{E1}}^{T_{E2}} c_{SC,APP} dT \quad (6.24)$$

Given the assumed linear endothermic absorption over the lipid melting region E, the fraction of lipid melt is represented as:

$$\varphi = \begin{cases} 0 & : T < T_{E,1} \\ \frac{T - T_{E,1}}{T_{E,2} - T_{E,1}} & : T_{E,1} < T < T_{E,2} \\ 1 & : T_{E,2} < T \end{cases} \quad (6.25)$$

The electrical conductivity of the *SC* is several orders of magnitude lower than the underlying composite layers. The lamellar structure of the bi-layer matrix greatly reduces ionic permeability. With the fluidization the lamellar lipid extracellular sheets in phase transition E comes increased *SC* ionic permeability. Electrical resistance has been shown to drop by two orders of magnitude in the temperature range of this phase change [53] which also suggests the barrier function of the *SC* is compromised in transition E.

To model the increase in electrical conductivity with lipid restructuring, the local *SC* effective electrical conductivity is related to lipid melt fraction by the relations:

$$\sigma_{SC}^* = \sigma_{SC} + \varphi(\sigma_{SC,MELT} - \sigma_{SC}) \quad (6.26)$$

where σ_{SC} is the normal electrical conductivity, and $\sigma_{SC,MELT}$ is the electrical conductivity associated with the *SC* after full lipid melting. In this study a value of $\sigma_{SC,MELT} = 0.001 S/m$ is used.

Similarly enhanced electrokinetic mobility and diffusion coefficient of the *SC* are related to lipid melt fraction by the relations:

$$m_{SC}^* = m_{SC} + \varphi(m_{SC,MELT} - m_{SC}) \quad (6.27)$$

where m_{SC} is the unperturbed mobility of the *SC*, $m_{SC,MELT}$ is the mobility associated with the *SC* after full lipid melting; in this study a value of $m_{SC,MELT} = 5 \times 10^{-11} m^2 / Vs$ is used.

$$D_{SC}^* = D_{SC} + \varphi(D_{SC,MELT} - D_{SC}) \quad (6.28)$$

where D_{SC} corresponds to the initial diffusion coefficient of the solute in the *SC*, $D_{SC,MELT}$ is the diffusion coefficient associated with the *SC* after full lipid melting; in this study a value of $D_{SC,MELT} = 10^{-13} m^2 / Vs$ is used.

In [21], [54] it is noted that long pulse duration is linked to increased post-pulse electrical resistance recovery times ($> 5 min$). In [55] the electric and mass transport barrier properties of *SC* regions experiencing lipid thermal phase transition by electroporation show very long recovery times ($> 30 min$). *SC* lipid structural changes associated with localized Joule heating during electroporation remain for some time after cooling of the *SC* [21]. Polarized light thermal microscopy of *SC* and extracted lipid samples heated to $130^\circ C$ show that as the *SC* samples return to room temperature, the restructuring of the lipids shows evidence of aggregate variation [46]. Because of these findings and in light of the relatively short time periods that transience is modeled (4s), upon cooling this study does not consider lamellar

restructuring of the lipid bilayers and subsequent electrical recovery. Thus Eq. (6.19) is recast as:

$$\varphi^{t+1} = \begin{cases} \max(\varphi^t, 0) & : T < T_{1,E} \\ \max\left(\varphi^t, \frac{T - T_{E,1}}{T_{E,2} - T_{E,1}}\right) & : T_{E,1} < T < T_{E,2} \\ 1 & : T_{E,2} < T \end{cases} \quad (6.29)$$

where the superscript t and $t + 1$ refers to the previous and current time steps, respectively.

6.4 Discretization and solution procedure

The computational domain is discretized using a variable cylindrical grid consisting of 34,100 cells. A fully implicit finite volume approach is used to solve Eqs. (6.1-6.3), (6.10), and (6.17). Temperature, T , and Joule heat, Q_J , are evaluated at the cell centers while the electric potential, ϕ , is evaluated at the cell edges. The driving potential gradient, $\nabla\phi$, of the electrophoretic term in Eq. (6.17) is evaluated at the midpoint of the cell faces. A global 1ms time step is used for transient Eqs. (6.1-6.3) and (6.17). Interface conductivity is represented by a harmonic mean similar to that described in [56], while a resistor representation is used between the cell center and the convection boundary condition (6.8). The SC specific heats, mobility, diffusion coefficient, electrical conductivity and lipid melt fractions of Eqs. (6.21-6.29) are updated at each time step using the local temperature solution of the previous time step. Variables T , ϕ , and C are solved using an implicit point iterative method. Since it is expected that between time steps dramatic solution changes occur not over the entire solution domain, but locally within and about the target region (and within the effective pore radius), a localized convergence measure is used between iterations.

The convergence criterion is defined as $\varepsilon = |T_{i,j}^{p+1} - T_{i,j}^p|$ where p is iteration and convergence is established when $\varepsilon < 10^{-5}$.

6.5 Code validation

To validate the numerical solution, comparisons with analytic solutions were made. The temperature solution of the one-dimensional steady form of Eq. (6.1) was obtained in the axial z -direction using parameter values: $h = 500 \text{ W/m}^2\text{K}$ and $T_\infty = 10^\circ\text{C}$, and setting a prescribed temperature at the target region half width $T(W/2) = T_a$. The one-dimensional solutions of the electric potential and Joule heat Eqs. (6.10) and (6.14) are obtained along the axial direction. A SC conductivity value of $\sigma_{SC} = 10^{-5} \text{ S/m}$ is used and the remaining composite tissue conductivity values are the same as in Table 6.1. Figures 6.2a-b show excellent agreement between computed and analytic solutions. Equation (6.17) is solved in a homogenous domain of diffusivity $D = 10^{-8} \text{ m}^2/\text{s}$ with zero applied potential and boundary conditions:

$$\left. \frac{\partial C}{\partial r} \right|_{r=0} = \left. \frac{\partial C}{\partial z} \right|_{z=W/2} = 0 \quad (6.30)$$

$$C|_{r=R_0} = C|_{z=0} = 1 \quad (6.31)$$

The solution results are compared to the analytic solution in Figure 6.2c which shows the L_2 root mean square error norm. Again excellent agreement between numerical code and the analytic solution is established.

6.6 Results and discussion

Solute concentration, electric potential, and thermal solutions of interest are highly localized within and about the *SC*, and therefore solutions are presented at locations near the *SC* and not over the entire computational domain.

6.6.1 Single pulse

In order to show the phenomenon associated with the thermal, electrical, and transport solutions during pore formation, comparisons between cases with the enhancer and without the enhancer are presented during a single 200ms pulse with an applied voltage of 275V. Pulsation influences will be presented in later sections. The potential solution in the half domain at the beginning and end of the pulse is shown for skin without enhancement (Figures 6.3a-b) and skin treated with the enhancer (Figures 6.3c-d). The local apparent *SC* electrical conductivity is shown in the small inserts in which the two σ_{sc}^* contours shown represent the electrical conductivity associated with lipid structures thermally unaltered (10^{-5} S/m) and the conductivity of lipid structures that have become completely fluidized (0.001 S/m). Early in pulse application, at $t = 20ms$ (Figures 6.3a,c), nearly the entire potential drop of the domain occurs across the *SC*. Even at this early time, the case with the enhancer shows evidence of increased pore development in the slightly more spread potential drops occurring at locations farther away from the pre-existing pore. Furthermore at this time for the enhanced skin case, the radial location of highest apparent *SC* conductivity is nearly double that of the untreated skin. At the end of the first pulse ($t = 200ms$) the potential solution of the untreated skin (Figure 6.3b) shows remarkably little change from the earlier solution reflecting that little thermal disruption of the *SC* lipid structures has taken place. This is

clearly not the case for the potential solution of the skin treated with the enhancer in Figure 6.3d. The radius of the border at which the *SC* conductivity is unaltered is nearly ten times larger than the untreated case. With this change in *SC* electrical property, Figure 6.3d shows a drastically reduced potential drop across the *SC* (especially in the region of lipid phase transition). Furthermore, the larger magnitude potential contours penetrate much lower into the tissue domain in the case with the enhancer. The electrical solutions reflect the thermal phase transition of the *SC* lipid structures, and specifically show that due to the lower transition temperatures, treating the skin results in a much larger radius in which the *SC* experiences electrical breakdown.

Transient thermal solutions of the 275V pulse are shown at representative times for the normal skin in Figures 6.4a-c and for skin treated with the enhancer in Figures 6.4d-f. The small inserts show a close-up of the *SC* in which the lipid melt fraction contours are depicted at low lipid melt fraction ($\phi = 0.1$) and nearly fully fluidized lipid structures ($\phi = 0.9$).

Recalling the results of the electrical solution, it is expected that the small effective pore radius (at $t = 20ms$) is associated in a large local current density which then results in large local Joule heating values and subsequent local temperature rises. This is confirmed for both treated and untreated skin in Figures 6.4a,d in which local temperatures exceed 100°C at the interface of the pre-existing pore and the epidermis. For the untreated skin over the course of the 200 ms pulse, the thermal profiles change little with the high temperature values exceeding 100°C and remaining focused about the pre-existing pore (Figures 6.4a-c). The inserts show that for the untreated skin, the lipid structures change very little over the course of the 200ms pulse and in fact at the end of the pulse, the lower level melt fraction contour reaching a radial distance less than 0.05mm. This is not the case for the enhanced skin, which

experiences the onset of lipid phase transition at a much lower value of 45°C . The results of the lowered transition temperatures are reflected in the inserts of Figures 6.4e,f which depict the effective pore radius extending beyond 0.3mm . A result of a larger effective pore radius is a drop in current density and resulting Joule heating. This is depicted in Figure 6.4e at 100ms , which shows the increase in the effective pore radius and resulting drop in the maximum temperature by about 50°C . As the lower contour levels propagate radially outward causing further lipid phase transitions and larger effective pore radius, the maximum temperature drops again to 75°C in Figure 6.4f.

Concentration solutions are discussed next. From the previous electrical and thermal solutions of untreated skin which show the highly concentrated profiles about the pre-existing pore, it is expected that the transport of solute concentration also remain in this vicinity. The concentration solution of solute transported through untreated skin is presented in Figure 6.5. Initially only low concentration level contours penetrate the *SC*, clearly following the path through the pre-existing pore (Figure 6.5a). After 100ms pulsing (Figure 6.5b), mid-level concentration level contours are seen penetrating through the *SC*. These seem to follow the large potential gradients within the *SC* and local regions of increased *SC* mobility values associated with the thermal phase transitions. A depletion of solute in the applicator gel is evident directly above this portion of the *SC*. At the end of the pulse (Figure 6.5c) a local accumulation of solute is seen in the higher concentration located at the lower *SC* - epidermis interface. This may be explained by recalling that the electrophoretic term of Eq. (6.17) is a function of the gradient in potential. Within the *SC* the apparent electrical conductivity values are much lower than those of the epidermis below. This results in a larger potential gradient within the *SC* compared to the epidermis below. Effectively the

electrophoretic driving forces carry the solute through the *SC* and then experience a sudden drop in magnitude. Due to the small effective pore radius associated with the untreated skin, even at the end of the $200ms$ pulse, concentrations of solute are restricted to the center of the domain and are not transported past the epidermal-dermal junction.

Figures 6.6a-d show the concentration profiles during a $200ms$, $275V$ pulse for skin treated with the enhancer. Similar to the untreated case, at early pulsing times only low concentrations penetrate the skin through the pre-existing pore. At $60ms$ pulsing, moderate concentrations have begun penetrating the *SC*. By $100ms$ a deposition of solute is seen along the interface of the *SC* and epidermis. In fact the concentration at this location exceeds the initial concentration of the gel (Figure 6.6c). This is attributed to the previously mentioned drop in electrophoretic forces as the solute moves from the *SC* into the epidermis. The lower temperature thermal behavior of the *SC*'s barrier lipids result in increased mobility at radial locations much greater than in the untreated skin. By the end of this pulse (Figure 6.6d), the upper and mid level concentration contours have reached $0.2mm$, a distance nearly ten times that of the untreated case. The concentration profiles of the treated skin are characterized by a shallow penetration distance coupled with a large radial distance.

6.6.2 Multiple pulses

Electroporation typically consists of multiple pulses spaced at regular intervals. In this study electroporation is modeled by four $275V$, $200ms$ long DC pulses applied at $1s$ intervals.

To compare the effects of the enhancer on transdermal delivery of solute, comparisons over the 4 pulse period are made between skin treated with and without the enhancer.

Figure 6.7 compares thermal pore formation of the normal to treated skin by showing the effective pore radius at the end of each pulse (Figure 6.7a) as well as the growth of the pore

during pulsing (Figures 6.7b-e). The effective pore radius is defined by the minimum radius at which lipids are unaffected by thermal phases change. In both cases the first pulse produces the greatest pore growth followed by much slower rate of growth in later pulses, although the treated skin experiences remarkably larger pore sizes. After the first pulse, the untreated skin experiences negligible growth since the growth of the effective pore radius in the first pulse drops the local current density to such an extent that in subsequent pulses the Joule heating is not sufficient to cause temperature rises required to increase the effective pore by lipid melting. Even though the current density and subsequent Joule heating and temperature rises decrease with increased pore diameter, for the skin treated with the enhancer, the lower phase transition temperatures of the lipids are reached, thus allowing for continued pore growth in subsequent pulses.

The previous results of the single pulse case clearly show that without the enhancer, the solute is delivered to a small region just below the *SC*. This is also the case when multiple pulses are applied. Figure 6.8 shows the concentration profiles at the end of each of the four pulses when the skin is untreated. As additional pulses are applied, the solute continues to accumulate at the lower *SC* epidermal border. The radial distance to which the solute penetrates into the *SC* does not increase with number of pulses administered. This is indicative of the limit of the thermal growth of the effective pore radius. An interesting artifact of this scenario is that as the electrophoretic forces continue to deliver solute through the small effective pore radius the solute concentrations wrap around the unperturbed *SC* as seen in the 0.1 concentration contour in Figure 6.8d. As pulsing continues, lower level concentrations penetrate the upper epidermis.

The delivery of solute into the untreated skin behaves much differently. Figure 6.9 depicts solute concentrations at the end of each pulse in treated skin. As expected from the previous results the lower lipid phase transition temperatures allow for a larger effective pore radius. In this case, as pulsing continues the solute penetrates the *SC* at increased radial distances, reflecting lipid melting by increased *SC* mobility. At the outer edge of the effective pore, the wrapping effect of the untreated skin case is not evident; in its place are contours that gradually slope toward the *SC* surface, indicating increased concentrations within the upper *SC* regions. The deposition of solute along the interface of the *SC* and epidermis continues to grow radially with pulsing. This region exceeds the initial concentration of the solute in the gel. It is interesting to note that the treated *SC* does not allow the solute to penetrate into the epidermis under the entire region of the effective pore, but only at locations near the axial center. This is a direct result of lower axial potential gradient that occurs as the *SC* becomes more electrically conductive by lipid melting.

6.7 Conclusions

A transient three-dimensional model of in vivo skin electroporation incorporating *SC* lipid melting has been developed in which electrical and transient thermal solutions are found. Large molecule transdermal transport is modeled by the Nernst-Planck equation with low mobility values. A comparison study is conducted between skin treated with the enhancer terpene d-limonene that acts to lower the lipid thermal phase transition temperatures and normal skin not treated with the enhancer. The lower lipid phase transition temperatures associated with the treated skin allow a larger effective pore size for given pulse parameters. Near the beginning of the first electroporation pulse the localized Joule heating is focused near the preexisting pore for both treated and untreated skin and local temperatures exceed

100°C. By the end of the pulse the maximum temperatures of the untreated skin remain at these high temperatures, while the lipids of the treated skin have melted to such an extent that the reduced local current density and Joule heating cannot sustain these temperatures and the maximum temperature is reduced to 75°C. Solute transport in the untreated skin is restricted to the center of the domain by the small effective pore radius. The lower temperature thermal behavior of the treated *SC* barrier lipids result in increased mobility, and consequently solute is transported through the *SC* at radial locations much greater than in the untreated skin. Transport solutions of both cases exhibit local accumulation of concentrations below the *SC*-epidermis interface which exceed concentration values initially contained within the applicator gel.

6.8 References

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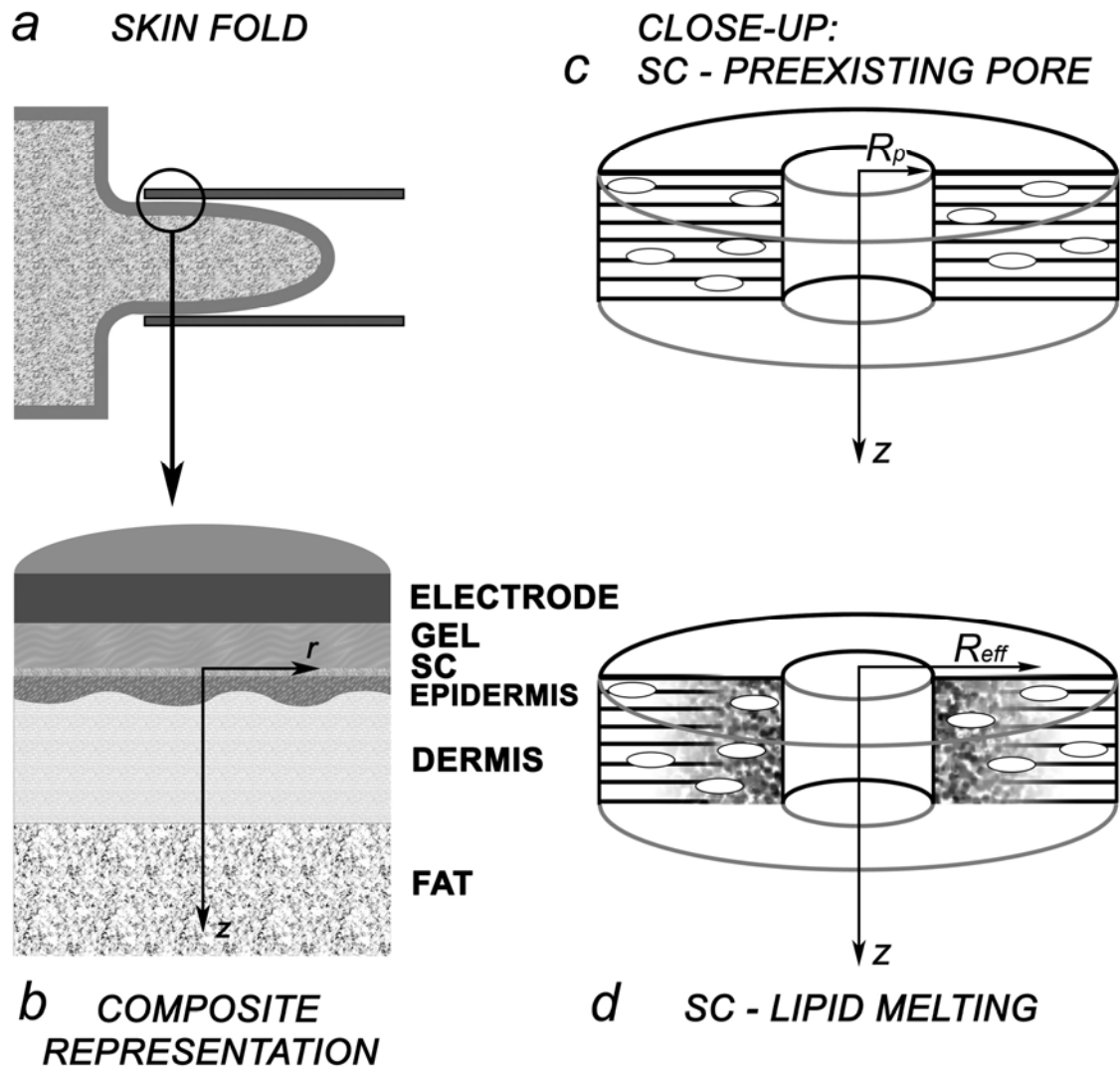


Figure 6.1 Physical model description: (a) skin fold, (b) cylindrical composite model, (c) trans- SC pore and lipid representation, (e) SC undergoing lipid phase transition.

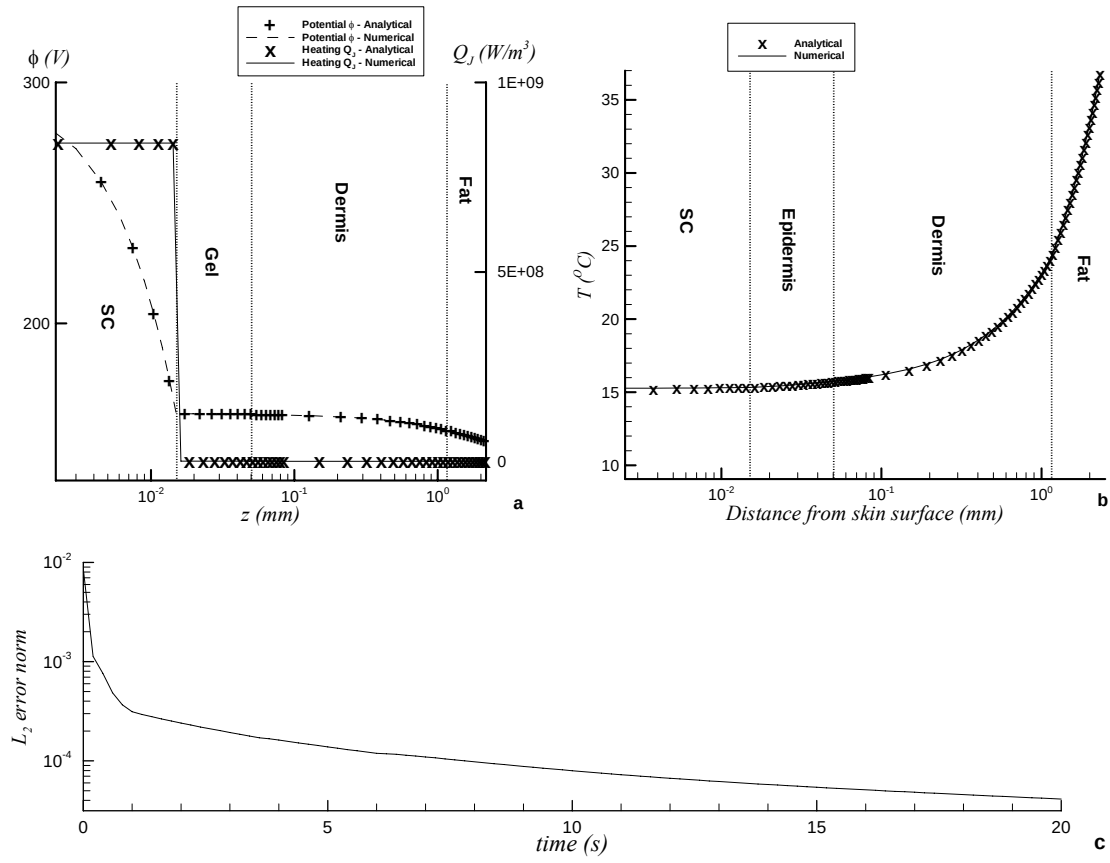


Figure 6.2 Comparisons between numerical code and analytic solution (one-dimensional): (a) Electric (potential and heating) in which the domain represents a vertical line between the electrodes using $V_{app} = 300V$. (b) Steady state temperature ($^{\circ}C$), in which the domain represents vertical depth below the skin's surface using convection coefficient $h = 500W/m^3$. Dotted lines separate composite sections. (c) L_2 normalized error of homogenous two dimensional solution comparison with analytical solution.

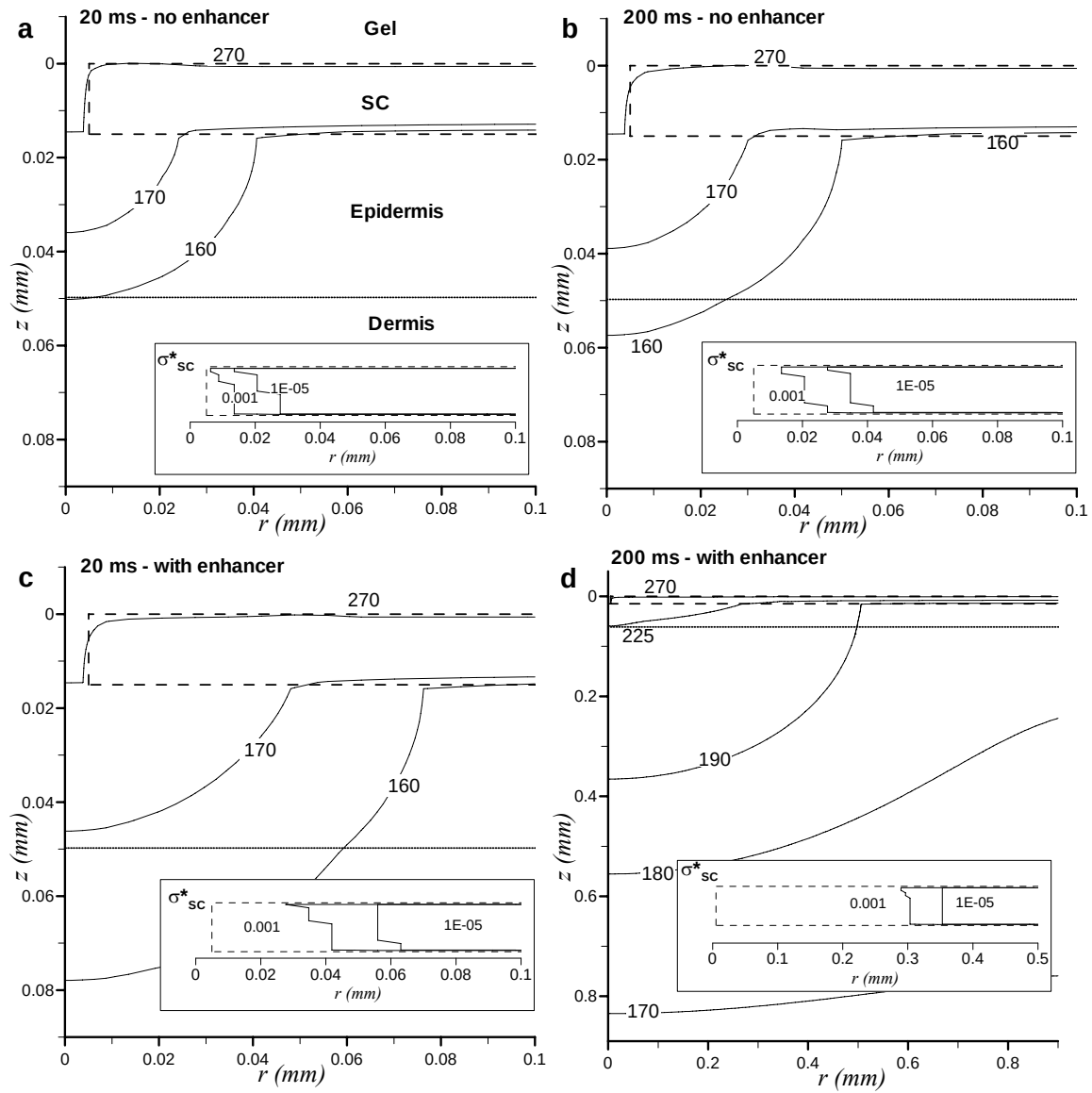


Figure 6.3 Potential solution at representative pulsing times of a single 275V pulse for cases without enhancer (a, b) and cases with enhancers (c, d). Dotted lines represent interfaces between composite layers. Dashed rectangle shows the boundary of the SC. Inserts represent a close-up of the SC with contours denoting apparent electrical conductivity (S/m).

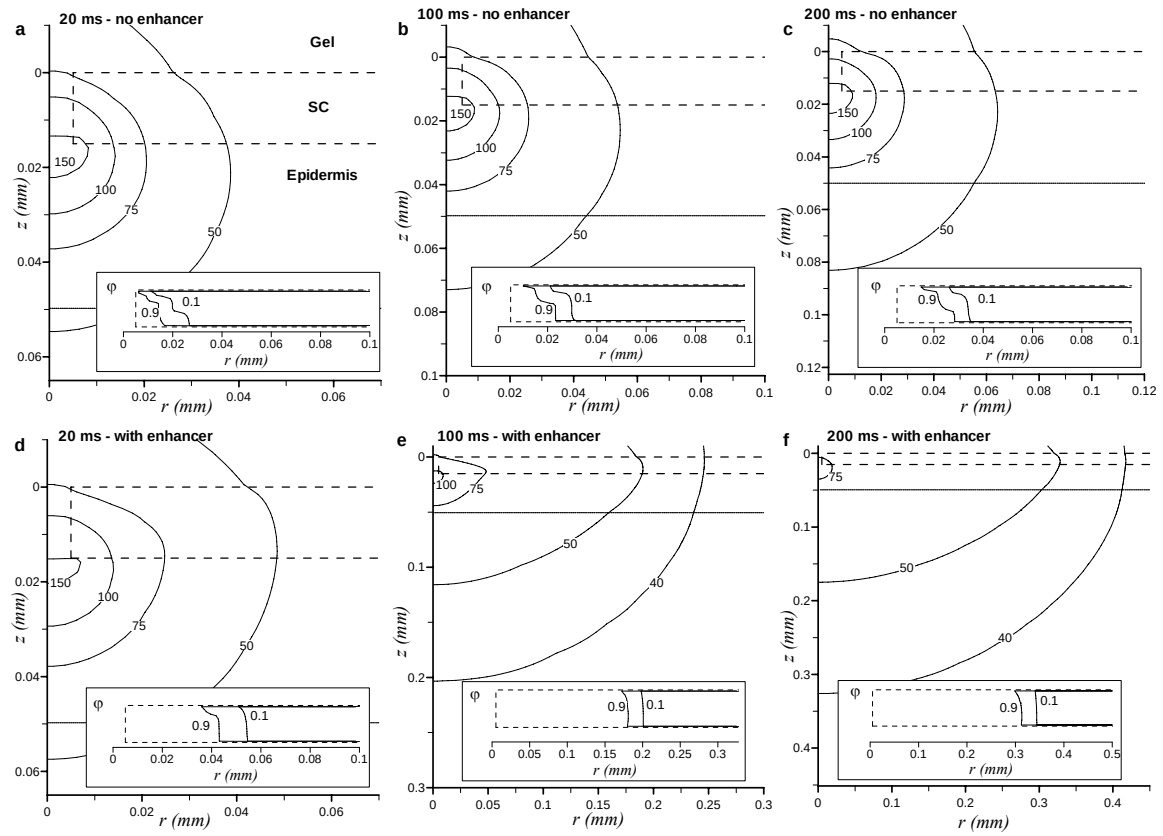


Figure 6.4 Transient thermal solution ($^{\circ}\text{C}$) at representative pulsing times of a single 275V pulse for cases: without enhancer (a-c) and cases with enhancers (d-f). Dotted lines represent interfaces between composite layers. Dashed rectangle shows the boundary of the SC. Inserts show lipid melt fraction contours in a close-up of the SC (dashed rectangle) using aspect ratios (insert e uses aspect ratio $x:y = 3:1$, insert f uses aspect ratio $x:y = 1:5$)

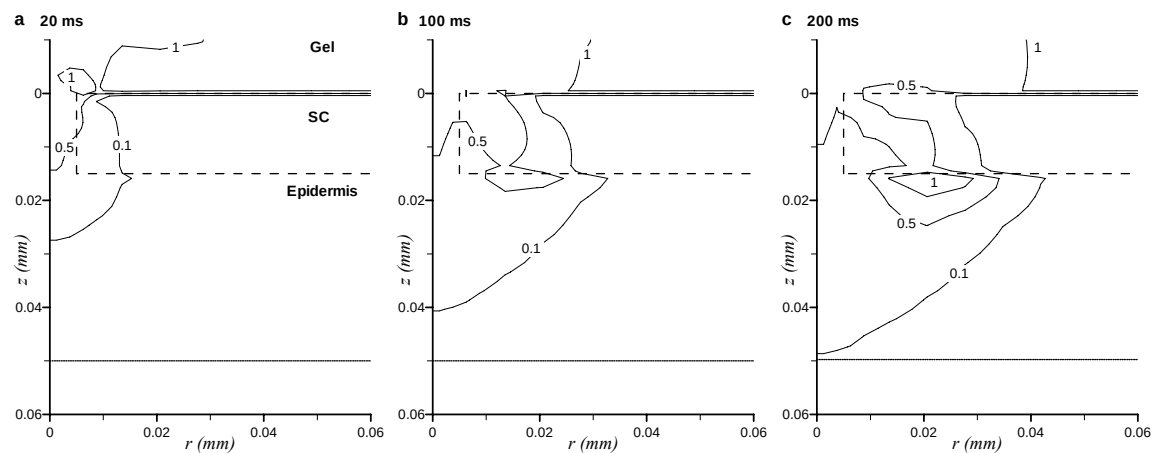


Figure 6.5 Transient concentration solution, C , of skin without enhancer treatment at representative pulsing times during a single 275V pulse. Dashed rectangle shows the boundary of the SC. Dotted lines represent interfaces between composite layers.

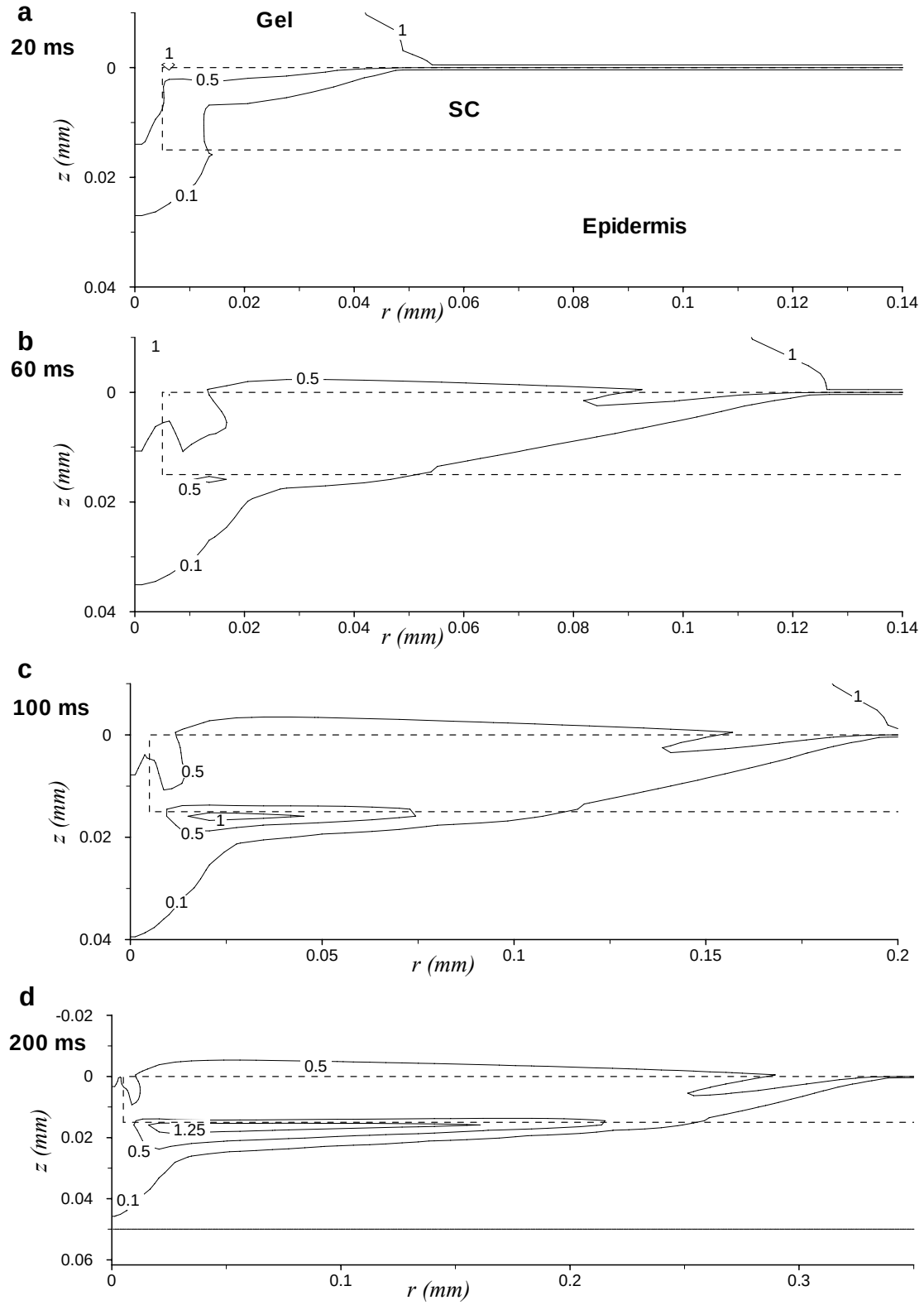


Figure 6.6 Transient concentration solution, C , of skin with enhancer treatment at representative pulsing times during a single 275V pulse. Dashed rectangle shows the boundary of the SC. Dotted lines represent interfaces between composite layers.

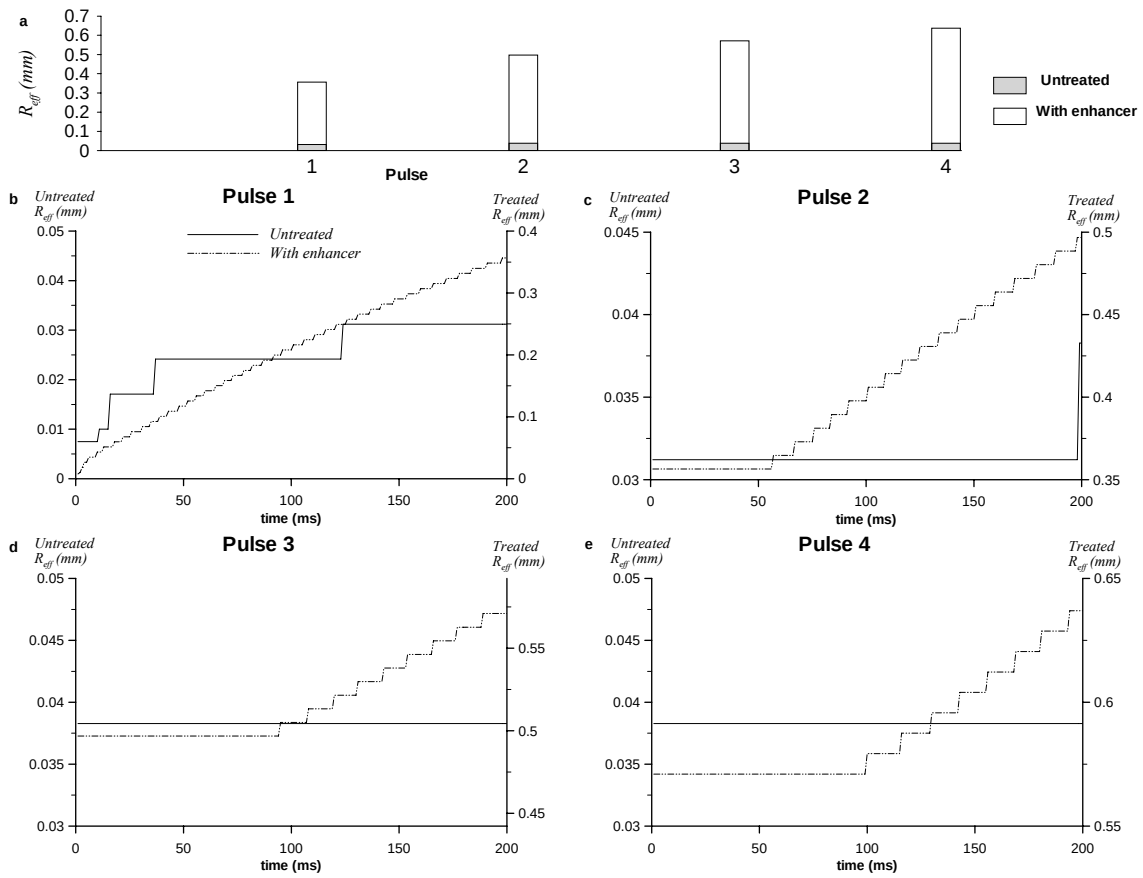


Figure 6.7 Effective pore radius growth comparisons between treated and untreated skin exposed to 200ms, 275V pulses spaced at 1s intervals. Effective pore radius is defined by the minimum radius at which lipids are unaffected by thermal phases change. In (b-e) effective pore radius for untreated skin shown on left axis, for skin treated with enhancer is shown on right axis.

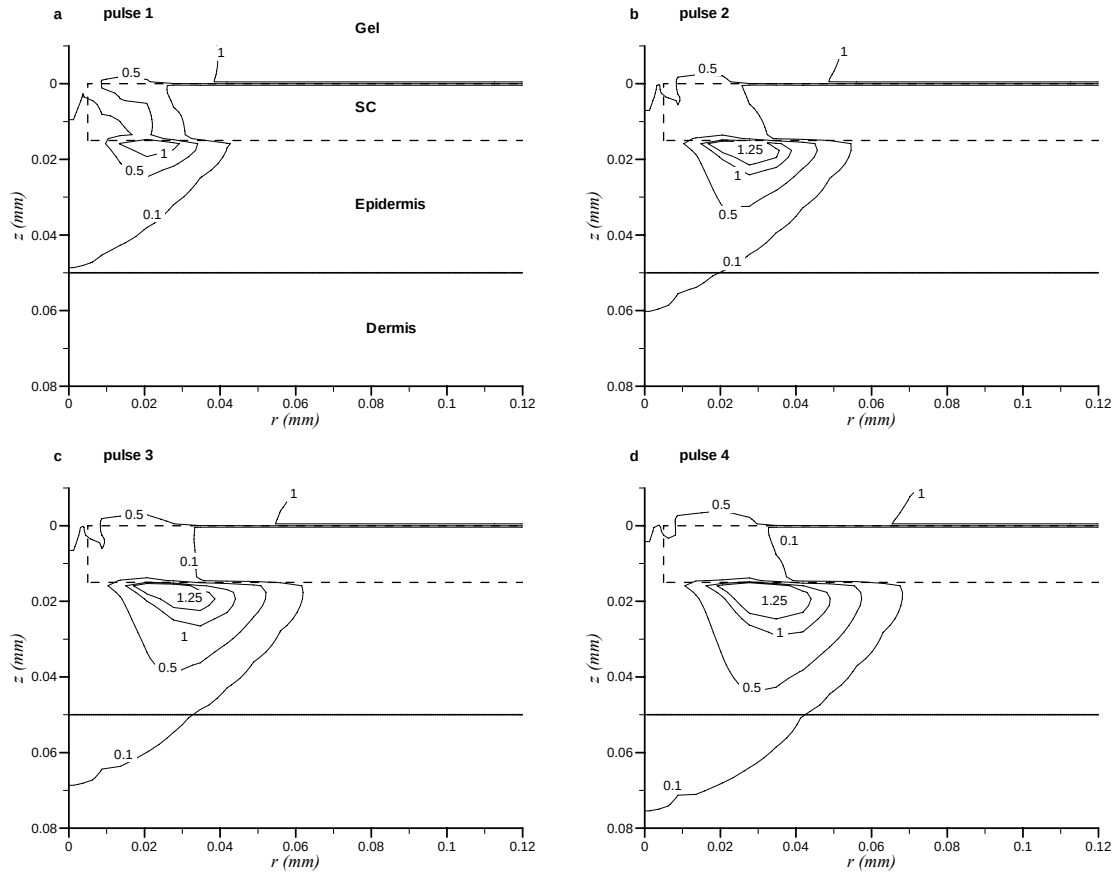


Figure 6.8 Concentration solution, C , of skin without enhancer treatment at the end of each of the four 275V, 200 ms pulses applied at 1s intervals. Dashed rectangle shows the boundary of the SC. Dotted lines represent interfaces between composite layers.

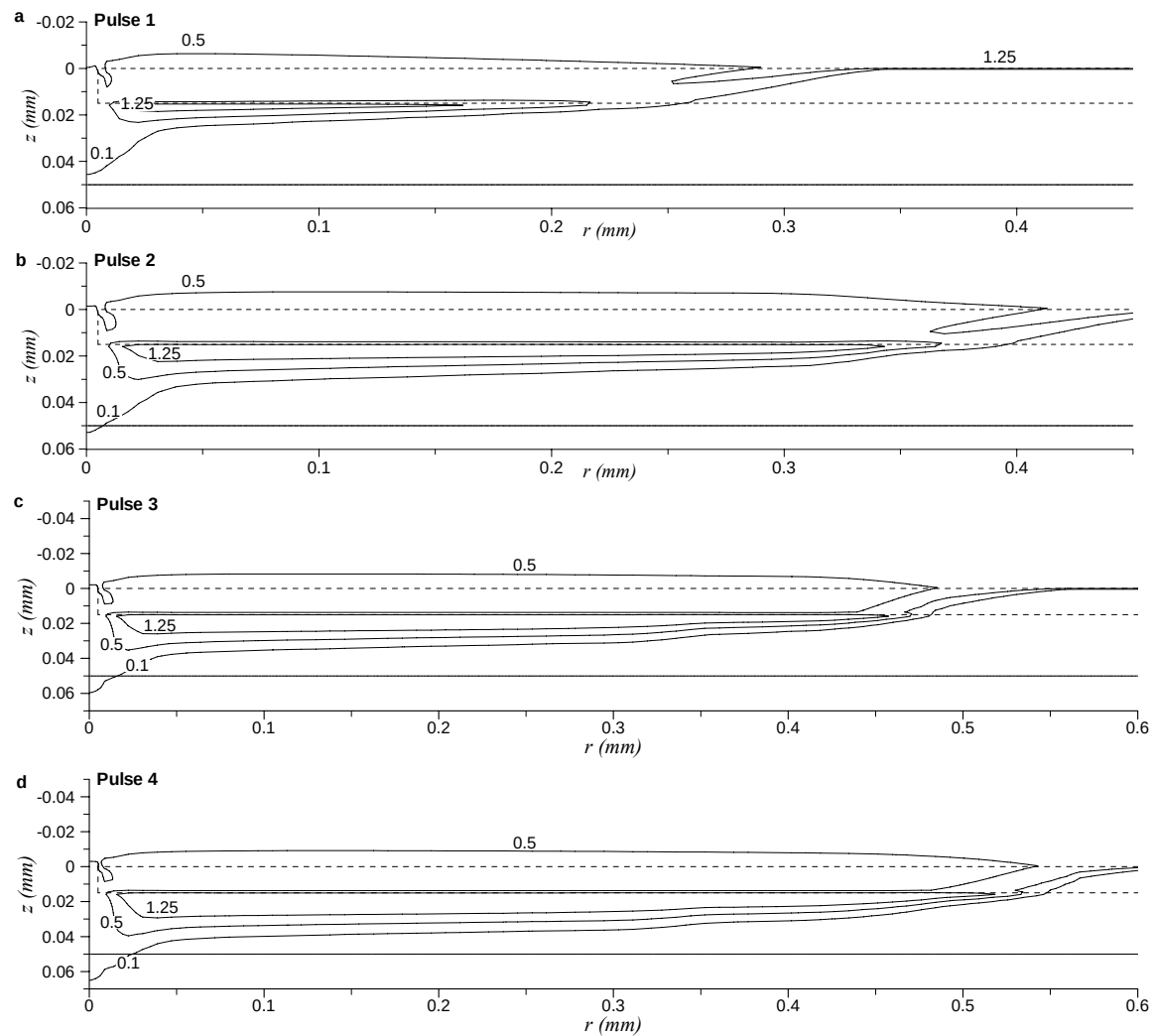


Figure 6.9 Concentration solution, C , of skin treated with enhancer at the end of each of the four 275V, 200ms pulses applied at 1s intervals. Dashed rectangle shows the boundary of the SC. Dotted lines represent interfaces between composite layers.

7 Conclusions

This work concerns the numerical modeling of *in vivo* plate electroporation.

In the second chapter, a parallel plate electroporation model of homogenous tissue is presented in which blood vessel, geometric, and electroporation pulse parameter effects on thermal dose magnitudes are investigated. Lower levels of thermal damage occur in the presence of a blood vessel passing through the axial center of the target region. For larger vessels ($R > 0.3$ mm) this effect is more pronounced. The vessel carries heat from the target region, cooling the tissue as the vessel blood flow enters the target region and heating the tissue as it exits the target region. The investigation of the effect of spacing between the electrode plates shows that increased plate spacing increases thermal damage: as the volume to surface area ratio increases, conduction and convection have relatively less area across which to move more thermal energy. The results of dividing a long pulse into shorter pulses of equal intensity and frequency were shown that increasing the time over which energy is introduced into the system results in lower maximum target region temperatures and significantly decreased thermal dose values. Similarly it was found that decreasing pulse frequency results in lower thermal damage and lower maximum temperatures.

The third chapter continues the thermal-electric study of electroporation, progressing from the homogenous tissue model to the more complex inhomogeneous tissues of the skin. A transient three-dimensional model of *in vivo* skin electroporation is presented in which the importance of representing the separate composite layers of the skin is established. The electrical solution of the skin composite model is strongly dependant on the electrical resistance of the *SC* and shows a strong one-dimensional tendency parallel to the induced electric field. The highly electrically resistive *SC* and epidermis layers receive the largest

Joule heating, while in the more conductive subcutaneous muscle tissue heating is negligible. The decrease in *SC* electrical resistance associated with skin electroporation significantly increases heating of the dermis and subcutaneous fat layers. (This chapter makes the assumption that the permeabilization of the *SC* is and occurs within the first 5 *ms* of pulsing.) The thermal effects of *in vivo* electroporation are characterized by a sudden jump in temperature followed by a much more gradual cooling process. The low heat capacity of fat promotes temperature rises associated with Joule heating. The dermis and subcutaneous fat layers are at great risk of thermal damage with the reduction of *SC* electrical conductivity. The fourth chapter, using the composite skin model, focuses on a parametric analysis of factors affecting thermal damage. A blood vessel running deep in the subcutaneous muscle tissue indirectly increases the maximum value of thermal damage by adding heat to the target region. Leaving the electrodes attached to the skin after electroporation promotes cooling, thereby reducing thermal damage of the outer composite layers. Also in this chapter a method is presented in which the target region is cooled prior to electroporation. Effectively cooling tissue prior to electroporation reduces the risk of thermal damage, allowing the experimentalist to use pulse parameters which increase the likelihood of successful drug delivery.

In the fifth and sixth chapters, the development of an electroporation pore during pulsing is investigated. A transient axi-symmetric model of *in vivo* skin electroporation incorporating *SC* lipid melting is developed in which electrical and transient thermal solutions are found. The electrical solution of the skin composite model shows potential drop across the *SC* decreases as the electroporation pore grows. This is a result of increased electrical conductivity with lipid phase transition. Heating and temperature rises are highly localized

occurring not over the entire target region, but within the vicinity of the electroporation pore. The very electrically resistive *SC* receives the largest Joule heating values which focus within the region of lipid phase transition. As a result of large Joule heating, lipid phase transition temperatures are attained during pulsing. The results show that *SC* lipid phase transition occurs in a pattern from the skin interior to the surface and radially outward. The rate of pore growth decreases with increased pore radius and pulsing time as a consequence of decreased current density. Longer pulse spacing allows for greater diffusion of heat between pulses thus lowering the maximum temperatures at the beginning of subsequent pulses. Electroporation pore growth is then inhibited by the resulting lower local maximum temperatures.

In the seventh chapter, mass transport is modeled and the effects of using a chemical enhancer to reduce the lipid phase transition temperatures are explored. Large molecule transdermal transport is modeled by the Nernst-Planck equation with low mobility values. The lower lipid phase transition temperatures associated with the treated skin allow a larger effective pore size for given pulse parameters. Near the beginning of the first electroporation pulse the localized Joule heating is focused near the preexisting pore for both treated and untreated skin and local temperatures exceed 100°C . By the end of the pulse the maximum temperatures of the untreated skin remain at these high temperatures, while the lipids of the treated skin have melted to such an extent that the reduced local current density and Joule heating cannot sustain these temperatures and the maximum temperature is reduced to 75°C . Solute transport in the untreated skin is restricted to the center of the domain by the small effective pore radius. The lower temperature thermal behavior of the treated *SC* barrier lipids result in increased mobility, and consequently solute is transported through the *SC* at radial

locations much greater than in the untreated skin. Transport solutions of both cases exhibit local accumulation of concentrations below the *SC*-epidermis interface which exceed concentration values initially contained within the applicator gel.