Experimental Factors to Be Considered in Electroporation-Mediated Transdermal Diffusion Experiments

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In this paper, we discuss some of the primary experimental factors that should be considered when interpreting and implementing the published results of skin electroporation studies concerning measurements of mass transport across the stratum corneum (SC) in the Franz cell. It is explained that the pulse magnitude should always be considered in the context of pulse shape and that transport measurements should always be presented in the context of the trans-SC potential difference (instead of the voltage between the electrodes). The condition of the SC prior to the application of the long-duration pulse strongly influences the evolution of the local transport region (LTR). This is quantified in a simple analytical investigation of the conditions that affect the thermodynamic response of the skin. [DOI: 10.1115/1.4031767]

Keywords: transdermal mass transport, thermally induced pathways, electroporation, square wave pulses, exponentially decaying pulses, Franz diffusion cells

1 Introduction

The skin's outermost layer, the SC, has an exceptionally low permeability to mass transfer and represents a formidable obstacle to the transdermal delivery of drugs. In order to increase the SC's permeability to molecular transport, both passive enhancement methods (which are chemically based) and active enhancement methods (which rely on an imposed physical force) have been extensively researched [1,2]. One of the active methods used to increase transdermal molecular delivery is electroporation. Electroporation of the skin uses intense electric pulses, which disrupt the multilamellar lipid bilayer structure of the SC thereby reducing the skin's resistance to molecular transport [3,4]. Depending on the electric pulse characteristics, the effects of this disruption can be observed to last from only a short period of microseconds up to several hours [5–8]. Longer lasting disruption of the skin's resistance allows for much enhanced transdermal diffusion.

The increase in the skin's permeability to transdermal delivery following skin electroporation is highly dependent on the electric pulse characteristics (i.e., amplitude, duration, spacing, and number). The field is in general agreement that there are different physical mechanisms responsible for these increases in skin permeability according to two primary pulsing regimes [4]:

(i) Short-duration high-intensity pulses result in an altered SC that is highly perforated with cell-sized aqueous pathways. These pathways are initiated as a result of the molecular scale interaction between the applied electric field and the dipole of individual water molecules that are located adjacent to the head groups of the lipid composition of the cell membranes within the first few microseconds of exposure to the electric field [9]. Pulses in this regime are referred to as "high voltage" (HV) pulses.
(ii) Long-duration low-intensity pulses occur on much longer timescales (up to hundreds of milliseconds) and result in regions of increased permeability within the SC, which are relatively large (up to hundreds of micrometer) but that occur at a much lower density (number of pathways per lateral SC surface area) [4,8]. These regions are referred to as LTRs. The development of the LTR is believed to be thermally related and associated with the thermal disruption of the SC lipid microstructure: within a temperature range around 60–70 °C, the SC lipid crystal structure experiences a fluidizing phase transition [10]. Long-duration pulses can result in resistive Joule heating, which has been documented under certain experimental pulse conditions to cause localized temperature rises of over 60 °C [7,11,12]. The pulses in this category are referred to as "low-voltage" (LV) pulses.

Even small variations in the experimental conditions can strongly influence the physics that result in these permeability increases. In this paper, we discuss some of the primary experimental factors that should be considered when interpreting and implementing the published results of skin electroporation studies. It is a discussion on already published work in the field by other authors as well as experimental and theoretical work by the authors of this paper and includes an analytical analysis of the LTR evolution.

In Sec. 2, a brief review is provided of studies implementing the most common pulse shapes (square wave (SW) or exponentially decaying (ED)) used in skin electroporation experiments. Studies of different pulse shapes report their results using identical jargon. The potential pitfalls of misinterpreting this identical language are discussed.

Franz diffusion cells are the standard experimental setup to measure the effect of electroporation on transdermal transport. The transport measured by the Franz cell setup is at the tissue level and not the cellular level. Because the system is very sensitive to the experimental parameters used, care should be taken when interpreting the results of experiments using this technology. This is discussed in detail in Sec. 3.

Finally, in Sec. 4, a simple analytical model is developed that can be used to highlight the sensitivity of the rate of LTR evolution on experimental parameters.

2 Pulse Shape

There are two primary pulse shapes that are employed in skin electroporation experiments. SW pulses use a constant voltage for

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Table 1 Pulse types and parameters used in the studies of transdermal molecular delivery

Study		Protocols	
References	Type of study[a]	Pulse type[b]	Pulse parameters or parameter ranges (number of pulses/voltage[c]/duration[d])
Prausnitz et al. [14]	TDD, EXP, in vitro, in vivo	ED	720/U_{skin}: 50–500 V/τ: 1–1.3 ms
Pliquett et al. [8]	Skin changes, EXP, in vitro	ED	up to 60/U_{skin}: up to 150 V/τ: 1.1 ms
Vanbever and Préat [15]	TDD, EXP, in vitro	ED	5/24–250 V/τ: 620 ms (changing voltage) 5/100 V/τ: 78–711 ms (changing duration)
Pliquett and Weaver [5]	TDD, skin changes, EXP, in vitro	ED	15–180/U_{skin}: up to 200 V/τ: 1.1 ms
Gallo et al. [16]	Skin changes, EXP, in vitro	SW	Up to several hundred/U: 5–500 V/Δt: 1–10 ms
Vanbever et al. [17]	EXP, in vivo, safety study	ED	15/U: 100 V/τ: 500 ms 15/U: 250 V/τ: 200 ms 60/U: 500 V/τ: 1.3 ms
Pliquett et al. [18]	LTR, EXP, in vitro	ED	5–60/U_{skin}: 20–100 V/τ: 1–300 ms
Chizmadzhev et al. [19]	EXP, in vitro, THEOR	SW	1/U: 10–60 V/Δt: 8 ms
Vanbever et al. [7]	TDD, EXP, in vitro	ED	240–720/U: 1000–1500 V/τ: 1 ms 20/U: 100–400 V/τ: 100–300 ms
Pliquett et al. [20]	TDD, EXP, in vitro	ED	30/U_{SC}: 75–90 V/τ: 1 ms
Martin et al. [21]	LTR, skin changes, THEOR	ED	1/U_{SC}: 70 V/τ: 1–10 ms
Pliquett et al. [22]	LTR, EXP, in vitro	ED	10/U: 80 V/τ: 100 ms 10/U: 105 V/τ: 360 ms
Dujardin et al. [23]	EXP, in vivo, safety study	SW	10/U: 1000 V/Δt: 100 μs 10/U: 335 V/Δt: 5 ms
Denet and Préat [24]	TDD, EXP, in vitro	SW	10/U: 250–400 V/Δt: 1, 10, 20, 200 ms
Pliquett and Gusbeth [11]	Skin changes, EXP, in vitro, THEOR	ED	60/U_{skin}: 100 V/τ: 1.1 ms
Pliquett et al. [12]	LTR, skin changes, EXP, in vitro	ED+SW	ED: U: 1000 V/τ: 1 ms ED: U: 100 V/τ: 300 ms SW: up to 1000/U: 10–150 V/Δt: 300 μs
Wong et al. [25]	EXP, in vivo, pain assessment	SW	60/U: up to 150 V/Δt: up to 1 ms
Zorec et al. [26]	TDD, EXP, in vitro, THEOR	SW	HV: 3/U: 500 V/Δt: 500 μs LV: 3/U: 45 V/Δt: 250 ms
Blagus et al. [27]	TDD, EXP, in vivo,	SW	24/U: 70–570 V/Δt: 100 μs

[a]TDD, transdermal drug delivery; LTR, study of local transport regions; EXP, experiments; and THEOR, theoretical study.
[b]ED, exponentially decaying pulse(s) and SW, square wave pulse(s).
[c]Pulse amplitude is usually reported as voltage between electrodes, however, voltage across skin sample for in vitro experiments is much lower. When voltage on skin is reported, the amplitude is indicated with either U_{skin} or U_{SC}. For ED pulses, voltage refers to the maximum voltage, at the beginning of pulse.
[d]Pulse duration for ED pulses is given as exponential decay time constant τ; for SW pulses, it is given as the duration of a single pulse (Δt).

the entire pulse duration in anticipation of a highly controlled and repetitive pulse protocol. ED pulses have been employed in skin electroporation experiments partly because the technology of capacitor-discharge devices required to deliver ED pulses is much simpler than that required by SW generators [13]. Furthermore, the long LV decaying tail of the ED pulse can provide an electrophoretic contribution to transport and under certain conditions it can result in Joule heating. The duration of the ED pulse is characterized by the exponential time constant: $V(t) = V_O \exp[-t/\tau]$ so that smaller values of τ correspond to more rapidly decaying pulses. A list of both, experimental and theoretical studies is provided in Table 1 that summarizes pulse types and their parameters used in the studies of transdermal molecular delivery reported in the literature.

As discussed in Sec. 1, the electroporation pulses are categorized as either HV or LV and each category has a different physics associated with the alteration of the SC barrier: HV pulses are associated with the disrupt of the lipid microstructure by a strong electromagnetic force and the LV pulses are associated with SC lipid thermal phase transition. Generally, the literature labels the pulse an HV pulse, when the amplitude between the electrodes is above 500 V (while their duration is much shorter: hundreds of microseconds) and as LV when the voltage between electrodes does not exceed 200 V (few to hundreds of milliseconds).

All studies, whether they use ED or SW pulse shapes, use identical terminology (HV and LV) to characterize the pulse regime. When referring to an SW pulse, the label HV or LV corresponds to the category of pulse amplitude during the entire duration of the pulse. However, when referring to an ED pulse, the pulse label HV or LV refers only to the initial pulse amplitude. Consider that the ED pulse inherently comprises a short but higher voltage component at the beginning and that this is followed by a lower amplitude decaying tail. The distinction between these can be seen in Fig. 1 in which both pulse shapes are superimposed for typical HV pulses (Fig. 1(*a*)) and for LV pulses (Fig. 1(*b*); note that the time scales differ by three orders of magnitude).

Note the potential discrepancy of using the same language (LV or HV) to characterize a pulsing regime for both SW and ED pulse shapes. Consider first Fig. 1(*a*) depicting the HV pulse. Clearly the entirety of the SW pulse shape lies only in the HV region. However, note that the tail of the ED pulse continues for a much longer time at a lower voltage. Thus, it is feasible (and likely) that the tail of the HV ED pulse could initiate physics that are normally only associated with pulses labeled LV. At early times, the decaying pulse initiates the high density distribution of cell-sized pathways through the SC, which is a purely electrical phenomenon. At later pulsing times, the same pulse causes microscale heating within these pathways that lead to the lipid thermal phase transition. While these two effects are traditionally attributed to two different pulse regimes, the pulse has been categorized as an HV pulse.

In contrast, in the LV range (Fig. 1(*b*)), the pulse *shape* may play a lesser role in LTR evolution (as long as the initial voltage of the ED pulse is not of a magnitude sufficient to cause the creation of new small aqueous pathways by electroporation). This is because the LTR growth is believed to result from the lipid thermal response to the local temperature rise which is proportional to

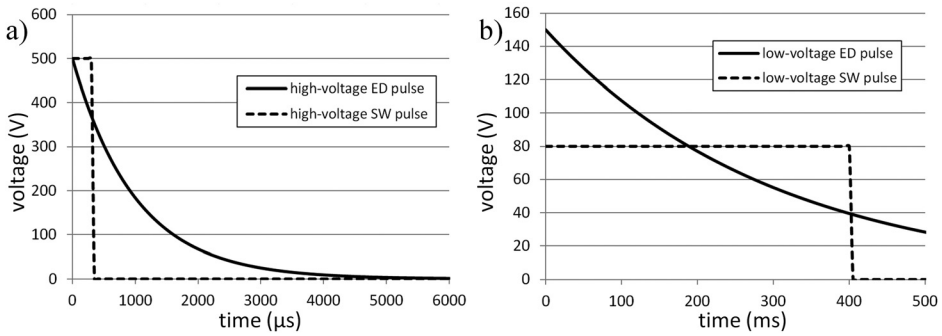

Fig. 1 A comparison between (*a*) typical HV ED and SW pulses and (*b*) typical LV ED and SW pulses. Note that the time scales differ by three orders of magnitude; also, the voltage scales are not the same for HV and LV.

the *amount* of energy dissipated within the LTR during the pulse. This is illustrated in Sec. 4 of this paper.

It is possible that the physics associated with the permeability increase of an HV ED pulse could have components that are normally associated with both HV and LV pulse types. This should be contrasted to the SW pulse which can only be either HV or LV but not both. Special attention should be made to the pulse shape when interpreting the label of the pulsing regime (HV or LV).

Having stated the above, it should be noted that in this discussion, the SW pulses are presented in their idealized form. Conversely, the pulses delivered with pulse generators always have nonzero rise and fall times and therefore also include exponential growth and decay components. However, the duration of these components is very short in comparison with the usable length of the pulse. Good SW pulse generators can produce HV pulses with fall times well below 10 μs (depending on the load) and even shorter rise times (a few microseconds), which is much shorter than true ED pulses and can be neglected for the purpose of this discussion. Still, a frequency analysis of both types of pulses would show an even more detailed comparison between typical SW and ED pulses.

3 Experimental Setup Considerations

The Franz diffusion cell is the setup of choice in most permeation studies to test the transdermal transport characteristics of molecules present in different formulations, such as creams, gels, or encapsulation systems. Diffusion cells consist of two buffer filled compartments: (1) a donor cell that contains the molecule to be delivered and (2) a receiver cell from which transport concentration values are measured. These two cells are separated by a skin sample. Because the transport of the molecule across the skin may be quantitatively measured, the Franz cell is the most widely accepted method of experimentally measuring passive transdermal diffusion (at the tissue level). However, there are several characteristics of the Franz diffusion cells with skin electroporation experiments that give rise to concern. In this section, we present some limitations of the use of Franz diffusion cells to model transport in skin electroporation studies that should be considered in order to avoid drawing erroneous conclusions.

Commercially available diffusion cells are designed for passive diffusion studies and therefore are often relatively small; the donor and receiver compartments may have an inner diameter of 1–2 cm. This makes fitting the electroporation electrodes onto the skin particularly challenging. To address this, instead of placing electrodes directly onto the skin, the electric pulses have been delivered into the buffer solution: one electrode in the donor solution and one in the receiver solution [5,7,8,11,12,14,15,18,19,22]. This can present a number of complications when implementing the results of published work.

The first concerns the interpretation of the magnitude of the pulse. From an electrical perspective, the buffer solution represents a non-negligible part of the whole system. It is not unlikely (as discussed in Ref. [26]) that a large portion of the total delivered voltage is lost to the resistance in the buffer solution itself: a much smaller voltage is delivered to the actual skin sample. When considering the published results of such electroporation studies, care must be taken not to interpret the voltage applied to the electrodes as the actual voltage delivered across the skin sample U_{skin}, which depends strongly on the placement of the electrodes in the donor and the receiver solution, and is not easily controlled. Therefore, at the very least, the U_{skin} needs to be measured, however, only some authors have actually done it [5,7,8,11,18].

The second complication concerns the electrical properties of the buffer solution itself. It has been established that the electrical system is highly sensitive to any minor changes in buffer solution composition or concentration [24,28,29]. Also, the electric pulses delivered to the skin indirectly via the donor and the receiver solution containing abundant ions bring along a number of electrochemical reactions that cannot be easily accounted for or controlled. Electric pulses will inevitably cause electrochemical reaction in the buffer solution that may influence the electrical characteristics of the buffer solution. In such a case, the shape of the electric voltage across the skin may not identically follow that of the pulse delivered to the electrodes. In this case, it is not only the magnitude of U_{skin} that needs to be recorded, but also its shape on the time interval *during* the pulse delivery.

Finally, when considering in vivo applications of skin electroporation, care must be taken when considering the results of the in vitro transport studies. This is because the in vitro experimental setup (electroporation through a Franz cell) differs greatly from the in vivo application. In an in vivo or clinical application of skin electroporation, variations of electrode configurations are used in which all of the electrodes are placed externally on the skin's surface [14,16,17,23,25]. The electric field associated with the Franz cell type setup (where one electrode is placed on the external side and one electrode is placed on the internal side of the skin) is unlikely to closely resemble the intended in vivo case. Because it is this electric field which is responsible for the creation of small pathways, for their expansion, as well as for the driving force, transport characteristics of the in vitro studies may differ greatly from the in vivo application, which they intend to represent.

4 Simplified Analytical Model of LTR Expansion

In the following discussion, a simple analytical model is developed to help quantify the influence that the pulse characteristics and the buffer solution have on the transport associated with electroporation. This will be done in the context of the temporal evolution of a thermally induced LTR.

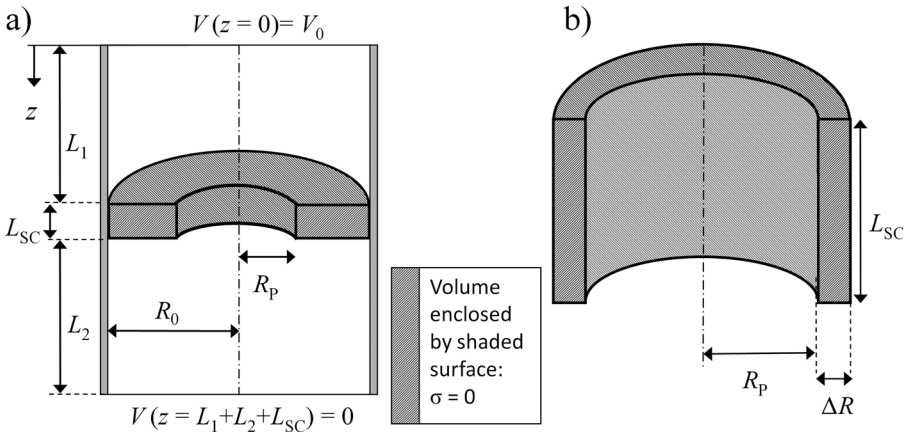

Fig. 2 (*a*) Schematic of the idealized LTR: a section of the SC surrounding the pre-existing pathway. The SC (thickness L_{SC}) extends to the outer domain boundary, R_0, and is traversed by a small cylindrical pore of radius R_P. (*b*) The expansion of the LTR: the small thin-walled cylindrical region of thickness ΔR represents the expansion of the pore due to the temperature rise.

Here, we consider a theoretically idealized and much simplified case representing the heating of a thin annular membrane of very low electrical conductivity (representing the SC) that is placed in an electrically conductive medium. The system is depicted in Fig. 2(*a*). The membrane (which is traversed by a small cylindrical pore of radius R_P) extends to the outer domain boundary, R_0, and has a thickness L_{SC}. We conducted a simple exercise that investigates how the parameters R_0 and R_P affect the Joule heating within the pore, and how they influence the time it takes to heat a small annular region (ΔR) surrounding the pore.

In general, biological materials are not purely resistive but have their capacitive or inductive component (reactance, frequency-dependent part). If the material is exposed to direct current, the frequency-dependent part—the reactance—plays no role when the system is in steady state, after all the transients have faded out. It does, however, dictate the course of the transient of the system. When using typical DC pulses to enhance transdermal transport with electroporation, electrical response of skin can be regarded as quasi-stationary, thus the capacitive effects and the finite propagation of the electric current in the biological tissue can be disregarded.

The outer radial boundaries are perfectly insulated (electrically) in order to represent the case when the SC is homogenously perforated by these pores. For simplicity, the entire domain has a uniform electrical conductivity, σ, everywhere except within the annular region occupied by the membrane $(L_1 < z < L_1 + L_{SC}$ and $R_P < r \leq R_O)$. The membrane electrical conductivity is so much lower than that of the surrounding medium that it is assigned a zero value.

The electric field will be approximated as one dimensional (in z) so that, for this exercise, any radial variations in the electric potential gradient are neglected. To represent an electric pulse that is applied to the upper boundary of the domain (at $z = 0$), an electric potential of $V(z = 0) = V_O$ is imposed. At the bottom domain boundary, the potential is held at a zero value: $V(L_1 + L_2 + L_{SC}) = 0$ for all times.

Recall that the evolution of the LTR is a phenomenon associated with the thermal state of the skin's microstructure. When the pulse is applied, the electric current follows the path of least resistance through the SC: any region traversing the SC that has a lower electrical resistance (for example, some small defect or appendageal pore) will experience a high current density. The electrical resistance in this localized region of high current density causes localized Joule heating. If the pulse is sufficiently long, the SC lipids may reach temperatures exceeding those required for thermal phase transition. The result is a greater permeability to mass transport in these regions of altered lipid microstructure.

We begin by considering the resistive Joule heating that occurs within the pore $(L_1 < z < L_1 + L_{SC}$ and $R_P < r \leq R_O)$. When a simple one-dimensional approximation is used, this Joule heating may be represented by

$$q_J = \sigma \left| \frac{\partial V}{\partial z} \right|^2 \tag{1}$$

This heat is responsible for the temperature rise of the membrane just around the pore that is made up of the small thin-walled cylindrical region of thickness ΔR as depicted in Fig. 2(*b*). We will consider the very special (purely theoretical) case in which all the resistive heating that takes place within the pore is instantly transferred to the heating of this thin-walled cylinder by making approximation that

$$\rho_{SC} c_{SC} \frac{dT}{dt} \cdot \mathrm{VOL}_{SC} = q_J \cdot \mathrm{VOL}_P \tag{2}$$

where the temperature is represented by T, the time by t, and the membrane density and specific heat are represented by ρ_{SC} and c_{SC}, respectively. The volume encompassed by the pore is $\mathrm{VOL}_P = \pi R_P^2 L_{SC}$ and the volume of the thin-walled cylindrical region of the SC is $\mathrm{VOL}_{SC} = \pi(2R_P \Delta R + \Delta R^2) L_{SC}$. While this simple representation clearly neglects the heat transfer in the axial direction, neglects any thermal gradients within the SC membrane, and neglects the thermal capacitance within the pore, it does serve to illustrate how the system parameters affect the minimum heating time required to raise the SC lipids by a temperature difference. This can be accomplished by integrating Eq. (2) over a time interval Δt. Doing this and rearranging the result, we can show that the maximum increase in temperature that can occur in the small cylindrical region during the time interval Δt is

$$\Delta T = q_J \frac{\Delta t}{\rho_{SC} c_{SC}} \left(\frac{1}{2\dfrac{\Delta R}{R_P} + \dfrac{\Delta R^2}{R_P{}^2}} \right) \tag{3}$$

where the ratio of the volumes $\mathrm{VOL}_P / \mathrm{VOL}_{SC}$ has been reduced to the term in parentheses. In order to evaluate the Joule heat of Eq. (3), we must find an expression for the electric field that influences the Joule heating of Eq. (1).

Consider that under the one-dimensional approximation, the total electric current, I, may be represented by an expression that capitalizes on the electrical resistances of each layer as

$$I = \int_0^{A_O} -\sigma \frac{dV}{dz} dA = \frac{\Delta V}{\sum R} = \frac{V_O}{L_1/\sigma A_O + L_{SC}/\sigma A_P + L_2/\sigma A_O} \tag{4}$$

Here, the cross-sectional area of the outer domain is $A_O = \pi R_O^2$ and the cross-sectional area of the pore is $A_P = \pi R_P^2$.

Using an equivalent length to represent the sum of the distances between the membrane surfaces and the electrodes ($L_{EQ} = L_1 + L_2$), the electric current may be expressed as

$$I = \sigma \frac{V_O}{L_{EQ}/A_O + L_{SC}/A_P} \tag{5}$$

Note that above and below the membrane, the current density is

$$J_O = \frac{I}{A_O} = \sigma \frac{V_O}{L_{EQ} + L_{SC}(R_O^2/R_P^2)} \tag{6}$$

where the term in parentheses is the ratio A_O/A_P.

Within the pore, the current density is

$$J_P = \frac{I}{A_P} = \sigma \frac{V_O}{L_{EQ}(R_P^2/R_O^2) + L_{SC}} \tag{7}$$

where the term in parentheses is the ratio A_P/A_O. It is interesting to note that within the pore traversing the SC, the current density decreases with the ratio R_P^2/R_O^2, while in regions above and below the SC, the current density increases with this ratio. It is the current density within the pore that is responsible for the resistive heating.

The gradient of the electric potential within the pore may be approximated as

$$\left.\frac{\partial V}{\partial z}\right|_{L_1<z<L_1+L_{SC}} = \frac{1}{\sigma} J_P = \frac{1}{\sigma} \frac{V_O}{L_{EQ} R_P^2/R_O^2 + L_{SC}} \tag{8}$$

Thus, the resistive Joule heating within the pore Eq. (1) can now be represented as

$$q_J = \sigma \left(\frac{V_O}{L_{EQ}}\right)^2 \frac{1}{(R_P^2/R_O^2 + L_{SC}/L_{EQ})^2} \tag{9}$$

Substituting the expression of the volumetric heating Eq. (9) into Eq. (3), the maximum possible temperature for the small region of SC surrounding the pore during a time period Δt is

$$\Delta T = \frac{\sigma V_O^2 \Delta t}{\rho_{SC} c_{SC}} \frac{1}{\left(2\frac{\Delta R}{R_P} + \frac{\Delta R^2}{R_P^2}\right)\left(\frac{R_P^2}{R_O^2} L_{EQ} + L_{SC}\right)^2} \tag{10}$$

In Sec. 3, concerns were raised regarding the importance of buffer solution electrical conductivity when Franz cells are used for skin electroporation studies. Equation (10) can be used directly to show that the temperature increase of the SC surrounding the defect is linearly proportional to the buffer electrical conductivity. Thus, when the buffer electrical conductivity is influenced by the electric field, the corresponding transient increase of the skin's mass permeability associated with the SC lipid structure's thermal alteration is influenced proportionally.

In Sec. 2, a discussion was presented that highlighted the difference between the terms HV and LV that are used in the context of an SW pulse compared to when they are used in the context of an ED pulse. During that discussion, it was stated that in certain circumstances, the physics that result from an ED pulse could have aspects that are traditionally associated with both HV and LV pulse types. For example, when the initial voltage of an LV pulse is in the HV range or when the tail of the exponential HV pulse lies in the LV range.

When an electroporation pulse (that is considered to be in the HV regime) is applied to the skin, conductive pathways (pores) are introduced into the SC. This electroporation of the SC is characterized by the actual size of these pathways (represented in Eq. (10) by R_P) and their density distribution (represented in Eq. (10) by R_O). Subsequent application of the pulse can result in the Joule heating and the temperature rise of Eq. (10). When this temperature is significant, lipid thermal phase transition within the SC region ΔR around the pore is initiated. This results in the evolution of the LTR in this region. Equation (10) has been developed in order to show the influence that the pathway density and size have on the subsequent temperature rises.

The density of pores per lateral surface area of skin is proportional to the square of the outer domain radius R_O. It is clear from Eq. (10) that the temperature rise increases with R_O: the derivative of the temperature rise with respect to the outer radius, $\partial/\partial R_O(\Delta T)$ is *always* positive. This implies that decreasing the number of pre-existing defects per cm^2 skin (increasing the magnitude of the outer radius) will *always* increase the temperature rise that contributes to SC lipid phase transition. The temperature rise, in fact, asymptotically approaches

$$\lim_{(R_P/R_O)\to 0} \Delta T = \frac{\sigma V_O^2 \Delta t}{\rho_{SC} c_{SC}} \left(\frac{R_P^2}{2R_P \Delta R + \Delta R^2}\right) \frac{1}{L_{SC}^2} \tag{11}$$

A depiction of Eq. (10) is plotted in Fig. 3 for the parameter values $\rho_{SC} = 1000\,\text{kg}\cdot\text{m}^{-1}$, $c_{SC} = 3600\,\text{J}\cdot\text{kg}^{-1}\,^\circ\text{C}^{-1}$, $\Delta t = 3 \times 10^2$ ms, $\sigma = 0.5\,\text{S}\cdot\text{m}^{-1}$, $V_O = 50\,\text{V}$, $L_{EQ} = 10\,\text{mm}$, $L_{SC} = 10\mu\text{m}$, and

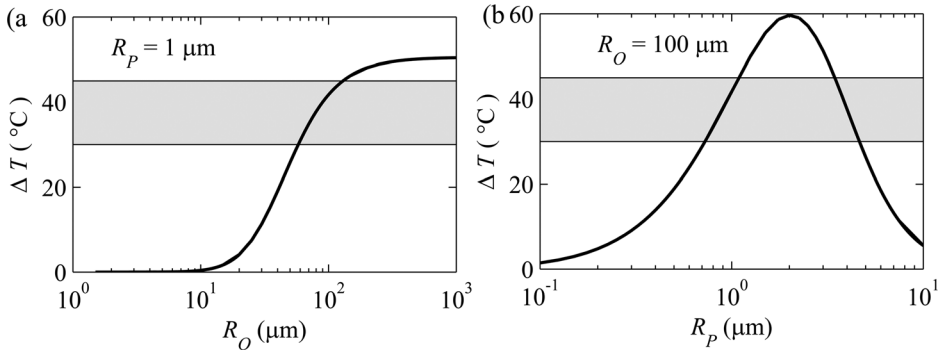

Fig. 3 (*a*) Temperature rise dependence on pore density (inversely proportional to R_O^2) and (*b*) nonlinearity in the dependence of temperature rise on pore size, R_P

$\Delta R = 0.75\,\mu$m. The thermophysical property values chosen here are representative of SC tissue and thickness, and the solution conductivity and the spacing between electrodes have been chosen to be representative of our own experiments [26]. The effect of the pore density is plotted in Fig. 3(*a*) for a pre-existing pore of outer radius $R_p = 1\mu$m. Increases in the outer radius (R_O) correspond to a lower density distribution of pre-existing pores. In Fig. 3(*a*), the shaded region corresponds to the temperature range in which the SC lipids experience thermal phase transition (LTR evolution) when the experiment is conducted at in vivo conditions (at 37 °C). The temperature is very sensitive to the density distribution of pores. Note that this example of the conditions required for LTR evolution considers temperature increases that, when sustained over extended periods, can lead to thermal degradation of living tissue.

The dependence of the temperature rise on the pore size is not as obvious. Consider that the derivative of required heating time with respect to pore radius $\partial(\Delta t)/\partial R_P$ of in Eq. (10) can change sign. The influence of pre-existing pore size on the temperature rise is plotted in Fig. 3(*b*) for $R_O = 100\mu$m.

The low temperature rises for very small pores are partly because the total heat generated within the pore is proportional to the pore volume (which scales with R_P^2) and partially due to the ratio of the volume of the SC cylinder to that of the pore. The lower temperature rises associated with very large pores are due to the inverse relationship between current density within the pore and pore size seen in Eq. (7).

While these results reaffirm the complexity of the dependence of LTR evolution on the structure of the SC, Eq. (10) also provides a method to quantify the influence of pore size and pore density. Establishing this sensitivity to these parameters is important to the discussion concerning the interpretation of the labels HV and LV in the context of pulse type.

5 Conclusions

The results of experimental studies characterize the pulsing regime in terms of the labels HV or LV regardless of pulse shape. It is possible that a single ED pulse type results in physical responses traditionally associated with HV type pulses as well as physical responses traditionally associated with LV pulse types. This should be contrasted to the SW pulse which results in a response that is attributed to either HV or LV but not both. In this case, our recommendation when interpreting the results of experimental studies is that the labels HV and LV always be considered in terms of the pulse shape.

When electroporation is conducted in Franz diffusion cells, geometric constraints make it necessary to apply the electric pulse into the donor and receiver solutions. In this case, the electric field behavior is very sensitive to the state of the buffer solution's electrical characteristics. Furthermore, the actual voltage across the skin can vary considerably from the voltage delivered to the electrodes. When interpreting and when presenting the results of these experiments, it is suggested to always consider the voltage across the skin sample in addition to the voltage delivered to the electrodes.

Future work should be done to reconsider the design of diffusion cell geometry in order to more closely match clinical applications. This could include a much larger donor side cell which would accommodate both anode and cathode.

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