

Advanced Drug Delivery Reviews 35 (1999) 61-76



A practical assessment of transdermal drug delivery by skin electroporation

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Abstract

Transdermal drug delivery has many potential advantages, but the skin's poorly-permeable stratum corneum blocks delivery of most drugs at therapeutic levels. Short high-voltage pulses have been used to electroporate the skin's lipid bilayer barriers and thereby deliver compounds at rates increased by as much as four orders of magnitude. Evidence that the observed flux enhancement is due to physical alteration of the skin by electroporation, as opposed to only providing an iontophoretic driving force, is supported by a number of different transport, electrical and microscopy studies. Practical applications of electroporation's unique effects on skin are motivated by large flux increases for many different compounds, rapidly responsive delivery profiles, and efficient use of skin area and electrical charge. Greater enhancement can be achieved by combining skin electroporation with iontophoresis, ultrasound, and macromolecules. Sensation due to electroporation can be avoided by using appropriate electrical protocols and electrode design. To develop skin electroporation as a successful transdermal drug delivery technology, the strong set of existing in vitro mechanistic studies must be supplemented with studies addressing in vivo/clinical issues and device design. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Electropermeabilization; Iontophoresis; High-voltage medical device

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PII: S0169-409X(98)00063-5

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1. Introduction

1.1. Transdermal drug delivery

Transdermal drug delivery offers many potential advantages over conventional methods, such as pills and injections [1-8]. Administration across skin avoids gastro-intestinal and first-pass liver degradation associated with oral delivery, provides a userfriendly approach, and can be formulated to deliver a drug in a continuous or controlled manner. However, most drugs diffuse across skin too slowly for medical applications. The skin's impressive barrier properties are due primarily to the outer 10-15 µm, called stratum corneum, which contains keratin-filled cells surrounded by an extracellular milieu of lipid organized in multilamellar bilayer structures [9,10]. It is these stacks of intercellular lipid bilayers which limit transdermal transport and must be overcome when delivering drugs across skin.

To increase rates of transdermal delivery a number of different enhancement methods have been studied, including chemicals [5], liposomes [11], ultrasound [12,13], microneedles [14] and electricity [15,16]. The mechanism by which these enhancers work involve one or both of the following two aspects: (1) changes in the skin's chemical and/or physical environment which allow more drug to penetrate into the stratum corneum and (2) application of a driving force which induces transport across the stratum corneum.

Chemical enhancers [5] can alter the chemical and physical environment within the skin so that drug more effectively partitions into the tissue. This involves increasing drug solubility in the stratum corneum and/or disrupting lipid structure. The mechanism of action for liposomes [11] remains somewhat unclear, but probably involves enhanced partitioning into the skin by changing the local

chemical environment. Ultrasound has been used both at high frequency [12], where enhancement is largely due to a pressure-driven driving force possibly coupled with physical disruption of lipids, and at low frequency [13], where ultrasound-induced cavitation causes extensive physical disruption of stratum corneum microstructure and also provides a pressure-driven driving force. Recently, arrays of micron-sized microneedles [14] which cross stratum corneum but do not stimulate nerves in deeper tissue, have been shown to create physical conduits for transport across skin. Finally, electricity has been used both at low voltage [15], where iontophoresis drives molecules across the skin and secondarily induces physical changes in skin microstructure, and at high voltage [16], where electroporation both rearranges the structure of stratum corneum's lipid bilayers and provides an electrophoretic driving force. This final method involving electroporation of skin using high-voltage pulses is the subject of this review.

For transdermal delivery of small, lipophilic drugs which are effective at low doses, no enhancement methods are needed. For other small compounds (e.g., more hydrophilic and/or those requiring larger doses), methods which provide an added driving force (e.g., iontophoresis, high-frequency ultrasound) or make changes in the skin's chemical environment (e.g., chemical enhancers, liposomes) have been shown to be helpful. However, for larger compounds (e.g., macromolecules) or those with especially challenging delivery requirements (e.g., large dose, pulsed or variable delivery), methods which physically disrupt the skin (e.g., electroporation, lowfrequency ultrasound, microneedles) are needed [7]. This review summarizes the evidence which shows how electroporation physically disrupts the skin and assesses the prospects for its application to transdermal drug delivery.

1.2. Electroporation

To better understand electroporation of skin, a brief, general overview of electroporation follows, based primarily on experiments performed on single lipid bilayer systems such as cell membranes. Electroporation involves the creation of transient aqueous pathways in lipid bilayer membranes by the application of a short electric pulse [17-20]. Permeability and electrical conductance of lipid bilayers can be rapidly and reversibly increased by many orders of magnitude. Electroporation occurs when the transmembrane voltage reaches a few hundred millivolts for electric field pulses typically of 10 µs to 100 ms duration. Electroporation is known to occur in metabolically-inactive systems, such as synthetic lipid membranes [21] and red blood cell ghosts [22,23], as well as in living cells [17,18] and tissues [24,25].

Electrical studies have shown that membrane resistance can drop orders of magnitude on a time scale of microseconds or faster due to electroporation [26–28]. Typically, upon applying a pulse, the membrane charges and initially remains stable. Then, the membrane becomes unstable and electroporation occurs, resulting in dramatically-reduced membrane resistance, which can be reversible or irreversible, depending largely on pulse parameters and membrane geometry and composition.

Molecular transport across membranes also increases during electroporation. Large numbers of compounds ranging in size from small ions to large macromolecules can be introduced into cells [19,23,29]. Gene transfection can be accomplished when DNA is electroporated across cell membranes and incorporated into a cell's genetic material, a technique routinely used in molecular biology [17,18].

The mechanism of transport caused by electroporation is expected to involve diffusion and/or electrically-driven transport. During a pulse, transport has been shown to occur by electrophoresis and/or electroosmosis, depending on the experimental system [22,30,31]. For small compounds (e.g. $M_{\rm r} < 1000$ Da), significant transport can also occur by diffusion after a pulse due to long-lived changes in membrane permeability. Postpulse diffusion of macromolecules is generally much slower.

Although studied mostly in planar bilayer and isolated cell systems, electroporation has also been demonstrated in cell monolayers [32,33] and in tissues, including retinal explants [34], islets of Langerhans [35,36], rice [37] and maize [38] tissues, skeletal muscle [39], frog skin [40], a number of different tumors [41–43], and mammalian skin's dermis [44] and stratum corneum [16,45].

1.3. Scope of paper

Skin electroporation has been the subject of considerable research since its first report in 1993 [45]. In other articles found in this special issue of Advanced Drug Delivery Reviews, mechanistic studies, biophysical characterization and in vivo experiments of skin electroporation are reviewed in detail, in addition to the other reviews which discuss drug delivery by electroporation of tissues other than skin. As a companion to those articles, this paper first defines the distinguishing capabilities of skin electroporation and then focuses on those results which give insight into potential applications to give a practical assessment of both established capabilities and future prospects.

2. Evidence for skin electroporation

2.1. Electroporation criteria

To evaluate opportunities to use skin electroporation for transdermal drug delivery, we must first establish that skin can be electroporated and identify those features which characteristically differentiate skin electroporation from other methods of enhancement. From the classic single bilayer electroporation literature [17–20], there are three characteristic features of electroporation which can guide interpretation of skin experiments: (1) dramatic increases in transmembrane transport; (2) reversibility over a range of conditions and (3) evidence for structural changes in the membrane barrier. The third characteristic is the most difficult to assess, but has been addressed using a number of different approaches

Although it is tempting to look for the physical "pores" predicted by electroporation, it is not the most useful approach. While the observed changes in

membrane properties associated with electroporation have been explained with models involving transient aqueous pathways [46,47], microscopic visualization of these pores has not been reported in skin and is a matter of controversy in single bilayer systems [48]. This is primarily because electropores are believed to be small (<10 nm), sparse (<0.1% of surface area), and generally short-lived (microseconds to seconds), making their capture by any form of microscopy extremely difficult. For this reason, electroporation is defined as a characteristic set of phenomena which can be explained by the creation of transient pores. In skin, we should look to see if these same characteristic phenomena occur and not search for hypothetical pores.

2.2. Large, reversible flux increases

As evidence that skin electroporation occurs, application of millisecond pulses greater than about 50 V across the skin has been shown to increase transdermal transport by orders of magnitude (Fig. 1). These flux increases are completely reversible over a range of conditions and at least largely reversible under most conditions examined. In Fig. 1, transdermal flux across human cadaver skin is shown to increase as a strong function of voltage, reaching rates more than four orders of magnitude greater than without pulsing. In this study [45], millisecond-long pulses were applied at a rate of 1 pulse every 5 s over the course of 1 h. Also shown in Fig. 1 is the flux 1 h after pulsing, where full reversibility of the flux is seen for voltages below ~100 V; significant reversibility is seen at larger voltages. These data, as well as similar findings in other studies (e.g., Refs. [49-51]), appear to satisfy the first two above-mentioned electroporation criteria of dramatic flux increases and reversibility.

The third criterion, i.e., evidence for structural changes, is more difficult to address, but has been supported by a number of experiments. Fig. 1 gives a first piece of supporting evidence in that there is a range of conditions (i.e., >100 V) which are not fully reversible. These long-lived fluxes suggest long-lived changes in skin structure.

Fig. 2 gives additional information about skin microstructural changes by showing data obtained by varying the pulse polarity [52,53]. Because the

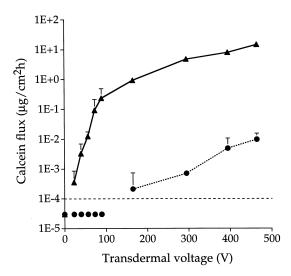


Fig. 1. Average transdermal flux of calcein caused by electroporation pulses of "forward" polarity (cathode in donor compartment) (\triangle) and 1 h after pulsing with "reverse" polarity (anode in donor compartment) (\bigcirc). These data show that transdermal flux increases up to 10 000-fold can be achieved which are partially or fully reversible. All experiments used human epidermis in vitro with 1 ms pulses at a rate of 1 pulse every 5 s for 1 h. Points below the dashed line indicate measurements below the detection limit on the order of $10^{-4} \, \mu \text{g/cm}^2 \text{h}$. Positive standard deviation bars are shown. Reproduced from Ref. [45], with permission.

model compound used in this study, calcein, carries a -4 charge, electric pulses applied with the cathode in the donor solution and the anode in the receiver solution are capable not only of causing skin electroporation, but should electrophoretically drive calcein across the skin as well. The large flux increases seen in Fig. 1 were achieved using forward polarity pulses. In Fig. 2, the same forward polarity data are plotted again, along with fluxes due to alternating polarity and reverse polarity pulses.

Even without a net electrophoretic driving force across skin, transdermal transport was enhanced during alternating and reverse polarity pulsing. Because electrophoresis cannot explain these observed flux increases, it is reasonable to expect that skin structural changes occurred. In alternating polarity pulsing, the polarity was alternated between forward and reverse with each pulse so that there was no net electrophoretic driving force [52]. Nevertheless, transdermal flux increased over three orders magnitude during alternating polarity pulsing. In reverse

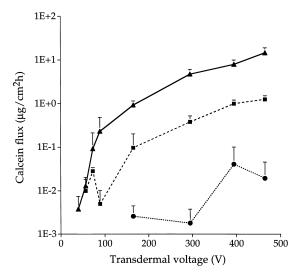


Fig. 2. Average transdermal flux of calcein caused by electroporation pulses of forward (\triangle), alternating (\blacksquare), and reverse (\bigcirc) polarity. These data show that while electrophoresis increases transport (forward polarity), flux increases are seen with no net electrophoresis (alternating polarity) or with electrophoresis in the "wrong" direction (reverse polarity). This indicates that changes in skin permeability occurred (see Section 2.2). All experiments used human epidermis in vitro with 1 ms pulses at a rate of 1 pulse every 5 s for 1 h. Positive standard deviation bars are shown. Reproduced from Ref. [53], with permission.

polarity pulsing, the polarity was always such that electrophoresis would oppose calcein transport across the skin. Again, significant flux increases were seen even though electrophoresis opposed transdermal transport of calcein. This increase can be explained by diffusion of calcein across permeabilized skin during the 5 s spaces between pulses.

2.3. Electrophoresis, electroosmosis, and diffusion

To further understand the roles of physical disruption of lipid bilayer structure and electrical driving forces for transport, we can assess the relative importance of electrophoresis, electroosmosis, and diffusion during skin electroporation. Considering the transport of highly-charged calcein, Fig. 2 indicates that flux enhancement was largely by electrophoresis (as opposed to diffusion), as shown by the large differences in transport for different pulse polarities. Transport could not have been by electroosmosis, because during forward polarity pulses,

electroosmosis would flow from the receiver chamber to the donor chamber and thus could only oppose transdermal calcein transport. Fig. 2 also suggests that diffusion was enhanced, albeit to a lesser extent, especially evident between pulses when electrophoresis does not occur.

Additional perspective on driving forces for transport comes from studies with fentanyl [54,55], a more lipophilic compound with a + 1 charge. In contrast to calcein, these studies showed that increases in fentanyl transport were only weakly dependent on pulse polarity, which indicates that neither electrophoresis nor electroosmosis contributed significantly to transport, since both phenomena depend on electric field orientation. Moreover, when rates of fentanyl transport measured during pulsing were compared to transport for 6 h after pulsing, much more fentanyl crossed the skin after pulsing, further supporting a diffusion transport mechanism. Related studies with sulforhodamine, another lipophilic compound with a -1 charge, further support the importance of diffusion for small, weaklycharged molecules enhanced by skin electroporation [50].

High-voltage pulses also increase transport of neutral molecules, such as mannitol, independent of pulse polarity (data not shown) [56]. This indicates that electroporation-enhanced mannitol transport occurred predominantly by diffusion through permeabilized skin, because electrophoresis cannot affect uncharged compounds and electroosmosis could not be significant since pulse polarity did not affect flux. This combined set of experiments indicate that while electroosmosis is generally not important to electroporation-mediated transdermal transport, electrophoresis and diffusion can both be important, where diffusion is dominant for uncharged or weakly charged molecules (e.g., mannitol, fentanyl) and electrophoresis gains importance for compounds with greater charge and increased hydrophilicity (e.g., calcein).

2.4. Transport number and electrical impedance analysis

Electrically-based analysis can give further insight into mechanisms of skin electroporation. Transport by electrophoresis is governed by the number of charges moved across the skin by the electric field. Ideally, every charge carrier would be a drug molecule. However, other charged compounds, such as sodium and chloride ions, are also present and compete with the drug to carry charge. The efficiency with which a given electrical exposure transports drug relative to other ions can be expressed as a transport number [57]. For electrophoresis through an unaltered membrane, the transport number should remain constant, independent of changes in electrical conditions. If there are changes in transport number, it indicates changes in membrane permselectivity, which in turn suggests changes in the membrane's physical structure.

Fig. 3 shows the dependence of transport number on transdermal voltage for a broad range of electrical exposures which vary in length from 20 µs to 1 ms, pulse rate from 100 pulses per second to 12 pulses per hour, and waveform including both square and exponential-decay [52]. For an unchanged barrier, as voltage increases, the flux should increase but the transport number, or efficiency, should remain constant. Fig. 3 shows that transport number increases strongly with increasing voltage, which suggests that high-voltage pulses induce changes in skin perm-

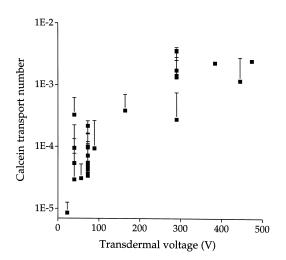


Fig. 3. Calcein transport number for transport across human epidermis electroporated in vitro under a broad range of electrical conditions. Transport number measures the efficiency of calcein transport relative to transport of other ions by electrophoresis. Calcein transport number increases with voltage, suggesting that larger pathways exist at higher voltages. Positive standard deviation bars are shown. Reproduced from Ref. [52], with permission.

selectivity in a voltage dependent manner. This cannot be explained just by an increased number of transport pathways, but requires the creation of pathways which more easily permit transport of calcein. Such pathways could be enlargements of existing pathways and/or creation of new, larger pathways for transport. This mechanism would be consistent with creation of "electropores" in skin.

Further evidence for changes in skin microstructure come from electrical impedance studies. Fig. 4 presents skin resistivity as a function of pulse voltage and shows that electroporation can decrease skin resistance by as much as three orders of magnitude (skin resistivity before pulsing is typically 100 k Ω cm 2 or more) [58]. These measurements were taken 20 μs after the onset of each high-voltage pulse, indicating that the large drops in skin resistance occurred on the microsecond time scale or faster.

The magnitude of electroporation-induced changes in skin electrical properties and the time scales over which they occur are summarized in Table 1 [50,52,58,59]. Both skin's resistance and capacitance are attributed largely to stratum corneum lipids. Thus, changes in skin electrical properties suggest changes in lipid structure. Table 1 indicates that

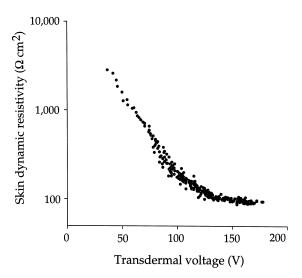


Fig. 4. Skin dynamic resistivity at $20~\mu s$ after the onset of a high-voltage pulse applied to human epidermis in vitro. Skin resistance very rapidly drops by as much as three orders of magnitude below prepulse values. Reproduced from Ref. [58], with permission.

Table 1 Summary of changes in skin electrical properties during and after electroporation^a

Time scale	Skin resistivity $(\Omega \text{ cm}^2)$	Skin capacitance (nF/cm ²)	
Before pulse	100 000	10	
μs	100	≤ 100	
ms	$\leq 10~000$	10	
s/min	$\leq 100~000$	10	

^a Data are from Refs. [50,52,58,59].

within microseconds skin resistivity drops by about 1000-fold and skin capacitance increases by up to a factor of 10. Then, within milliseconds after the pulse stops, skin resistance increases by at least an order of magnitude and capacitance returns almost to its prepulse value. After seconds to minutes, skin resistance increases further and reaches a final value sometimes equal to prepulse resistance, but often up to an order of magnitude less. Skin capacitance shows complete recovery in all reported experiments. These very fast and extensive changes in skin electrical properties have not been reported at low voltage (e.g., iontophoresis) or for ultrasound, chemicals or any other types of enhancement [7] and indicate the occurrence of significant changes in skin structure which are largely or completely reversible.

2.5. Microscopic visualization of transport pathways

As a final method of characterizing changes in skin caused by high-voltage pulses, fluorescence microscopy can be employed to visualize transdermal transport pathways used during different electrical exposures. To provide a basis for comparison, passive diffusion across stratum corneum is believed to occur largely by a tortuous intercellular path that travels within the lipids and does not involve keratinocyte cells [60,61]. In contrast, transport during iontophoresis often follows the "shunt" pathways of hair follicles and sweat ducts which bypass the bulk of the stratum corneum barrier [62–64]. Fluorescence microscopy displays this as brightly stained hair follicles and sweat ducts following iontophoresis of a charged dye [65].

Fig. 5 shows a fluorescence micrograph of skin exposed to high-voltage pulses in the presence of

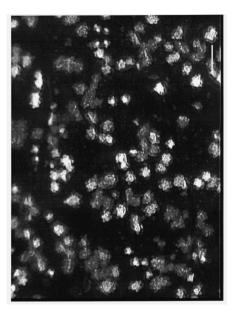


Fig. 5. Light micrograph of human stratum corneum showing fluorescence of calcein transported by high-voltage pulsing in vitro. Sites of fluorescence can be interpreted as sites of transdermal calcein transport. Skin was electroporated by applying 10 pulses of 157 V across the skin and 1 ms in duration. Scale bar equals 200 μm. Reproduced from Ref. [65], with permission.

calcein. Sites of bright staining are evident, which have been shown to correspond to sites of transdermal transport [65]. Unlike iontophoresis, the sites of transport during high-voltage exposures are not associated with hair follicles or sweat ducts and unlike passive diffusion, they represent pathways which travel straight through the bulk of stratum corneum with no evidence for tortuosity [65–67]. This pathway would be expected if intercellular lipid bilayers were electroporated and molecules could follow the path of least resistance directly across the tissue through electropores.

2.6. Summary of evidence for skin electroporation

To identify if skin electroporation presents opportunities for transdermal delivery it is essential first to determine if skin electroporation actually occurs and second to identify the mechanisms by which it enhances transport. Based on the three characteristic features of electroporation found in classic single bilayer literature, evidence for skin electroporation is

supported by large and reversible transdermal flux increases which appear to require changes in skin microstructure. The observed results cannot be explained by a mechanism based solely on an electrophoretic driving force. The long-lived permeability which persists well after the electric pulse and the observed enhancement with alternating and reverse polarity pulses involve phenomena other than electrophoresis.

Charged compounds are transported by a mixture of electrophoresis and enhanced diffusion, where electroosmosis does not appear to play a significant role. Transport of uncharged compounds can also be increased by enhanced diffusion. Supported by transport, electrical and microscopy data, the large flux increases caused by high-voltage pulses can be best explained by the creation of new transport pathways which are larger than preexisting pathways and are created on the microsecond timescale or faster. These paths appear to travel directly across the bulk of stratum corneum.

3. Applications to transdermal drug delivery

A number of characteristic features of skin electroporation are described above and distinguish

electroporation from iontophoresis and other enhancement methods. Although these distinctions are important for understanding mechanisms, they are also useful for developing medical applications. A practical assessment of using electroporation's unique characteristics for transdermal drug delivery is discussed below.

3.1. Large flux increases for many different compounds

Skin electroporation has been shown to enhance transdermal transport of a broad range of compounds ranging from small drugs to macromolecules to microspheres (Table 2) [68–74]. Flux enhancement of between one and four orders of magnitude has been reported for molecules ranging in molecular mass from 18 to 12 000 Da, in charge from neutral to heavily charged (both positive and negative), and in hydrophilicity from water-soluble to oil-soluble. Nano- and microspheres have been transported into the skin as well [66,75].

Macromolecules have been delivered transdermally and shown to retain biological activity. As shown in Fig. 6A, biologically-active heparin was transported across human skin in vitro at rates relevant to current clinical applications [76]. Biologically-active

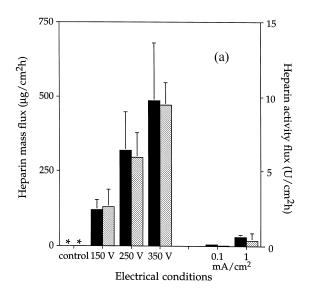
Table 2					
Compounds	delivered	across	skin	bv	electroporation ^a

Compound	Molecular mass	Charge	Solubility ^b	Log enhancement ratio ^c
Water	18	0	W	1
Mannitol	182	0	W	2
Metoprolol	267	+ 1	o/w	3
Alnitidan	302	+1/+2	o/w	2
Fentanyl	336	+ 1	0	2
Domperidone	426	+ 1	0	2
Lucifer yellow	457	-2	W	4
Sulforhodamine	607	-1	W	3
Calcein	623	-4	W	4
Erythrosin derivative	1 025	- 1	W	4
LHRH	1 182	+ 1	W	4
Cyclosporin-A	1 201	0	0	1
Oligonucleotide	4 800	- 15	W	1
Oligonucleotide	7 000	-24	W	1
Heparin	~ 12 000	~ - 76	W	2
Nano/microspheres	$10 \text{ nm}{-}45 \mu\text{m}$	Highly –	-	_

^a This table was kindly provided by Rita Vanbever.

^b Solubility in water (w) or oil (o).

^c Enhancement ratio is defined as the transdermal flux due to electroporation divided by the passive control flux under the same conditions.



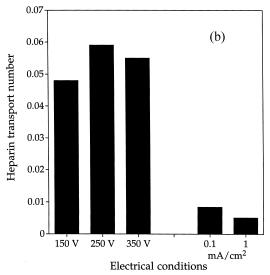


Fig. 6. Transdermal heparin transport across human epidermis in vitro during electroporation and iontophoresis. (A) Large amounts of biologically active heparin can be delivered across skin. (B) Higher heparin transport numbers during electroporation suggest the presence of larger transport pathways. Electroporation pulses were 2 ms long and applied at a rate of 1 pulse every 5 s. Iontophoresis was applied as a continuous constant current. Heparin mass flux was determined by assay of radiolabelled heparin, while heparin activity flux was determined using a blood clotting time assay. The symbol (*) indicates a flux below the detection limit. Positive standard deviation bars are shown. Reproduced from Ref. [76], with permission.

fentanyl has also been delivered to rats by skin electroporation and shown to produce a strong analgesic effect [77].

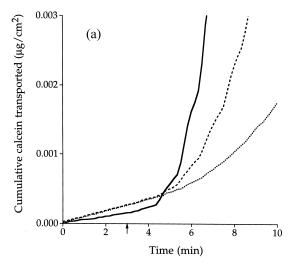
3.2. Rapidly responsive drug delivery

Skin electroporation is capable not only of delivering a lot of drug, but can do so with a very short lag time. Fig. 7A shows the amount of calcein transported across skin at the beginning of an electroporation protocol. After 3 min, the first pulse was applied and additional pulses were given once per minute. After just three pulses (i.e., at 5 min), the rate of calcein transport increased significantly [78]. In similar experiments, when the pulse rate was increased to 1 pulse every 5 s, enhanced transport was still observed within 10 to 20 s, i.e., within 2 to 4 pulses [78]. This extremely rapid onset has also been demonstrated during in vivo skin electroporation [77], but has not been reported for any other method of transdermal delivery.

Fig. 7B shows transdermal flux over the course of a 1 h electroporation protocol and further demonstrates how rapidly skin electroporation acts. In this example, steady state flux was achieved within 20 min; faster pulsing rates can reduce this time to less than 5 min [78]. Moreover, protocols which initially apply pulses rapidly to "prime the pump" and then continue pulsing more slowly have been shown to achieve steady state flux within just 1 min [78]. Fig. 7B also shows that the flux drops immediately after pulsing stops, which is important for precise control of delivery rates. Finally, steady oscillations in flux are observed in Fig. 7B at a rate of one oscillation per minute. These oscillations vary in frequency with the pulsing rate (data not shown) and are interpreted as rapidly responsive increases in flux at the time of each pulse followed by lower flux between pulses [78,79].

3.3. Efficient use of skin area and electrical charge

Conventional methods of transdermal drug delivery make inefficient use of skin area for transport. This results not only in small fluxes, but may cause irritation due to locally large drug concentrations at sites of transport [80]. For example, passive transder-



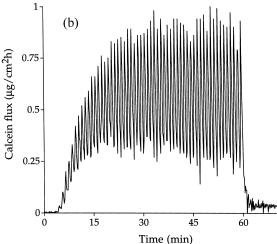


Fig. 7. Real-time measurement of calcein transport across human epidermis in vitro using a flow-through apparatus. Pulsing was initiated at 3 min and continued for 1 h at a rate of 1 pulse per minute using 1 ms pulses. (A) Independent of transdermal voltage, an increased rate of transport was observed after three pulses (e.g., at 5 min): 270 V (solid line), 135 V (dashed line), 115 V (dotted line). (B) Steady state flux was achieved within approximately 20 min, was maintained throughout pulsing, and dropped rapidly after pulsing was stopped. The peaks and valleys in flux during pulsing occur at a rate of one per minute, suggesting that the effects of each individual pulse are being measured. Transdermal voltage was 270 V. Reproduced from Ref. [78], with permission.

mal diffusion usually occurs exclusively in the intercellular spaces and does not involve transport through keratinocytes. As a result, only a tiny fraction of the stratum corneum is used for transport.

Similarly, electrically-enhanced transport by iontophoresis drives drugs largely across the skin through hair follicles and sweat ducts, which occupy only about 0.01% of skin area [9].

Transport by skin electroporation uses skin area more efficiently. By creating new pathways across the bulk of stratum corneum and through keratinocytes, a greater fraction of the skin is used for transport, estimated to be up to 0.1% of skin area [52,81,82]. Although this is still only a small fraction of the total skin available, it is nevertheless much more than that used by other methods.

In addition to transporting more molecules across a greater fraction of the skin, electroporation drives charged drugs across the skin more efficiently than conventional iontophoresis. Based on analysis using transport numbers, which measure the efficiency of drug transport relative to transport of competing ions (e.g., sodium and chloride), efficiency of calcein transport increased by more than two orders of magnitude as voltage was increased over the range studied (Fig. 3). In a direct comparison between skin electroporation and iontophoresis [76], electroporation was shown to increase heparin transport an order of magnitude more efficiently than iontophoresis (Fig. 6B). These protocols each transported about the same number of total ions across the skin; for electroporation, ten times more of those ions were heparin molecules.

3.4. Electroporation combined with other enhancers

Skin electroporation has been combined with other enhancement techniques to provide synergistic effects. In combination with iontophoresis, skin was electroporated with a single initial pulse and then exposed to iontophoresis to drive luteinizing hormone releasing hormone molecules across permeabilized skin (Fig. 8) [83,84]. Iontophoresis preceded by electroporation resulted in fluxes about five times greater than those of iontophoresis alone.

Skin electroporation has also been combined with high-frequency therapeutic ultrasound [85]. Under the conditions used, ultrasound alone did not enhance transdermal transport, but when it was applied simultaneously with electroporation, greater fluxes were observed than for electroporation alone and the

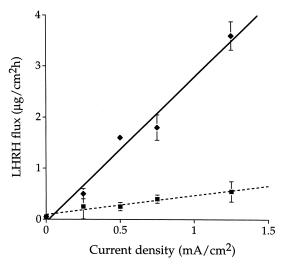


Fig. 8. Transdermal delivery of luteinizing hormone releasing hormone (LHRH) across human epidermis in vitro using iontophoresis for 30 min alone (■) or preceded by a single 5 ms electroporation pulse of 1000 V applied across the transport chamber (the transdermal voltage was much less) (♦). At the same iontophoretic current density, much more LHRH was transported when an electroporation pulse was used to permeabilize the skin first. Standard deviation bars are shown. Reproduced from Ref. [83], with permission.

apparent threshold for electroporation effects was reduced (Fig. 9). This synergistic interaction might be caused by ultrasound partially disorganizing stratum corneum lipids, thereby making them more susceptible to electroporation.

As chemical enhancers which only enhance electroporation-mediated transport, macromolecules have been used to increase skin electroporation fluxes [86,87]. Although macromolecules do not enhance passive diffusion or transport by iontophoresis, they can significantly enhance transport during electroporation (Fig. 10). Macromolecule enhancement is proposed to act by sterically stabilizing "electropores" created in the skin.

3.5. Avoiding sensation and tissue damage

Application of hundred-volt pulses to the skin can induce painful sensation and cause injury. However, when applied correctly, evidence suggests that such pulses can electroporate the skin without dangerous or unpleasant side effects. Sensation is caused pri-

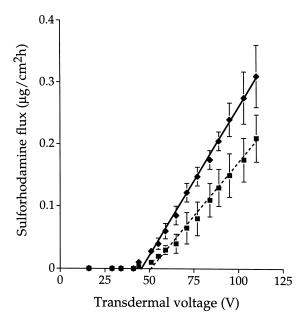


Fig. 9. Sulforhodamine flux across human epidermis in vitro using 1 ms electroporation pulses applied alone (■) or in combination with continuous ultrasound at 1 MHz and 1.4 W/cm² (♦). The concurrent application of ultrasound lowers the apparent threshold for skin electroporation and increases the transdermal flux. Standard deviation bars are shown. Reproduced from Ref. [85], with permission.

marily by direct excitation of nerves by the applied electric field [88]. Indeed, protocols similar to those of interest for skin electroporation are used clinically to stimulate nerves for diagnostic or therapeutic purposes (Table 3) [89]. For drug delivery applications, sensation can be reduced and possibly eliminated by applying pulses shorter than 1 ms [89], designing closely-spaced electrodes which localize the electric field within the stratum corneum [90,91] and using other approaches, as described in the article by Vanbever and Préat appearing in this issue of Advanced Drug Delivery Reviews.

Concerns about tissue damage caused by skin electroporation are partially addressed by experiments in the literature, but require further study. As shown in Fig. 1, the effects of electroporation on skin barrier properties are largely or completely reversible, indicating that stratum corneum does not suffer lasting damage. In addition, electrical studies show that the skin resistance drop which remains a few minutes after skin electroporation (even at the

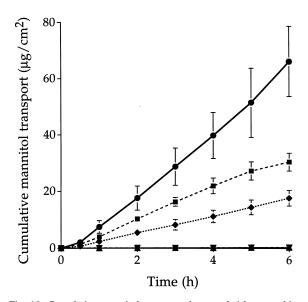


Fig. 10. Cumulative mannitol transported across hairless rat skin in vitro using five electroporation pulses of 150 V (applied across the transport chamber) and 180 ms duration followed by passive diffusion for 6 h: (\blacktriangledown) passive diffusion, no macromolecule present; (\blacktriangle) passive diffusion, 10 kDa dextran sulfate in the donor solution; (\blacklozenge) electroporation, no macromolecule present; (\blacksquare) electroporation, 12 kDa heparin in the donor solution; (\spadesuit) electroporation, 10 kDa dextran sulfate in the donor solution. The addition of macromolecules during pulsing increased flux. Bars indicating the standard error of the mean are shown. Reproduced from Ref. [86], with permission.

highest voltages) is always less than the drop in skin resistance caused by inserting and removing a 28-gauge needle (data not shown) [59]. This indicates that the "damage" caused by electroporation is less than that of a small conventional needle. Moreover, a needle leaves a large hole which is much bigger than bacteria, while electroporation makes many submicroscopic holes [17,18] which should not pose risk of infection.

Animal studies similarly suggest than electroporation does not damage the skin [77,84,90,92,93]. As described in the article by Vanbever and Préat in this journal, measurement of transepidermal water loss and erythema in hairless rats shows only small, transient increases which are similar in magnitude and duration to those caused by conventional iontophoresis [93]. Because these effects are short-lived and no more severe than those associated with iontophoretic conditions widely believed to be safe [80], this suggests that skin electroporation may be safe as well.

Clinical studies of electroporation applied to the skin for enhanced local chemotherapy of cutaneous tumors are described in the article by Heller et al. in this journal and similarly indicate that electroporation is safe [24,94–96]. For example, a phase I/II study involving about 100 tumors on 25 patients found no long-term side effects of electroporation

Table 3 Representative current (I), voltage (V), pulse length (t), and pulse rate (r) of existing clinical procedures using medium or high voltage protocols^a

	I	V	t		
	(mA)	(volts)	(msec)	(pps)	
Iontophoresis	≤ 25	_	d.c.	cont	
Transcutaneous electrical nerve stimulation	≤ 200	_	≤ 6	≤ 200	
Functional electrical stimulation	≥ 30	_	100-300	≥ 20	
Electromyography	_	~ 300	0.1 - 3	~ 50	
Somatosensory evoked potential testing	≤ 125	~ 400	0.05-1	≤ 100	
Treatment of ununited fracture	5-10	~ 5 (a.c.)	(60 kHz)	cont	
Electrotactile speech processing	1–12	≤ 100	0.01 - 1	≤ 500	
Electroanesthesia	50-250	15-30	_	cont	
Electroconvulsive therapy	550-800	50-300	1-2	40-90	
Cardiac pacing	≤ 200	_	5-55	30-180	
Ventricular defibrillation	$\leq 50~000$	_	4–30	_	

^a Abbreviations: pps = pulses per second, a.c. = alternating current, d.c. = direct current, cont = continuous. All numbers are approximate values selected from Ref. [89] to indicate the order of magnitude of electrical properties used.

typically applied with eight pulses on the order of 1000 V and 100 µs each [43,97]. The only short-term side effects were a strong muscle contraction with each pulse observed in all subjects and mild, local muscle fatigue reported by about a quarter of subjects, presumably caused by the muscle contraction during the pulse. In these electrochemotherapy studies, the electric field had to penetrate well past the stratum corneum to reach the tumor, making it impossible to avoid nerves. However, for transdermal drug delivery, the use of closely-spaced electrodes which localize the electric field in the stratum corneum could eliminate muscle contractions.

Finally, some of the other high-voltage clinical procedures used for routine diagnostic and therapeutic purposes (Table 3) almost certainly electroporate skin as a side effect. Many of these procedures are in widespread use and are considered to be safe.

4. Conclusion

Human skin presents a formidable barrier to transdermal drug delivery, due largely to the intercellular lipid bilayers of stratum corneum. Transport, electrical, and microscopy experiments suggest that these bilayers can be electroporated by short, high-voltage pulses. As a result, transdermal transport is dramatically increased for a broad range of different compounds including macromolecules; can reach steady state and be controlled for rapidly responsive delivery patterns with time resolution as short as minutes; uses skin area and electrical charge in an efficient manner; and can be synergistically combined with other enhancers, including iontophoresis, ultrasound, and macromolecules.

Although in vivo efficacy and evidence for safety have been demonstrated in a limited number of experiments, most skin electroporation studies have examined mechanistic issues and have been performed in vitro. Other than a very small pilot study [98], no clinical work has been done. Some efforts have addressed engineering issues associated with developing a small, wearable device which delivers pulses in a painless but effective manner, but not enough has been done and even less has been published. Building off the relatively strong scientific base provided by single bilayer and skin electropora-

tion studies, there is significant opportunity to study in vivo/clinical and engineering issues of skin electroporation and to develop its unique capabilities for transdermal delivery applications with strong potential for clinical and commercial success.

Acknowledgements

I thank Uwe Pliquett, Rita Vanbever, James Weaver and Veronique Préat for helpful discussions. This work was supported in part by NSF Career Young Investigator Award BES-9624832 and the Emory/Georgia Tech Biomedical Technology Center

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