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Skin electroporation for transdermal and topical delivery

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Abstract

Electroporation is the transitory structural perturbation of lipid bilayer membranes due to the application of high voltage pulses. Its application to the skin has been shown to increase transdermal drug delivery by several orders of magnitude. Moreover, electroporation, used alone or in combination with other enhancement methods, expands the range of drugs (small to macromolecules, lipophilic or hydrophilic, charged or neutral molecules) which can be delivered transdermally. Molecular transport through transiently permeabilized skin by electroporation results mainly from enhanced diffusion and electrophoresis. The efficacy of transport depends on the electrical parameters and the physicochemical properties of drugs. The in vivo application of high voltage pulses is well tolerated but muscle contractions are usually induced. The electrode and patch design is an important issue to reduce the discomfort of the electrical treatment in humans.

Keywords: Electroporation; Skin permeability; Transdermal delivery; Topical delivery; Mechanism; Safety

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1. Introduction

Transdermal drug delivery offers several advantages over conventional routes [1,2]. It avoids the first-pass metabolism and the gastrointestinal tract. Transdermal delivery has the potential for sustained and controlled drug release. Moreover, it is a non-invasive mode of drug delivery with no trauma or risk of infection. Patient compliance may be improved by this user-friendly method.

In spite of the advantages of the transdermal delivery, only a small percentage of drugs can be delivered transdermally due to the barrier properties of the skin: only small potent lipophilic drugs can be delivered at therapeutic rates by passive diffusion [3]. Moreover, transport of most drugs across the skin is very slow and lag-times to reach steady state fluxes are in hours. Achievement of a therapeutically effective drug level, is therefore, difficult without enhancing skin permeation. A number of approaches have been developed to enhance and control transport across the skin, and expand the range of drugs delivered. These involve chemical and physical methods, based on two strategies: increasing skin permeability and/or providing a driving force acting on the drug [4,5].

Electroporation or electropermeabilization is the transitory structural perturbation of lipid bilayer membranes due to the application of high voltage pulses. This phenomenon occurs in different kinds of lipid bilayer membranes: artificial (liposomes), cellular (bacteria, yeast, plant, mammalian cell) or in a more complex structure (stratum corneum). Hence, electroporation has been used for different applications. Electrical exposures typically involve electric field pulses that generate transmembrane potentials of 0.5–1.0 V and last for 10 μs to 10 ms. Reversible electrical breakdown and high molecular transport are observed, resulting from structural rearrangements of the cell membrane [6]. It has been hypothesized that these rearrangements consist of temporary aqueous pathways, with the electric field inducing pore formation and providing a local driving force for molecular transport. The first use of electroporation was to introduce some DNA materials into cells in vitro. Due to its application as a method of DNA transfection, electroporation has been applied in tissues (e.g. for gene therapy) and shown to reversibly permeabilize them. One interesting application of tissue electroporation is electrochemotherapy, which consists of applying high voltage pulses to permeabilize tumor cells to an impermeable cytotoxic drug [7]. Electrochemotherapy has been shown to be more efficient than the chemotherapy alone in eliminating local tumors, e.g. in the skin [8].

About 10 years ago, the use of electroporation for transdermal delivery was suggested (Fig. 1) [9]. Although the technique is normally used on the unilamellar phospholipid bilayers of cell membranes, it has been demonstrated that electroporation of skin is feasible, even though the stratum corneum contains multilamellar, intracellular lipid bilayers with few phospholipids [9–12]. Hence, electroporation has taken its place among the physical techniques of transdermal drug delivery, like ultrasound and iontophoresis.

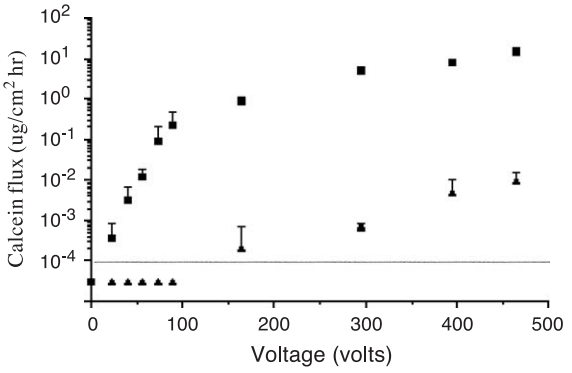


Fig. 1. Average transdermal fluxes of calcein due to exposure of human skin to different electrical conditions: application of forward-polarity pulses (■) and 1 h after pulsing in the reverse direction (▲). Donor solution: calcein 1 mM in PBS buffer. From Prausnitz et al. [9].

The concept of skin electroporation and the supporting preliminary data have motivated a number of subsequent studies, mainly in vitro but also a few in vivo in animals and in humans. The combination of electroporation with other enhancement methods open new perspectives [5,13,14].

In this paper, studies on transdermal and topical drug delivery using electroporation are reviewed with emphasis on potential clinical applications. Efficacy of transport by skin electroporation alone or in combination with other methods and safety issues are discussed.

2. Mechanism of transdermal drug transport by electroporation

2.1. Electroporation of the skin

Because the stratum corneum is the main barrier to transdermal transport, the disruption of the stratum corneum can dramatically influence overall skin permeability and it has been suggested that electroporation of its intercellular lipid bilayers might enhance percutaneous drug delivery. The biological composition and structure of the stratum corneum, the outermost layer of the skin, make it particularly attractive for electroporation. The stratum corneum contains approximately 100 bilayer membranes in series, and electrical breakdown associated with dramatic increase in transport has been observed for transdermal voltages of 30–100 V (100–1500 V applied voltages), which well corresponds to the range of voltages used for electroporation in cells, i.e. 0.3–1.0 V per bilayer [9,15].

New aqueous pathways would be created within the stratum corneum due electroporation of its lipid bilayers [6,12,16]. Molecular transport through transiently permeabilized skin then occurs due to different mechanisms, mainly by electrophoresis and enhanced diffusion [10,16,17]. Thermal effects may be involved [11,18].

2.2. Pathways of transport

2.2.1. New aqueous pathways or electropores

Electroporation of lipid bilayers induces a dramatic and reversible increase in transmembrane transport and structural changes in membrane barrier [9]. Models to explain these dramatic changes in membrane properties associated with high voltage application involve the creation of "pores" or aqueous pathways. Evidence for the creation of these pores is still entirely indirect. The pores are believed to be small (<10 nm), sparse (0.1% of surface area), and generally short-lived (μs to s) [12,19,20].

Consistent with the electroporation features of single lipid bilayers, experimental and theoretical data support the hypothesis that application of high voltage pulses to skin also induces the creation of new and/or the enlargement of existing aqueous pathways in the stratum corneum [12]. The skin resistance drops by several orders of magnitude during pulsing and is partly reversible. In vitro transport increases by up to four orders of magnitude compared to passive diffusion. The effective fractional area for small ion transport during electroporation is approximately 0.1% [21–23]. For the same amount of transferred charges, the drug transport is higher for electroporation than iontophoresis, suggesting that electrophoresis alone cannot explain drug transport by skin electroporation and that skin structure must be altered.

2.2.2. Localization of transport

Whatever the electroporation protocol used, the transdermal transport by high voltage pulses has been shown to occur through highly localized transport

regions (LTR) of the stratum corneum, covering between 0.02% and 25% of the skin surface [21,24].

The permeabilization of the stratum corneum is not homogeneous within a LTR. The current density and hence drug transport is maximal at the center of the LTR. Moreover, it has been reported that LTR are surrounded by localized dissipation regions (LDR), presenting a low resistivity where transport of only small ions can occur (Fig. 2) [25]. The size and the number of the LTR are dependent on the pulsing protocol. The size of LTR increased with the duration and the number of pulses, and it ranged between 0.1 and 0.2–2.5 mm in diameter for short and long pulses, respectively. In contrast, the number of LTR increased with the pulse voltage: while LTR number ranged between 2 and 10 per 0.1 cm^2 for long duration medium voltage pulses, it increased to 20–100 per cm^2 when short duration high voltage pulses were applied [24–26].

Within a LTR, molecular transport appears to occur through intercellular and/or transcellular pathways [21,24,25]. The contribution of each transport is dependent on the pulse voltage. While molecular transport seems to be transcellular in the case of short high voltage pulses, it seems to be intercellular with possible implication of appendages when decreasing the voltage and lengthening the pulse duration [25,26]. Theoretical models suggest that appendageal macropores are possible pathways for electrical current at moderate voltage [27,28]. The permeabilizing effect of electroporation on epidermal cells could be potentially interesting for a targeted topical treatment [29,31].

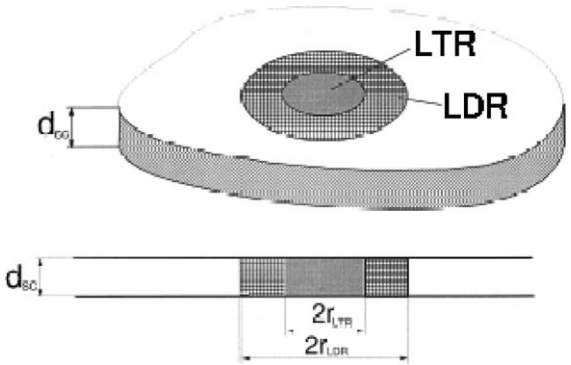


Fig. 2. Idealized drawing of the outer layers of the skin, showing the hypothetical structure of a LTR contained within a LDR. The LTR is represented by a cylindrical region, with a thickness of the SC (d_{sc}=10–20 μm) and a radius (r_{LTR}=10–100 μm), which is strongly dependent on high voltage pulse duration. The larger concentric cylinder represents an idealized LDR, whose sizes also depend on pulse length. From Pliquett et al. [25].

The molecular weight is another parameter influencing the route of transport: the smaller the molecular weight, the more intracellular the penetration. Lombry et al. [30] showed that FITC molecules (low molecular weight) penetrated in the keratinocytes, while FITC–dextran molecules of 38 kDa was mainly around the keratinocytes with only a small fraction entering the cell.

Electroporation of skin is associated with a temperature rise within the highly LDR. When an electric field is applied to the skin, it dissipates energy and heats up (Joule heating). This local temperature rise may affect the barrier function of the skin if the temperature rise induces a phase change in the sphingo-lipids of the stratum corneum, increasing the permeability of skin. Indeed, dramatic decrease of the skin resistance occurs when 65–70 °C are reached [18,32]. As revealed by temperature sensitive crystals, during the pulse, the temperature did not rise instantaneously over the entire skin surface but started at small spots, resulting in a propagating heat front [11,18]. Hence, the interior of the LTR was heated above the phase transition temperature, while the LDR was also heated but did not reach the phase transition temperature. As the transdermal voltage and pulse time constant increased, the temperature tended to a plateau but did not reach the phase transition of the water [33]. During cooling, the multilamellar system could not re-establish, leaving the existence of water-rich domains, which provided aqueous pathways even long after the pulse [18,33].

The biological significance of heating of the stratum corneum above the phase transition temperature of sphingo-lipids is not yet understood. However, in vivo experiments using hairless rats showed no significant skin irritation for short and long pulses [34].

2.3. Mechanisms of molecular transport

Molecular transport through transiently permeabilized skin by electroporation results from different mechanisms at different times. Enhanced diffusion, during and after pulses, and electrically driven transport during pulses, i.e. electrophoretic movement and

very slightly electroosmosis, are the main mechanisms of transport. The contribution of electrophoresis and diffusion are dependent on the physicochemical properties of the molecule.

2.3.1. Electrophoretic movement

During high voltage pulses, the main driving force for transport of charged molecules is electrophoresis [9,10,35,36]. Evidence for this major contribution of electrophoresis is the drop in the drug transport with reverse electrode polarity opposing electrophoresis.

2.3.2. Diffusion

Molecular transport through skin highly permeabilized by electroporation is also due to enhanced passive diffusion. Although much higher skin permeability is achieved during the pulse, prolonged permeabilization and thereby transport occur after pulsing, lasting for hours in in vitro studies. Evidences for the contribution of enhanced post-pulse diffusion arise from the increased transport seen (i) with reverse electrode polarity, (ii) with neutral molecules, and (iii) when the drug is added after application of the pulses [35–37].

2.3.3. Electroosmosis

In contrast with iontophoresis, the contribution of electroosmosis during high voltage pulses is low. The short time of current application (few s) limits the role of electroosmosis in drug transport by skin electroporation. Further evidence comes from the similar anodic and cathodic transport of neutral molecules [37].

3. Parameters controlling drug delivery by electroporation

Electrical parameters of the pulses, physicochemical properties of the drug and formulation of the drug reservoir can affect and allow control of transdermal drug delivery by electroporation. These parameters are summarized in Table 1.

Table 1
Parameters affecting drug transport by skin electroporation (adapted from Préat and Vanbever [38])

Parameters	Increase in	Effect
Electrical parameters	Pulse voltage	+
	Pulse number	+
	Pulse length	+
Physicochemical properties of drug	Charge	+
	Molecular weight	−
	Conformation	?
	Lipophilicity	−
Formulation of drug reservoir	Competitive ions	−
	Ionization (pH)	+
	Viscosity	−

+, Positive effect; −, negative effect.

3.1. Electrical parameters

Electrical pulses are characterized by their electrical parameters: waveform (exponential decay or square wave), voltage (50–1500 V), duration (few μs to ms), and interval between pulses (few s to min). These electrical parameters can be optimized, depending on experiment requirements and clinical application of electroporation [35].

3.1.1. Pulse waveform

Two kinds of pulse generators are usually employed to transport molecules by electroporation: there are generators which can deliver exponentially decaying pulses [9,10,39] or square wave pulses [7,8,40]. Both are used for different applications, i.e. drug delivery, electrochemotherapy and gene therapy. Due to its long voltage tail profile, the main potential advantages of exponentially decaying pulses are to maintain or expand the high permeability state of the skin induced by electroporation and to promote the electrophoretic movement. However, because the duration of exponential decay pulses depends on the resistance of the skin and the electroporation system (electrodes, conducting medium), the reproducibility of the pulse conditions for clinical use could be a problem. In contrast, voltage and duration of square wave pulses remain constant whatever the skin or drug reservoir. Hence, square wave pulses should be used to have a better control and reproducibility of drug transport. Until recently, the square wave pulses were exclusively used for electrochemotherapy and DNA electrotransfer, whereas exponentially decaying pulses were restricted to transdermal drug delivery to take advantage of the long voltage tail.

3.1.2. Pulse voltage, duration, number and rate

Control on drug transport by skin electroporation can be achieved by controlling the pulse voltage, duration, number and rate. The effect of varying these electrical parameters on transdermal transport has been extensively studied in vitro [9,10,29,35,41–43].

Because significant voltage drop occurs within the electroporation system, the transdermal voltage is only a fraction (ca. 10–50%) of the voltage applied across the electrodes, depending of the relative resistance of the skin and the drug reservoir. Flux rate always enhances when electrical pulse conditions strengthen: when the number, the voltage or the duration of the pulses increase, the transdermal drug transport increased (see Figs. 1 and 3). With increasing voltage of pulses, the transdermal flux increases but less steeply at high voltages [9,35]. When the pulse duration and pulse numbers increases the drug transport often linearly increases [10]. Increasing the pulse rate increases transdermal flux as well [10,26,41].

The electrical parameters influence transdermal flux but also onset time for transport, which decreases with increasing pulse duration and rate, but is independent of voltage [26,41].

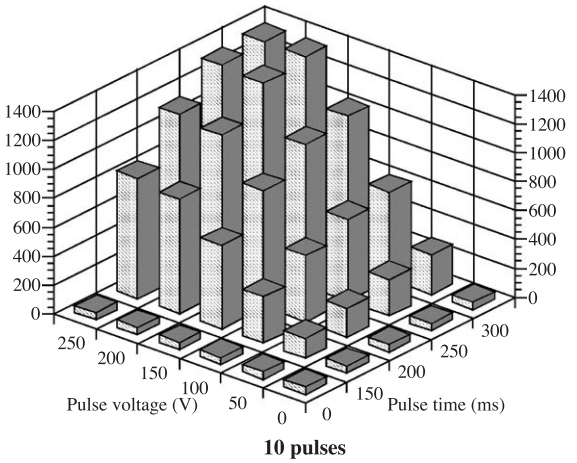


Fig. 3. Effect of pulse voltage and time on fentanyl transdermal transport by electroporation. The plot presents values calculated from a response surface equation obtained by factorial design. Donor solution: fentanyl 40 μg/ml in a citrate buffer (0.01 M, pH 5). From Vanbever et al. [35].

3.1.3. Pulsing protocol

Two different types of pulsing protocols are usually reported in the literature. They can mainly be distinguished by the pulse duration: (i) numerous (>100), short duration (1–2 ms), high voltage pulses; and (ii) a low number (<20), long duration (70–1000 ms), medium voltage pulses. In the case of exponentially decaying pulses, at the same total electrical charge transported through the skin, a few long pulses allowed generally higher molecular transport than many short pulses [26].

3.1.4. Electrode design

The design of the electrodes is still a critical issue, both in terms of efficacy of drug transport and tolerance. The early research on skin electroporation was performed in vitro with electrodes placed on both sides of the skin. If insights on transdermal drug delivery were gained, the position and design of the electrodes were not representative of in vivo conditions. Various electrodes and reservoir systems, e.g. plate electrodes with a skin fold [31,44], meander electrodes [44], have been designed for in vivo applications. The efficacy of drug transport is influenced by the electrode design because the distribution and intensity of the electrical field in the skin are affected [45,46]. The simplest configuration to generate a more or less uniform electric field is parallel plate electrodes in the form of calipers. However, underlying nerves and muscles could be subjected to electrical stimulus and superficial skin burning could be observed. The meander electrodes consist of an array of interweaving electrode fingers, allowing the electric field to be mostly localized within the superficial layers of the skin, thereby avoiding undesirable effects in underlying tissues.

As the reaction is extremely fast, inert electrodes, e.g. platinum, are preferred to active electrodes, e.g. silver/silver chloride electrodes. As oxydoreduction occurs at the electrodes, hydrogen and hydroxyl ions are produced and could lead to a pH shift in the reservoir.

3.2. Physicochemical properties of the drug

In addition to the electrical parameters of the pulses, the physico-chemical properties of drug can affect the transdermal drug delivery by electroporation.

3.2.1. Charge

Because the electrophoretic movement is the main mechanism of transport for charged molecules through a highly permeabilized skin by electroporation, the pK_a of the drug and the pH of the delivery solution are essential parameters influencing the electric charges of the molecule to deliver. Increasing the charge of the permeant enhances its transport. Hence, the pH of the drug reservoir, which affects drug ionization, will influence the efficacy of drug delivery. The transport of neutral molecules is also enhanced by electroporation, due to passive diffusion through the permeabilized skin [37]. This transport of neutral molecules is lower, compared to the transport of charged molecules during pulses.

At physiological conditions, the skin is negatively charged, presenting a better permselectivity to the cations. However, due to the short duration of current application, the contribution of electroosmosis is limited [37], suggesting that the skin permselectivity is not as important as for iontophoretic transport.

3.2.2. Lipophilicity

The influence of the partition coefficient of the permeant has not been systematically investigated. In contrast to passive diffusion, increasing the lipophilicity of the drug tends to decrease the enhancement ratio. The transdermal fluxes of nalbuphine and lipophilic prodrugs were enhanced by electroporation, as compared to passive diffusion. The total amount of nalbuphine and its prodrugs were similar but the enhancement ratio decreased as the lipophilicity increased [43].

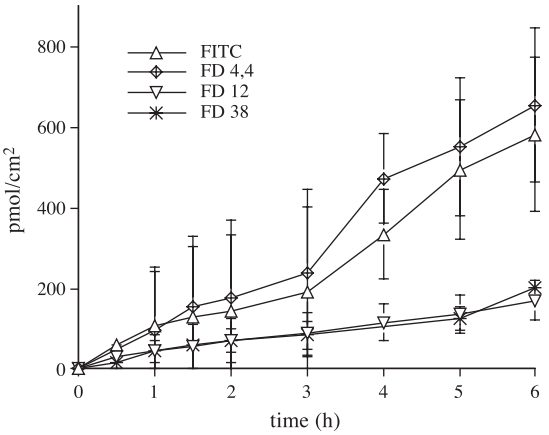
3.2.3. Molecular weight

Another physicochemical property of the drug influencing the transdermal transport by skin electroporation is its molecular weight. Using FITC–dextran of increasing molecular weight, Lombry et al. [30] showed that significant transport and intracellular penetration in the skin were detected after high voltage pulse application (Fig. 4). The greater the molecular size, the lower the transdermal transport. The absence of molecular weight cut off (at least up to 40 kDa) suggested that electroporation could be useful for macromolecule delivery.

3.2.4. Formulation of drug reservoir

Because the drug concentration affects the transdermal transport of drug by electroporation, the choice of drug concentration in the reservoir could allow control on drug delivery. The higher the drug concentration, the higher the transport. However, a non-linear relationship between the quantity of drug delivered in the skin and the drug concentration of the reservoir has been reported [29,47].

The ions present in the drug reservoir (buffer ions, counter ions, ions from the skin) compete with the delivered drug for the electrophoretic movement. Hence, the selection and optimization of the reservoir



Molecules	Flux (pmol/cm²/h)
FITC	95,86 ± 4,94
FD 4,4	108,25± 16,68
FD 12	18,30 ± 3,39
FD 38	20,52 ± 0,63

Fig. 4. Cumulative quantities and fluxes of FITC and FITC–dextran (FD) of increasing molecular weight (4.4, 12 and 38 kDa) detected in the receptor compartment as a function of time, after exponentially decaying electroporation (10 pulses of 150 V, 150 ms), donor solution: 250 μM. From Lombry et al. [30].

composition must take into account the drug ionization (pH), the pH shift induced with inert electrodes (presence of a buffer required), the presence of competitive ions (ionic strength and composition of the solution) and the conductivity (high conductivity compared to the skin). Hence, the composition is a critical parameter to enhance drug transport. Even though theoretical inputs help in selecting appropriate pH (drug ionization), buffer composition, buffer capacity (number of charged transferred) and buffer conductivity, the optimization of the reservoir formulation remains a ''trials and errors'' optimization [10,35,40].

An increase of the viscosity of the drug solution has been reported to decrease drug transport by skin electroporation [47].

4. Potential clinical applications of skin electroporation

The dramatic and reversible increase in skin permeability caused by electroporation indicates that drugs might be delivered transdermally at significantly enhanced rates. Especially for macromolecules, such as proteins and gene-based drugs, electroporation-mediated transdermal drug delivery could be a promising route of administration. Electroporation can also be used for topical delivery.

Extensive work on molecular transport by skin electroporation has been performed in vitro. Essential features of transport include (i) high fluxes for many different compounds, (ii) rapidly responsive molecular transport and (iii) modulation of transport by controlling the electrical parameters and the physicochemical properties of drug and reservoir.

4.1. Transdermal drug delivery

Application of high voltage pulses has been shown to increase transport across and/or into the skin for compounds ranging in size (i) from small, e.g. fentanyl, timolol [35,40]; (ii) to moderated-sized molecules, e.g. calcein [9]; (iii) to macromolecules, e.g. LHRH, calcitonin, heparin, FITC–dextran up to 40 kDa [30,48–50]. Orders of magnitude increase in transport has also been reported for lipophilic (e.g. timolol [40]) and hydrophilic (e.g. metoprolol [10]) molecules, for charged (e.g. heparin [50]) and neutral (e.g. mannitol [37]) molecules (Table 2).

The in vitro transport of drugs by skin electroporation has been shown to increase by up to four orders of magnitude with lag-times of only seconds to minutes, indicating that molecules rapidly respond to electric pulses. The enhancement ratio and the onset time for transport depend on the electrical parameters of the pulses and the physicochemical properties of the drug as well as on the experimental model (e.g. skin model). Hence, the control of drug transport by skin electroporation can be obtained by the selection of these parameters.

The in vitro features of transport have been shown to translate in vivo, in support of the potential therapeutic applications. In vivo data have especially

Table 2
In vitro studies on transdermal drug delivery by electroporation (adapted from Préat and Vanbever [38])

Compound	Molecular weight	Charge	Log enhancement ratio	References
Water	18	0	1	[37]
Mannitol	182	0	2	[37]
Atenolol	266	+1	2	[51]
Metoprolol	267	+1	3	[10,47]
Tetracaine	301	−1	1	[52]
Alnitidan	302	+1/+2	2	[42]
Timolol	316	+1	2	[40]
Methylene blue	320	+1/+2		[53]
Fentanyl	336	+1	2	[17,35]
Na nonivamide acetate	350	−1	1	[54]
Nalbuphine	357	+1	1	[43]
FITC	390	−1		[30]
Domperidone	426	+1	2	[55]
Lucifer yellow	457	−2	4	[56]
Terazosin	460			[57,58]
Buprenorphine	504	+1	1	[59]
Sulforhodamine	607	−1	3	[22,26,56]
Calcein	623	−4	4	[9,22,56]
Erythrosin derivative	1025	−1	4	[9]
Cyclosporine A	1201	0	1	[60]
Salmon calcitonin	3600	+1	2	[49]
Dextran sulfate	5000	Highly −	2	[61]
Heparin	12,000	Highly −	2	[50]
Defibrase	36,000	Highly −		[62]
FITC–dextran	4–38,000	−		[30]
Nano-microspheres	10 nm–45 μm	Highly −	−	[21,63,64]

underlined the enhanced transport and the rapid onset for transport. In the first in vivo study, calcein transport by skin electroporation was assessed in rats serum: fluxes of calcein were greater than two orders of magnitude than the control (Fig. 1) [9]. Another in vivo study using hairless rats showed that fentanyl rapidly responded to electric pulses: very rapid transdermal delivery of fentanyl (within 15 min) at therapeutic level were obtained by skin electroporation, inducing a deep analgesia lasting for about an hour (Fig. 5) [65].

The in vitro and preclinical in vivo data indicate that electroporation could be used to enhance transdermal drug delivery and expand the range of compounds delivered to hydrophilic, charged and even macromolecular substances. Compared to other transdermal delivery methods, electroporation could be more adapted for a rapid and pulsed delivery and/or macromolecule delivery.

4.2. Topical drug delivery

The rationale for using electroporation for topical delivery is based on the permeabilizing effect of high voltage pulses on lipid bilayers: besides the permeabilization of the main skin barrier, the stratum corneum, application of high voltage pulses to the skin can also enhance the permeability of the cells underlying the stratum corneum. The first evidence for this cellular permeabilization came from electrochemotherapy that consists in treating subcutaneous tumor by the injection of a non-permeant cytotoxic bleomycin followed by the local application of high voltage pulses to permeabilize the cells exposed to the electrical field [7]. Hence, to target impermeable substances to the keratinocytes or to dermal cells, electroporation could be particularly promising, specially for macromolecules (see Table 3). Enhancement of topical delivery of both lipophilic and hydrophilic drugs by electroporation has been achieved. Electroporation enhanced by one order of magnitude the topical delivery of cyclosporine A formulated as a coevaporate to increase its water solubility [68]. It also increased by the quantity vitamin C in the skin with an efficacy depending on the formulation [66].

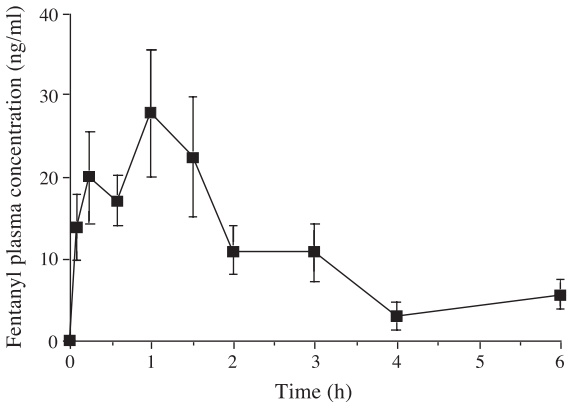


Fig. 5. Fentanyl plasma concentrations as a function of time after transdermal delivery using electroporation. Electroporation was carried out using 15 exponentially decaying pulses of 250 V and 200 ms, applied from time 0 to 5 min in hairless rat skin in vivo. Foams at the cathode and anode were soaked with a solution of fentanyl (400 μg/ml in a citrate buffer 0.01 M at pH 5). From Vanbever et al. [65].

Table 3
Topical delivery of drugs and macromolecules into the skin using electroporation

Permeant	Model	Skin	References
Vitamin C	In vitro	Human	[66]
Lidocaine	In vivo	Human	[67]
Cyclosporine A	In vitro	Hairless rat	[68]
Oligonucleotide	In vitro	Hairless rat	[36,69,70]
Antigens	In vivo	Mice	[71]
Ovalbumin	In vivo	Mice	[72]
DNA (plasmid)	In vivo	Mice, rat	[31,73]

The topical delivery of macromolecules can be enhanced by electroporation. Electrical parameters of pulses and oligonucleotide concentration control the amount of oligonucleotide delivered by electroporation in the viable tissue of the skin [36,69]. Unlike iontophoresis, which does not permeabilize keratinocytes, electroporation induces a rapid delivery of oligonucleotide in the keratinocytes [69]. Electroporation also enhances the topical delivery of DNA in the epidermis, inducing an intracellular delivery of the plasmid within several hours and an enhanced gene expression (see Fig. 6) [31]. The expression of the reported gene in the epidermis lasted for 7 days.

As the skin is an immuno-competent organ, immunization through intradermal or topical route are under investigation. Passive diffusion of an antigen applied topically elicits an immune response when an adjuvant such as cholera toxin is used [74]. Electro-

Fig. 6. *XY*-planar CLSM section showing the localization of the fluorescent labelled plasmid in the epidermis 8 h following skin electroporation (10×1000 V, 100 μs). The image was acquired at depth 12 μm below the surface of stripped skin. Scale bar: 50 μm. From Dujardin et al. [31].

poration was tested to achieve a needle free, adjuvant free and non-invasive immunization with antigen. As it increases the penetration of antigens into the skin, an electrically-assisted delivery of antigens, e.g. myristilated peptide, dipheleria toxoid and ovalbumin, elicites a higher antigen specific IgG response in the plasma, mainly through a Th2 response [71,72].

5. Combinations of enhancing methods

In addition to electroporation, various physical and chemical methods have been used for enhancing transdermal drug transport by different mechanisms: (i) increasing skin permeability (chemical enhancers, ultrasound and electroporation) and/or (ii) providing a driving force (ultrasound, iontophoresis and electroporation) [14]. While all these enhancers have been shown to increase drug transport, their combinations have been hypothesized to be more effective compared to each of them alone and to increase the safety.

5.1. Electroporation and chemical enhancers

The synergistic effects between electroporation and pre or co-treatment with chemicals enhancers have been reported [14,75–77]. These chemicals are different than those mentioned commonly in the literature and include polysaccharides (heparin and dextran), urea, sodium thiosulfate and phospholipids. In contrast with traditional chemical enhancers for passive transdermal delivery, an effective chemical enhancers for electroporation does not need to disrupt lipids, but should stabilize the transient disruptions created by electroporation. The hypothesis for the combination of these two methods is to create enlarged aqueous pathways and/or to prolong the lifetime of the electropores. Vanbever et al. [76] and Weaver et al. [78] showed that macromolecules (heparin or dextran-sulfate) increased transdermal transport of mannitol by electroporation. No enhancement was observed during passive diffusion or iontophoresis, suggesting that macromolecules interact specifically with transport pathways created at high voltage. The persistent low post-pulse electrical resistance could result from the insertion of these linear macromolecules in the aqueous pathways. Zewert et al. [75] showed that significant macromolecule transdermal fluxes occurred when sodium thiosulfate was present, supporting the hypothesis that enlarged aqueous pathways or microconduits were created allowing large quantities of macromolecules to be transported through human skin. Anionic phospholipids were found to enhance the transdermal transport of FITC–dextran up to 40 kDa by electroporation, probably by interacting with the lipids of stratum corneum [77].

5.2. Electroporation and ultrasound

Due to their similar mechanisms of action, the combination of ultrasound and electroporation could be less promising. However, synergy between ultrasound and electroporation has been reported by Kost et al. [79]. A simultaneous application of ultrasound and electroporation enhanced transdermal calcein transport. The application of ultrasound also reduced the threshold voltage for electroporation.

5.3. Electroporation and iontophoresis

The rationale for the combination of iontophoresis and electroporation is based on the difference between the mechanisms of action of these enhancers. Specifically, electroporation may disorder the lipid bilayers of the skin and create new transport pathways into the skin, thus facilitating passage of current during subsequent iontophoresis and resulting in increased transdermal transport [14]. The application of electroporation before iontophoresis has been reported either to increase drug transport and/or shorten the lag-time,

Table 4
Effects of electroporation on iontophoretic transport

Effect of electroporation on iontophoretic transport	Drugs	References
Transport ↑	LHRH, PTH, dextran sulfate, defibrase, atenolol	[48,49,51, 61,62]
Lag-time ↓	Sodium nonivamide acetate	[54]
Transport ↑ + lag time ↓	Salmon calcitonin	[49]
Transport=	Salmon calcitonin (when low voltage)	[49]
Transport ↓	Buprenorphine Timolol	[51,59]

or in some cases to have no effect on iontophoretic flux (Table 4). Bommannan et al. [48] studied the synergistic effect of iontophoresis and electroporation on transdermal delivery of LHRH in vitro. Application of a single electroporation pulse prior to constant intensity iontophoresis consistently yielded 5–10-fold higher fluxes. The increased efficiency of electroporation+iontophoresis was attributed to the reduced impedance and size-selectivity of the skin. Chang et al. [49] studied the effect of electroporation and iontophoresis on transdermal transport of salmon calcitonin and parathyroid hormone through human epidermis (Fig. 7). Pulsing at low voltages (<120 V) followed by iontophoresis did not result in increased transport over iontophoresis alone. The transdermal transport of salmon calcitonin by pulsing 15 exponentially decaying pulses (1 ppm) of 500 V to 200 ms followed by iontophoresis led to a quick input and high flux. Fang et al. [54] showed that pulses of high

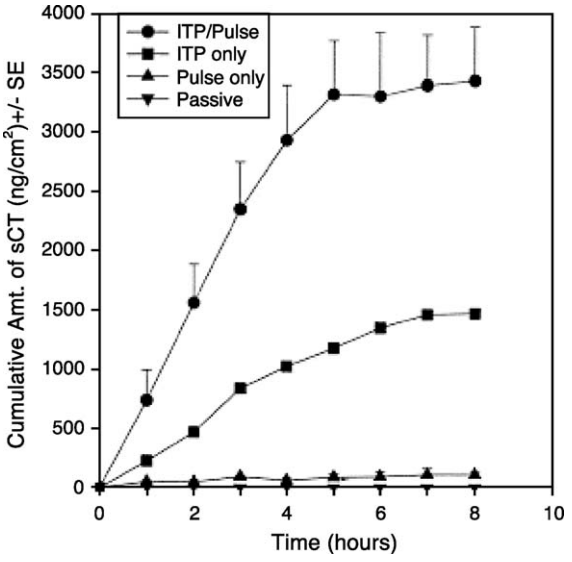


Fig. 7. Delivery of salmon calcitonin (sCT) through human skin by passive diffusion and different electrical conditions. ITP, 4 h of iontophoresis with a current of 0.5 mA/cm^2. Pulse, six pulses of 120 V, 10 ms. ITP/pulse, electroporation followed by iontophoresis. Donor solution, salmon calcitonin 50 μg/ml in a citrate buffer (50 mM, pH 4). From Chang et al. [49].

Table 5
Effects of electroporation on the skin (adapted from Jadoul et al. [80])

Investigation method	Observations	References
Visual aspect	Mild reversible erythema	[81]
Impedance	↓ Resistance (up to three orders of magnitude on a time scale of microsecond)	[9,11,15, 26,81,82]
FTIR	=fluidity of the lipid alkyl chain, ↑ hydration	[83]
DTA	=T° (T2 and T3+4), ↓ in enthalpy→disordering	[84]
Xray scattering	↓ lamellar ordering, ↓ intralamellar packing	[83]
FFEM	Spherical deformations, rough surfaces disorganization of the lamellae, appearance in a network-like structure	[84,85]
TEWL	Mild reversible increase (impairment in barrier function)	[34,82]
LDV	Mild reversible increase in blood circulation, no significant modification in blood flow	[34,82]
LDI	Reversible (10 min) decrease in blood circulation	[82]
Chromameter	Mild reversible erythema, no erythema	[34,82]
Clinical evaluation	No skin irritation, Electrical sensation well tolerated by most patients	[73]

FTIR, Fourier transform infrared spectroscopy; DTA, differential thermal analysis; FFEM, freeze-fracture electron microscopy; TEWL, trans epidermal water loss; LDV/LDI, laser Doppler velocimetry/imaging.

voltages followed by iontophoresis did not result in increased transport of sodium nonivamide acetate over iontophoresis alone, but shortened the lag-time. The effect of the physicochemical properties of the drug on transdermal transport by combination of electroporation and iontophoresis was recently highlighted [51,59]. In the case of buprenorphine, a very lipophilic drug, electroporation failed to enhance iontophoretic transport through human epidermis [59]. Denet et al. [51] compared the effect of electroporation on iontophoretic transport of a lipophilic and an hydrophilic cationic β-blockers, i.e. timolol and atenolol. Contrary to atenolol, a lower transport of timolol was observed with the combination of electroporation and iontophoresis than with iontophoresis alone. This negative effect of electroporation on iontophoretic transport of timolol was explained by the accumulation of positively charged timolol in the skin by electroporation inducing a decrease of electroosmotic flux during iontophoresis.

In conclusion, besides the electrical parameters, the physicochemical properties of the drug are important parameters able to affect the electrotransport of the drug.

6. Safety issues associated with skin electroporation

A major aspect in the clinical acceptability of transdermal drug delivery by electroporation is its effect on the skin and underlying tissues. Different methods have been used to assess the skin tolerance to electric pulses. Visual examination, non-invasive bioengineering methods, measurement of skin electrical properties, histological and ultrastructural studies as well as clinical studies have been performed (Table 5). Overall alterations of the skin following high voltage pulses are mild and reversible but muscle contractions are usually induced [23,80]. When high voltage pulses are applied to the skin, its electrical resistance is dramatically decreased to a very low resistance [9,15]. For large pulses and/or multiple pulse protocols, this drop in skin resistance is partially reversible. Structural changes persist within the LTR, due to combined electrical and thermal effects [33]. As the stratum corneum has a much higher electrical resistance than underlying skin and deeper tissues, an electrical field applied to the skin will concentrate in the stratum corneum and will be much lower in the

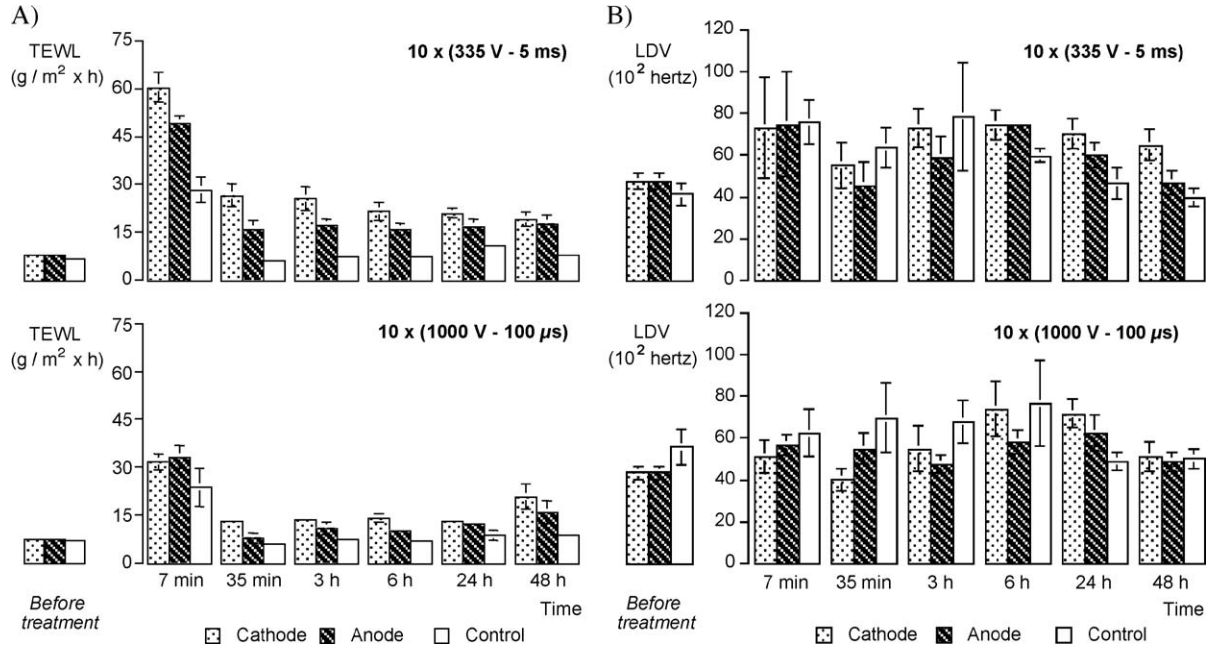


Fig. 8. (A) TEWL after rat skin electroporation using square wave pulses. (B) Laser Doppler velocimetry (LDV) after rat skin electroporation using square wave pulses. From Dujardin et al. [82].

viable tissues, which could be protected from adverse effects.

A sensation or pain during electroporation has been reported, due to the current applied on the skin which causes a direct excitation of underlying nerves and muscles. Increase in pulse rate, duration or voltage tends to enhance levels of sensation like itching, tingling, pricking, muscle contractions and outright pain [21].

Besides a decrease in skin resistance, electroporation with exponentially decaying pulses was reported to induce (i) an increase in skin hydration; (ii) a disorganization of the stratum corneum lipid bilayers; (iii) a transient impairment of the barrier function (increased transepidermal water loss); (iv) a transient increase in the blood flow. These changes were partly reversible [80,84].

More recently, the effect of square wave pulses on skin integrity in vivo was studied [82]. The effects induced by square wave pulses on the skin were also found mild and reversible. Square wave pulses induced a mild impairment of the barrier function of the skin, i.e. a dramatic decrease in skin impedance and an increase in transepidermal water loss (TEWL) rapidly reversible and a transient (<10 min) decrease in blood flow (Fig. 8). Even though these perturbations of the skin were observed, they did not alter the viability of the skin and confirm the tolerance of the skin to square wave pulses in vivo.

Clinical precedent for safety applying electrical pulses exists with the use of techniques such as transcutaneous electrical nerve stimulations and electrochemotherapy. Indeed, pulse application in patients for electrochemotherapy (8×1000 V/cm, 100 μs with plate electrodes) indicated that these pulses were well tolerated. The immediate effects were marks of the electrodes in skin that disappeared after a few minutes, and unpleasant sensation predominantly caused by muscle contraction. The patients did not require special pain control as the bearable pain dissipated immediately after application of the electric pulses [86]. Hence, application of high voltage pulses seems to induce minor and temporary adverse effects that consist mainly in muscle contractions during pulsing and transient erythema.

The design of electrodes, as well as pulsing protocols, may reduce these unwanted side effects. By confining the electrical field to the stratum corneum, the muscle contraction and potential pain could be reduced. Zhang et al. [73] showed that with meander electrodes, effective drug delivery without pain might be achieved even in the sensitive penis area in the absence of anesthesia. The design of electrodes and electrical delivery conditions should be optimized depending on the disease target and medical requirements, without discomfort of the treatment.

7. Conclusions

Electroporation is an efficient method for enhancing transdermal drug delivery in vitro and in vivo, and expands the range of compounds delivered transdermally. It could be a promising alternative as a non-invasive delivery of macromolecules (up to at least 40 kDa) and, fast and/or pulsatile transdermal delivery. Combined with other enhancing methods, electroporation can provide modulated and adequate delivery according to the treatment. Pulse protocol and electrode design need to be optimized to reduce the main adverse effect, i.e. muscle contraction.

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