Assessing the potential of skin electroporation for the delivery of protein- and gene-based drugs

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Although transdermal drug delivery has many potential advantages, the permeability of skin to macromolecules is extremely low. However, the application of short, high-voltage pulses to electroporate skin has recently been shown to make it reversibly permeable. A number of studies have demonstrated that electroporation-mediated transdermal delivery of peptides, polysaccharides, oligonucleotides and genes may be possible at clinically relevant rates, leading to the current commercial development of electroporation techniques.
Factors affecting delivery by electroporation

Electrical and other parameters determine the rates of transdermal transport by electroporation. Key variables include the voltage, duration, and number of the pulses applied. Usually, the goal is to maximize drug delivery using a protocol that will be acceptable for in vivo human studies; that is, one that will neither cause pain nor damage the skin. A typical applied transdermal voltage is 50–150 V (Ref. 8), although higher voltages have been used during in vitro transdermal studies. For studies with calcine, a model fluorescent compound, transport increased almost linearly with transdermal voltage above a threshold of about 80 V, level- ing off at higher voltages (Ref. 14). Some studies have suggested that a few low-voltage, long-duration electroporation pulses (e.g. 50 V, 200 ms) may deliver drugs across skin more efficiently than a large number of high-voltage short-duration pulses (e.g. 100 V, 1 ms). However, long-duration pulses cause significant local heating within the skin and could be associated with increased skin irritation and sensation. Finally, both exponential-decay and square-wave pulses have been used to electroporate skin. One study suggested that exponential-decay pulses may be more effective than square-wave pulses when applied at the same total energy. In a study that combined the use of electroporation and ultrasound, it was found that the application of ultrasound reduces the threshold voltage required to electroporate skin.

Electroporation for cancer chemotherapy

Electroporation can be applied to tissues other than skin, most notably to tumors. This novel form of cancer treatment, called electrochemotherapy, has utilized a combination of electroporation and chemotherapeutic agents. The technique typically involves the systemic or local administration of an anticancer drug followed by the delivery of electrical pulses to the tumor. The rationale for this approach is that many cancer drugs penetrate tumor cells poorly and that permeabilizing the tissue by electroporation can increase drug uptake, thereby making therapy more effective. In one study with six patients, electrochemotherapy was used for the delivery of bleomycin to a total of 18 tumor nodules; three of the patients had malignant melanoma, two had basal-cell carcinoma and one had metastatic adenocarcinoma. Eight electroporation pulses were administered to the tumors after intravenous administration of bleomycin. Five of the six patients responded positively to the treatment, with responses (partial to complete regression) seen in 13 of the 14 nodules. Nodules exposed to the same systemic bleomycin dose but not treated with electrical pulses showed no regression. A control of electric pulses alone was not possible in this trial because of the systemic administration of bleomycin.

Even better results have come from ongoing clinical trials, in which the treatment of approximately 100 cutaneous tumors on 25 patients yielded a 92% complete-response rate; nodules left untreated as controls showed no regression. To avoid systemic exposure for localized drug therapy, bleomycin can be injected directly into tumors for electrochemotherapy, as is done in the current clinical protocol. Studies in mice have shown that intratumoral injection of bleomycin in combination with electrical pulses is effective, with complete regression being the outcome in 60% of the animals. By contrast, no regression was seen when bleomycin alone or pulses alone were used, which indicates that the pulses are required to permeabilize the tumor for effective penetration of bleomycin. The response rate of tumors that have responded only partially to single electrochemotherapy treatments can be further improved by using multiple treatments.

The delivery of peptides

Electroporation, either alone or in combination with low-voltage electrophoresis (iontophoresis), can be
used to expand the scope of transdermal delivery to include larger molecules such as therapeutic peptides and proteins. Electroporation has been shown to increase the flux of luteinizing-hormone-releasing hormone (LHRH) significantly and reversibly through human skin in vitro (Fig. 1). The application of a single pulse prior to iontophoresis increased the flux by five to ten times over that achieved by iontophoresis alone 22. Using a porcine-skin-flap model, it was found that the application of a single pulse immediately prior to 30 min of iontophoresis almost doubled the LHRH concentration in the perfusate, while the application of a pulse every 10 min tripled it 22. By using repeated applications of the iontophoresis protocol, it was shown that electroporation could repeatedly enhance LHRH transport, thus demonstrating pulsatile delivery of a therapeutic peptide 22.

Although iontophoresis can be used to provide baseline levels of transport, electroporation pulses can be applied to provide rapid infusions. This and more complex delivery profiles have also been shown for the transdermal transport of calcim across human skin by changing the pulse parameters 13. The delivery of the peptide cyclosporin A by electroporation has also been reported. This drug is useful for the treatment of psoriasis but is toxic if given systemically at the levels needed to treat this condition (in contrast to its routine use at lower levels to prevent graft rejection). However, some of these drawbacks may be overcome by transdermal administration. Skin electroporation has been successfully used to transport 15-mer and 24-mer antisense oligonucleotides across human skin in vitro with fluxes on the order of 10 pmol cm–2 h–1, a rate of delivery that may be sufficient for some applications. Transport was found to increase significantly with transdermal voltages greater than about 70 V but then plateaued at higher voltages (>110 V) 28. In another study, 15-mer phosphodiesters were shown to be better delivered to hairless-rat skin by electroporation. Owing to improved stability against skin nucleases, the 3'-protected phosphodiesters were shown to be better for topical delivery to the skin than unprotected phosphorothioate oligonucleotides 29.

The delivery of poly saccharides

Polysaccharides are an emerging class of biotechnological therapeutics that could benefit from transdermal delivery. Transdermal transport of heparin across human skin in vitro has been shown to be feasible at therapeutic rates (<1–10 U cm–2 h–1) by applying short, high-voltage pulses (Fig. 2). Heparin retained biological activity as it was transported across the skin but, as smaller heparin molecules appeared to be preferentially transported, the anti-coagulant activity per unit heparin transported was lowered 25. Moreover, in a direct comparison between electroporation and iontophoresis that transferred the same amount of charge across the skin, it was shown that heparin was transported about ten times more efficiently by electroporation than by iontophoresis 25. It was also noted in this study, that examined further in subsequent studies, that heparin (and other long, linear compounds such as dextran and polylysine) could enhance and prolong the permeability of skin to other cotransported molecules (e.g. glucose). It has been suggested that this was due to these macromolecules entering and becoming stuck in ‘electropores’ within the skin, preventing their closure.

The delivery of oligonucleotides

Conventional delivery routes, such as the oral route, are not suitable for oligonucleotides, owing to their poor cellular uptake, biological instability, rapid plasma elimination and the difficulty of targeting their delivery. Some of these drawbacks may be overcome by transdermal administration. Skin electroporation has been successfully used to transport 15-mer and 24-mer antisense oligonucleotides across human skin in vitro with fluxes on the order of 10 pmol cm–2 h–1, a rate of delivery that may be sufficient for some applications.

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The delivery of genes

Topical diseases and conditions such as lupus vulgaris, fungal infections and herpes-simplex-virus infections can benefit from gene therapy targeted to the skin, which, because of its accessibility, is also an attractive target tissue. Although most work in the field of gene therapy has focused on viral gene transfer, problems with viral delivery have prompted the development of nonviral approaches, such as lipid- or liposome-stabilized transfer 26. Electroporation provides an alternative nonviral approach that already enjoys a strong precedent as a method of in vitro gene transfer into mammalian and other cells. The usefulness of electroporation for the gene therapy of skin has been shown in an in vivo study in which lacZ DNA was delivered to hairless mice. Three days after treatment, the skin was removed and stained with X-gal (a colorogenic substrate for the LacZ enzyme). Extensive expression of the LacZ gene was observed in the dermis, including the hair follicles 11,13. The technique of electroporation can also be used for introducing plasmid DNA into skin cells in vivo. A mixture of two supercoiled plasmid DNAs were

![Figure 1](image-url)

Transdermal delivery of luteinizing-hormone-releasing hormone (LHRH) across human epidermis in vitro using iontophoresis for 30 min, either alone (m) or preceded by a single electroporation pulse of 1000 V and 5 ms (L). 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. 22 with permission.)
injected subcutaneously into newborn mice and, after 10–60 min, the pleat of skin was exposed to two high-voltage pulses. Fibroblast cells were then removed from the treated skin and, after 2–3 weeks of culture, clones of stably transformed mouse fibroblasts were obtained. This approach could lead to the use of electroporation for DNA vaccination.

**Commercial and clinical development of electroporation**

One potential benefit of electroporation is that it might expand the scope of transdermal delivery to include larger molecules than those that can be delivered by passive diffusion or using chemical enhancers or iontophoresis; if the delivery of particles becomes feasible, then molecules of essentially any size could be delivered through the skin. The commercial development of electroporation for the transdermal delivery of drugs is still in its early stages, but a number of companies have expressed interest in the technology and performed small-scale, in-house research. However, only two companies have discussed their work publicly: Genetronics has developed a palm-sized generator that can deliver pulses to a medication patch attached to the skin, and has a number of patents in the area of electroporation. Cygnus has a patent on the application of a driving force to assist the delivery of drugs through skin or tissue that has been electroporated—for example, iontophoresis can be used to drive a drug through permeabilized skin over an extended period of time, and intermittent pulses can be applied to make the skin permeable. Cyto-Pulse has also recently emerged as a player in this arena. Finally, the Massachusetts Institute of Technology has a number of important patents on skin electroporation.

Very few human studies have been carried out with electroporation, although the use of electrically assisted skin delivery via iontophoresis has been widely investigated and shown to be safe and well tolerated. In a small double-blind study, transdermal delivery of garlic juice was used as a simple model to demonstrate the feasibility of clinical skin electroporation. Electroporation or “sham” electroporation (i.e. no pulse) was applied to the arm, either with or without garlic juice. The intensity of tongue sensation and taste were scored by the subjects as a test of the systemic delivery of garlic-juice components. The controls did not yield significant sensation or taste, but electrical pulses applied with electrodes firmly pressed against the skin yielded positive responses.

**Safety**

Although only limited work has been carried out, a number of studies suggest that skin electroporation is safe. Studies with hairless rats have demonstrated that electroporation causes only mild, transient erythema and edema, which were reversed within hours and were comparable in severity to that caused by conventional iontophoresis. Experiments with pigs showed that, at both the gross and the light-microscopic levels, electroporation did not result in any skin changes not seen with iontophoresis alone under the conditions examined. For clinical electrochemotherapy, electric pulses greater than 1000 V have been applied to the skin to aid chemotherapeutic treatment of tumor nodules.

**Conclusions**

In summary, even though electroporation has been known for approximately 25 years, it has only recently been applied to skin. In a relatively short time, the field has blossomed, fueled by a range of mechanistic studies and coupled with demonstrated transdermal delivery not only of small drugs but also of macromolecules including peptides, polysaccharides, oligonucleotides and genes. These studies suggest that the delivery of protein- and gene-based drugs by skin electroporation could be an important vehicle for bringing more biotechnology products to the clinical market.

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D- and L-amino acids. Typically displaying relaxed substrate specificity, rapid reaction rates and lacking the need for cofactor regeneration, transaminases have been studied extensively since their discovery over 60 years ago and have frequently been investigated in biotransformation approaches for the production of natural amino acids and chiral amines. Recently, they have been widely applied in the large-scale biosynthesis of unnatural amino acids, which are in increasing demand by the pharmaceutical industry for peptidomimetic and other single-enantiomer drug applications. The existence of transaminases with broad substrate specificity for the synthesis of either D- or L-amino acids makes these enzymes attractive for this type of application, in which the desired products are structurally diverse. The process is often most effective as a biotransformation when the transaminase reaction is coupled to additional enzymatic steps through the use of multiple cloned genes combined in a single organism. Alternatively, it is possible to modify naturally occurring biochemical pathways to bring about the biosynthesis of unnatural amino acids using transaminases.