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Skin Permeabilization for Transdermal Drug Delivery: Recent Advances and Future Prospects

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Abstract

Introduction—Transdermal delivery has potential advantages over other routes of administration. It could reduce first-pass metabolism associated with oral delivery and is less painful than injections. However, the outermost layer of the skin, the *stratum corneum* (SC), limits passive diffusion to small lipophilic molecules. Therefore, methods are needed to safely permeabilize the SC so that ionic and larger molecules may be delivered transdermally.

Areas Covered—This review focuses on low-frequency sonophoresis, microneedles, electroporation and iontophoresis, and combinations of these methods to permeabilize the SC. The mechanisms of enhancement and developments in the last five years are discussed. Potentially high-impact applications, including protein delivery, vaccination, and sensing, are presented. Finally, commercial interest and clinical trials are discussed.

Expert Opinion—Not all permeabilization methods are appropriate for all applications. Focused studies into applications utilizing the advantages of each method are needed. The total dose and kinetics of delivery must be considered. Vaccination is one application where permeabilization methods could make an impact. Protein delivery and analyte sensing are also areas of potential impact, although the amount of material that can be delivered (or extracted) is of critical importance. Additional work on the miniaturization of these technologies will help to increase commercial interest.

Keywords

Cavitation; Drug Delivery; Electroporation; Immunization; Iontophoresis; Microneedles; Microprojections; Penetration Enhancers; Permeabilization; Sonophoresis; Transcutaneous Immunization; Transdermal; Ultrasound; Vaccination

1. Introduction and Background

1.1. Importance of Transdermal Drug Delivery

The efficient delivery of drugs into, and across, the skin has been a goal of researchers for decades. The skin is an attractive area for delivery due to its prevalence and ease of access. However, because of the barrier posed by the skin, most compounds are administered with a hypodermic needle [1], [2]. While common, injections have two serious disadvantages: i)

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pain and needle phobia, and ii) transmission of infectious diseases through needle reuse and unintentional injury.

Injections are unpleasant and painful, which increases needle phobia, resulting in decreased compliance. The significance of this is highlighted by a study, which suggested that 30% of adults suffer from needle phobia [3]–[6]. Even more significant is the risk of unintentional needle injuries to both the patient and physician. This results in hundreds of millions of dollars-worth of additional medical treatment, and increases the transmission of infectious diseases [7]–[10]. In 1999, the World Health Organization estimated that 1.3 million deaths were caused by unsafe needle practices [9]–[11]. In developing countries, it was estimated that 50% of the injections are unsafe. In the United States, there are 800,000 reported cases of needle injuries by medical professionals every year [9], [12], [13].

Despite these disadvantages, needles are required to overcome the barrier properties of the skin. The main barrier to therapeutic delivery is the outermost layer of the skin, the *stratum corneum* (SC). As a result, various methods of skin permeabilization have been explored for their ability to enhance the transport of drugs across the SC.

1.2. Skin Architecture and Barrier Function

Skin has been widely studied and its structure is well understood. The first layer of the skin is the epidermis and encompasses the SC. In spite of being only 15–20 μm thick, the SC provides the majority of the barrier function of the skin [7], [14], [15]. It is comprised of densely packed, dead corneocytes filled with keratin, surrounded by a lipid matrix (see Figure 1) [16], [17]. This lipid matrix is primarily composed of ceramides (50%), cholesterol (25%), and other free fatty acids, and is estimated to be less than 100 nm wide, limiting passive diffusion to small, lipophilic molecules [18]–[20]. Because of the long, flat, tile-like shape of the corneocytes, the SC is often described as having a “brick-and-mortar” structure [15], [21]. This region is indicated in Figure 1.

The epidermis itself is approximately 100 μm thick and is composed of keratinocytes below the SC [22]–[24]. These cells continually proliferate, pushing older cells to the surface where they undergo keratinization and programmed cell death, forming the SC [12], [25]. The live keratinocytes directly below the SC also serve a protective function. Upon insult, keratinocytes are able to secrete cytokines and chemokines to stimulate immune function at the site of infection [26]. This signaling can recruit patrolling dendritic cells. Additionally, upon injury, keratinocytes migrate to the site of the wound and form a protective cover over it [1], [6]. The deepest layer of the skin is the dermis, which is between 1,000–2,000 μm in thickness [11], [13]. It is made up of connective tissue, including collagen and elastic fibers, and has macrophages, fibroblasts, and adipocytes throughout [12], [15].

Upon removal of the SC, the epidermis becomes the rate-limiting barrier to transdermal drug delivery (TDD) [3]–[5]. Removal of the viable epidermis and SC resulted in an order-of-magnitude increase in delivery over removal of the SC alone [3], [10]. This is an important finding because drugs must reach the capillaries found in the dermis for systemic delivery [8], [10].

2. Techniques for Skin Permeabilization

Disruption of the SC allows for a broader class of materials to be delivered into the skin. Additionally, physical insult can activate the immune system at that site, an important feature for vaccination, discussed in Section 3.2 [14], [17]. The methods for skin permeabilization discussed in this review include LFS, MNs, and iontophoresis and electroporation. With the exception of iontophoresis, these methods physically disrupt the

SC. While there are several review articles discussing any one particular method, this review aims to discuss those methods regarded as most important, so that comparisons and differences may be highlighted.

2.1. Low-Frequency Sonophoresis

LFS employs the use of ultrasound (US) to permeabilize the SC. US is a longitudinal pressure wave with frequencies $> 20\text{kHz}$, the upper limit of hearing [16], [19], [20]. US is typically divided into three frequency ranges: low-frequency ($<100\text{kHz}$), therapeutic (0.7–3MHz), and high-frequency ($>3\text{MHz}$) [21], [23], [24].

The primary mechanism for LFS-enhanced tissue permeability is transient cavitation. When applied to a coupling fluid, LFS nucleates bubbles in solution. The bubbles become unstable as a result of large local pressure gradients, and implode. This results in a jet of liquid that can penetrate the SC [25], [26]. Cavitation effects are inversely correlated with US frequency, making LFS the most efficient for permeabilization [27].

LFS is typically applied to the skin together with a chemical penetration enhancer (CPE), which, together, have been shown to act synergistically [28]. Treatment results in two distinct regions in the skin: localized transport regions (LTRs) and non-localized transport regions (non-LTRs), both with different levels of permeability. LTRs are regions in the skin in which a high degree of fluidization of the SC has taken place due to high concentrations of CPE [29]. The transient cavitation events near the skin surface physically disrupt the skin, while actively pushing CPEs into the skin in those regions. Non-LTRs, while more permeable than native skin, experience fewer cavitation events, and therefore, their CPE content is lower, resulting in lower permeability compared to LTRs [29]–[31].

Current research focuses on two areas: i) enhancing the skin permeability achieved, and ii) miniaturization and optimization of the equipment. With regards to the first, the formation of LTRs is chaotic, and typical treatments only result in 5–10% LTR formation [32], [33]. Therefore, advances in LFS have focused on maximizing LTR formation. One such approach is the simultaneous application of low- and high-frequency US [34]. Introduction of high-frequency US was found to nucleate additional bubbles that collapse under the influence of LFS. This dual-frequency treatment resulted in 40-fold larger LTRs than those achieved with LFS alone (see Figure 2). It also resulted in an order-of-magnitude enhancement in the delivery of the permeants that were tested under certain conditions [34]. Achieving greater skin permeability has positive implications on the types and sizes of molecules that can be delivered.

Important advances are also being made in the portability and power requirements of the US equipment. New construction techniques have allowed for the replacement of traditional piezoelectric systems that are bulky and require excitation voltages in excess of 100 V [35]. Today, low-profile cymbal transducer arrays are being used. These transducers take advantage of the flexural mode of vibration, allowing for more modest excitation voltages to be used. In addition to significantly reduced power requirements, these devices have low profiles ($< 10\text{ mm}$), affording greater portability and potential incorporation into wearable patches [35], [36]. Together, enhancements in permeability and further miniaturization of the electrical components could make US a clinically meaningful TDD method.

2.2. Microneedles

MNs are thin projectiles with lengths on the order of microns that are used to pierce the SC. Because of their length, the needles do not penetrate deep enough to stimulate nerves, making them painless. There are four types of MNs: i) solid MNs used for pretreatment of the skin followed by the application of a topical cream [37], ii) hollow MNs for infusion of

larger quantities of drug into the skin [38], iii) coated MNs where the drug to be delivered is coated onto the surface of solid MNs [39], and iv) dissolving MNs in which the needle itself is a dissolvable material encapsulating the drug [40].

Two main hurdles to clinical adoption of MNs are: i) the ability to scale-up production of these devices, and ii) the range of molecules and the amount that can be delivered. The latter has received particular attention and strategies to improve delivery depend on the type of MN being investigated. A common factor, however, is the ability to have all MNs penetrate the skin, which maximizes the amount of drug delivered. For dissolving MNs, another important criterion is the dissolution time. Achieving total dissolution of the needles enables less drug to be loaded into the patches and reduces biohazard waste when still intact needles are disposed of. One recent method that address both issues is the manufacturing of biocompatible polymeric MNs on water-soluble, flexible backings [41]. These patches were observed to completely dissolve in 5 minutes in water. In skin, because the backing is water-soluble, there is no need to remove the device, ensuring total dissolution while reducing biohazard waste [41]. Additionally, the flexible backing, shown in Figure 3, allows for the patch to contour to the skin, and the localization of insertion forces over fewer MNs compared to rigid backings, thereby increasing the ability of each MN to pierce the SC.

Another recent fabrication method for dissolving needles is the droplet-born air blowing method [42]. Patterned droplets of polymer can be stretched between two plates. Blowing air between the two plates then shapes and cures the MNs. The advantages of this method are the mild temperature and pressure requirements and the short manufacturing time. It was found that MNs fabricated using this method dissolved in 60 minutes *in vitro* [42].

When used as a pretreatment, solid MNs create long-lasting pores in the skin through which macromolecules can diffuse. This method involves application of a MN device to the skin, followed by removal of the device and placement of a medicated cream or patch over the site. The duration over which drug diffuses through the skin, however, depends on the lifetime of the pores. It has recently been shown that pores decrease significantly in size in only 15 minutes [43]. However, simple methods such as occlusion of the treatment site can be used to extend pore life to between 48–72 hours [44]. More advanced methods include the co-delivery of non-specific cyclooxygenase inhibitors. In guinea pigs, this has been shown to increase pore life to 7 days, and has recently been used in humans to deliver naltrexone continuously for 7 days [45], [46]. While promising, the safety of prolonged pore life must be investigated.

Despite these advances, challenges still exist. Dissolving MNs are limited by the practical size of the MN, which controls how much material can be delivered. There are also a limited number of therapeutics that can be coated on the surface of solid MNs [47], [48]. Nevertheless, this TDD method is making an impact in the area of vaccination. This application is discussed further in Section 3.

2.3. Electrical Techniques

The two primary means of electrically-facilitated TDD are iontophoresis and electroporation. Iontophoresis involves the application of electrical current to drive charged permeants into the skin through electrostatic effects [49], [50]. Typical currents range from 0.1–1.0 mA/cm² [49], [51]. While uncharged species can also be delivered through electroosmosis, fluxes observed are low, limiting the utility of this method [50]. Electroporation also uses electricity, albeit to disrupt cellular membranes [52]. Electric pulses of hundreds of volts, lasting for 10 μs–10 ms, are typical and result in the formation of aqueous pores in the lipid bilayers of the SC, as well as in the reversible disruption of cell membranes [53], [54]. While the electric field does provide a driving force, delivery is

primarily by passive diffusion through the long-lived pores due to the short duration of the pulses (typically milliseconds) [55]. Iontophoresis, by comparison, has a negligible effect on skin architecture at short treatment times. This is because of the low-voltage nature of the applied electric current. Only at long treatment times do morphological changes start to take place as a result of resistive heating [56]. These two methods are sometimes coupled to achieve greater drug delivery.

Recent studies have demonstrated the utility of these methods for the delivery of a broad range of therapeutics from small molecules, to larger proteins (discussed in Section 3.1) [49], [52], [57]. There has also been work on the miniaturization of the necessary equipment, making it battery-powered and portable [58]. However, there are several concerns surrounding the clinical applicability of electrical techniques. For example, only charged molecules experience significant enhancement in delivery as a result of this method. With regards to electroporation, its application can rapidly change the electrical resistance of the SC [55]. When this occurs, the pulses are no longer confined to the SC, and may stimulate the lower lying nerves and motor neurons, eliciting pain and muscle contraction. For example, electrophoretic treatments that do not elicit pain can still invoke twitching of the muscles [52]. These concerns have tempered research into the use of electroporation for TDD. Instead, it has found use in other delivery applications. In the field of vaccination, its use in achieving intracellular uptake of DNA-based vaccines by skin resident dendritic cells is an active area of research [53], [59].

2.4. Combination Methods

Combinations of skin-permeabilizing methods have also been investigated. Most studies focus on the combination of a skin permeabilizing technology with an active driving force, i.e., MNs and electrical techniques, MNs and US, or US pretreatment followed by iontophoresis. Here, recent developments in the combination of MNs and US with other methods are discussed, followed by an examination of the future prospects of these methods.

2.4.1. Microneedles Combined with Electrical Driving Forces—The combination of MNs with either electroporation or iontophoresis is straightforward. The MNs create additional aqueous pores in the skin through which the applied current is conducted. Typically, skin is pretreated with MNs, and then the drug of interest is applied with simultaneous iontophoresis for 4–6 hrs. [60], [61]. Two advantages of this delivery method are the ability to control the flux of drugs through the modulation of the applied current, as well as a reduction in the lag time needed for drugs to penetrate the skin [62]. However, in the case of small molecules, this method only yields additive benefits at best relative to the use of either method alone. This is because little disruption of the SC is necessary to enhance the delivery of small molecules [60]. Finally, the sequential and time-consuming nature of the treatment detracts from its ease-of-use.

Other studies, however, have investigated the simultaneous use of both TDD methods for the delivery of larger molecules. One such study investigated the simultaneous application of MNs and electroporation as a pretreatment before passive delivery of dextran (4.3 kDa) [63]. This study showed a 140-fold enhancement in delivery over control samples, and an almost 7-fold enhancement over electroporation alone, the TDD method that showed the next-best delivery. Additionally, the treatment only utilized 10 pulses over a 10 second period, making treatment rapid [63]. The delivery of proteins has also been investigated and these studies are presented in Section 3.1.

The safety of these TDD methods remains to be investigated. The puncture of the skin with the MNs may allow the current to reach the lower layers of the skin faster, which could stimulate nerves and motor neurons. However, if proven safe and painless *in vivo*, these

combination methods could be useful, particularly those utilizing simultaneous treatments [63], [64].

2.4.2. Ultrasound Combined with other Transdermal Drug Delivery Methods—

Several reports exist on the combined use of MNs and US for drug delivery [65]–[67]. Here, the MNs are used to penetrate the SC and then US is used to further permeate, and actively push drug into the skin [67]. The most interesting is the simultaneous use of both methods. One group constructed a MN device with a 20 kHz piezoelectric crystal such that each MN also was able to conduct US into the skin [67]. The simultaneous treatment and integration into a single device simplifies this method's use. The authors found that simultaneous treatment yielded an enhancement in the delivery of calcein and bovine serum albumin over either method alone [67].

There have also been some studies on the use of US and iontophoresis [68], [69]. Hikima et al. investigated the combined use of 300 kHz US with iontophoresis for molecules with a range of molecular weights (122–1485 Da). Similar to the combined use of MNs and iontophoresis, they found that only larger molecules experienced synergistic enhancement [68]. They did, however, note that US treatment followed by iontophoresis yielded larger fluxes of material than the simultaneous use of both. This is most likely due to permeabilization of the SC by US before the iontophoretic driving force is applied [68]. This suggests that lower US frequencies, when combined with iontophoresis, may yield greater enhancements.

2.4.3. Future Prospects of Combination Methods—

While the combination methods discussed above show some benefit in delivery over the individual methods, there are challenges associated with their implementation. First, the treatments must be simple to use. This requires that a single device can apply all of the methods used without continual intervention by the patient or physician. Second, safety studies are needed to assess the potential for pain, especially for those methods utilizing electrical modalities. Finally, the specific application should be carefully considered as not every TDD method, or their various combinations, will yield appropriate delivery. Indeed, while each TDD method typically yields an enhancement in delivery relative to passive diffusion for a broad class of drugs, this is not necessarily the case for the enhancement in delivery when using combination methods, relative to each method alone.

3. Areas of Potential Impact

Skin-permeabilizing methods have matured to the point where they may now make a positive impact on the treatment and prevention of diseases. Discussed here is the application of skin-permeabilizing methods to the areas of protein delivery, vaccination, and sensing.

3.1. Protein Delivery

The potential advantages of TDD are especially pertinent for protein delivery. Because of their degradation and low absorption in the gastrointestinal tract, proteins cannot be administered orally. Additionally, certain proteins, such as interferons, must be delivered in a sustained manner, which the transdermal route could allow for [70]. As a result, transdermal protein delivery has been a goal of researchers for years [70]. The delivery of human erythropoietin alfa (35 kDa) coated onto a MN patch, for example, was investigated in rats and found to achieve comparable pharmacokinetics to subcutaneous injection, the standard delivery method [71]. Additionally, the kinetics of insulin delivery to mice from dissolving MNs has also been shown to be comparable to subcutaneous injection [42]. Addressing the issue of what can be coated onto MNs, other studies have examined coating

formulations to deliver the model proteins bovine pancreatic ribonuclease A (13.7 kDa) and chicken ovalbumin (45 kDa) [72], [73]. Both studies found that the model proteins could be delivered into skin *in vitro*, with dosage and release kinetics dependent on the coating chemistry.

US and iontophoresis have also been investigated for protein delivery. US has previously been shown to enable the delivery of insulin (5.8 kDa), interferon gamma (17 kDa), and erythropoietin (35 kDa) [70]. More recently, it has been shown to effectively deliver a synthetic cytokine (12.7 kDa) and achieve serum levels comparable to those obtained by the combined use of MNs and iontophoresis [69]. Studies utilizing iontophoresis alone have shown the delivery of ribonuclease A as well, in addition to cytochrome c (12 kDa), ribonuclease T1 (11.2 kDa), and oligonucleotides for the stimulation of anti-tumor activity [49], [51], [74]. Interestingly, delivery was shown to be due to electromigration for all proteins, despite electroosmosis being thought to be the dominant contribution to the flux of larger molecules [75]. The delivery of ribonuclease A was found to be greater than that of ribonuclease T1, even though it is larger and has a lower net charge. This suggests that more than simply a molecule's charge to mass ratio influences its delivery by iontophoresis [74]. It should be noted that in all studies, treatments were carried out for up to 8 hours. It remains to be seen whether the cumulative dose, delivered over such a period of time, is clinically meaningful.

Combination methods have also been investigated for their ability to deliver proteins. One study tested the delivery of both bovine insulin (5.8 kDa) and bovine serum albumin (66 kDa) using the simultaneous application of MNs and iontophoresis [64]. *In vitro* results showed a synergistic enhancement in the cumulative amount of each protein delivered using the combination, compared to either method alone [64].

3.2. Vaccination

There has been considerable interest on the use of skin-permeabilizing methods for vaccination. The dendritic cell-rich environment present in the epidermis makes the skin an attractive site for vaccination. Specifically, Langerhans cells (LCs), epidermal resident immune cells, cover 25% of the skin's area [76]. Second, immunizations administered transdermally typically require minimal amounts of antigenic material to be delivered in order to elicit an immune response. Further, delivery into the LC-rich environment can be dose-sparing compared to traditional intramuscular immunizations that deliver antigenic material to the muscle where much lower populations of immune cells reside [77].

Current needle-based vaccinations have two major limitations: First, for non-replicating vaccines, adjuvants or viral vectors are necessary to induce robust, long-lived immune responses. Both are important for activating the immune system but have serious disadvantages, including their inherent immunogenicity and systemic toxicity responsible for many of the side effects of vaccinations [78]. Furthermore, non-replicating vaccines cannot elicit cytotoxic T cells, which are thought to be essential for clearing viral infections and killing cancer cells [79]. Transdermal-based technologies have the potential to solve these serious side effects.

US, for example, has been investigated for its ability to elicit immune responses using the model antigen tetanus toxoid [7], [9]. The US-based treatments were found to effectively permeabilize the skin and allow diffusion of the tetanus toxoid. This resulted in comparable antigen-specific serum antibody titers to those elicited using intramuscular injection. More significant was the fact that US allowed for an order-of-magnitude dose sparing [9]. This is due to the activation of LCs after US application. It was found that even in the absence of antigen, LCs were activated in the epidermis as a result of the US treatment alone,

suggesting that the US treatment itself is an immunization adjuvant [9]. This was further confirmed by a different research team, who found that antibody titers did not correlate with the extent of skin permeabilization, and therefore, with the amount of antigen that was delivered [7]. With relatively few research reports in this area, the use of US for immunization is a rich area for study, and it remains to be seen whether this treatment protocol can be used to tune the specific response achieved *in vivo*.

Electroporation has seen broader use in the area of immunization, particularly with DNA-based vaccines. DNA-based vaccines are gaining in importance, particularly because of their favorable safety profile compared to vector-based methods. DNA vaccines, however, have traditionally been unable to elicit potent immune responses because they were not internalized by the cell nucleus [59]. By creating transient pores in the cellular membrane, electroporation enables the efficient uptake of the DNA material (see Figure 4). One recent study found comparable titer levels induced through the use of electroporation and anthrax protective antigen compared to an FDA-approved anthrax vaccine [59]. More interestingly, it was found that the electroporation method induced the largest levels of anti-antigen antibodies one year after immunization [59].

Electroporation has also been used to vaccinate human subjects with an HIV-1 DNA vaccine [80]. Cell-mediated immunity was found to be increased over 70-times compared to vaccination without electroporation. In addition to more robust responses, electroporation also facilitated a dose-sparing effect. Local pain and tenderness were found to be greater in the groups receiving electroporation. Despite the positive immune response, the pain and bulky equipment required will most likely limit the impact electroporation has, especially in the developing world.

MNs have attracted the most attention due to their ease-of-use. Many groups have examined the use of MNs for immunization against a range of diseases. For example, superior titers and dose-sparing over traditional injection have been reported for measles [81], rotavirus [82], and various strains of influenza [83]–[85]. For influenza immunization, one study utilized dissolving MNs to deliver inactivated influenza virus (strain A/PR/8/34, H1N1) [85]. The authors found that MNs afforded superior responses compared to intramuscular injection in mice, including conferring protection against a lethal challenge. Additionally, challenges three months post immunization resulted in more efficient viral clearance in the MN group.

MNs have also been used for the delivery of DNA-based vaccines [86], [87]. While electroporation is considered to be the gold-standard for DNA vaccination, one group tested the method of DNA-containing polymer films loaded onto MNs [87]. This allowed for the DNA to be reproducibly delivered into the epidermis and released by the MNs. Adjustment of the polymer formulation allowed for control over the duration of the film persistence [87]. Using model HIV antigens, the group found that the use of their “multilayer tattooing” resulted in a strong T-cell response, with antigen specific CD8⁺ T cells exceeding 5% of the total circulating population. This was comparable to the response seen for electroporation [87]. While adjuvants and immunostimulatory molecules were used to elicit responses, this is an exciting result, which, if proven safe in clinical trials, could greatly simplify DNA-based vaccines.

3.3. Sensing

In addition to delivery, skin-permeabilizing technologies can also be used to extract material for sensing. There is considerable interest in non-invasive sensing of metabolites, especially glucose, for diabetic populations [88]. The two main methods employed are US and MNs, because both can provide a high degree of skin permeabilization, maximizing the amount of

material that is extracted. Iontophoresis has also been used for the extraction of a number of analytes, including glucose and potassium [89], [90]. However, because of its limited impact on skin permeability, it is more limited in the types of analytes that can be detected.

US has seen significant use for skin permeabilization for the detection of interstitial glucose, with the first human trials occurring in the early 2000's [88]. These trials used an off-the-shelf transducer to permeabilize the skin for subsequent glucose detection. The authors showed good correlation between blood glucose levels and those found in interstitial fluid. Additionally, readings could be taken for over 15 hours while the skin remained highly permeable [88]. While this study utilized bulkier equipment, other studies have focused on the use of smaller equipment affording personalized calibration of skin permeabilization [91]. Chuang et al. showed the utility of non-invasive glucose monitoring in a variety of patient populations. More recently, cymbal transducer arrays have been integrated with glucometers to make wearable monitors [92].

Microneedles have similarly been investigated for their ability to extract and sense analytes. Recent studies have focused on the use of hollow MNs with various electrodes loaded within the MN [93], [94]. These detection methods use electrochemical reactions, in which the electrodes have been chemically modified to react with the metabolite of interest, generating a current. This method has been used for the detection of hydrogen peroxide, ascorbic acid, and lactate [93], [94]. The hollow MNs were shown to remain intact within the skin and the electrodes exhibited specificity for the metabolites of interest.

Other studies have utilized solid MNs with functionalized surfaces. One study looked at the use of conducting polymers for the sensing of glucose [95]. The polymers are loaded with glucose oxidase and then coated onto metal MNs. The authors found that their stand-alone patch had acceptable sensitivity over the entire physiological glucose range, and was stable in wet or dry conditions for prolonged storage times [95]. Other studies have examined the detection of large antibodies [96]. By functionalizing the surface of the MNs with influenza strains, the authors were able to show detection of antibodies *in vivo* after animals were vaccinated against those strains [96]. While the detection of large molecules is interesting, this technology is in its infancy. It requires significant post-processing to quantify the extent of capture by the MNs, and is limited to a "yes" or "no" response, rather than to real-time concentration-dependent monitoring.

Two important issues surrounding non-invasive sensing are the amount of material that can be extracted over a given period of time and the correlation of interstitial analyte levels with those of blood. A lag-time has been determined to exist between blood glucose levels and those found in interstitial fluid, for example [88]. As a result, there has been interest in the non-invasive extraction of blood for collection and later testing. Indeed, there are reports of the use of high-aspect ratio MNs for blood extraction [97], [98]. There is also commercial interest in this area (see Table 2).

4. Commercial Interest

There is growing commercial interest in the area of skin permeabilization for the purpose of delivering therapeutic substances. As shown in Figure 5, there are currently a total of 22 drugs FDA-approved for transdermal delivery. Drugs approved since 2007 are listed in Table 1, with pre-2007 approvals listed elsewhere [2]. The fact that most of these drugs were previously approved for other routes of administration highlights the growing interest in transdermal delivery. Controlled release patches can reduce the pain and frequent dosing regimens associated with other delivery methods, thereby increasing patient preference and

compliance. This also allows pharmaceutical companies to reformulate drugs to receive additional periods of exclusivity.

While the majority of currently approved drugs for transdermal delivery rely on passive delivery or a moderate level of permeabilization using chemical penetration enhancers, there are a few drugs already approved for administration using iontophoresis and US [2], [99]. With the advances discussed above, increasingly more drugs will be formulated for use with these permeabilization methods. Specifically, advances in miniaturization, converting many of these technologies into wearable patches, has particularly increased the commercial interest. This is evident in the number of companies focusing on developing permeabilization technologies (see Table 2). Several of these technologies are in Phase 2 clinical testing, and NuPathe Inc. recently won FDA approval for Zecuity®, a wearable iontophoretic patch for the treatment of migraines.

Much of this interest is a result of vaccination and the benefits discussed in Section 3.2. Of the companies listed in Table 2, half have programs testing their technology for vaccination. Many vaccines are currently in clinical trials against diseases ranging from various cancers to HIV (see Table 3). These technologies are allowing for vaccination against diseases which are currently lacking any vaccine and further miniaturization and ease of use may even make vaccines self-administrable. These attractive features will continue to draw commercial interest.

5. Conclusion

TDD is an advantageous route of drug and vaccine administration. It offers the potential to be pain free and minimize first-pass metabolic effects associated with oral delivery. As a result, significant attention has been paid to developing methods to overcome the barrier function of the skin or to add a driving force for active delivery. LFS relies on the phenomenon of transient cavitation to painlessly erode the SC. These cavitation events can also provide an active driving force, pushing drugs into the skin. There are also electrical methods, including electroporation and iontophoresis. While the former creates transient pores in cell membranes, the latter, instead, primarily provides a driving force to enhance delivery. This driving force has made iontophoresis attractive for coupling with a technology that first permeabilizes the skin. MN devices are a power-free method of skin permeabilization. MNs rely on micron-scale needles to puncture only the upper layers of the skin. With recent advances, these technologies are getting smaller and less expensive to manufacture, resulting in considerable commercial interest, particularly in the area of protein delivery, vaccination, and sensing. Despite the maturity of these methods, there is still a need for further study and development, particularly in the areas of device miniaturization and the permeabilization that can be achieved.

6. Expert Opinion

The SC limits the number of drugs that can diffuse across the skin. As a result, many methods have been studied for their ability to permeabilize the SC. The three most common permeabilization methods are LFS, MNs, and electrical techniques. However, so far, none of these methods have made much of a clinical impact. This is primarily due to bulky equipment complicating use and patient compliance, expensive manufacturing methods, and safety concerns, in addition to the kinetics of delivery achievable with these methods.

LFS can permeabilize a large area of skin. However, treatment times can be long, and so far, the equipment required is bulky and expensive. Despite FDA-approval for the transdermal administration of lidocaine, it has seen relatively little use [100]. This highlights the need for focused studies on the use of LFS for specific applications where the technology would

solve an unmet need. Examples include delivery of small molecules with poor oral delivery or the delivery of larger molecules that require small doses. However, the kinetics of delivery must be considered in addition to the total dose achieved. It remains to be seen whether this method is appropriate for vaccination.

Regardless, LFS offers many advantages and deserves further investigation. First, LFS permeabilizes the largest area out of all of the permeabilization methods considered and specifically targets the top layer of the skin, making it painless. Second, further work into the miniaturization of this technology could enable wearable patches, reducing cost and increasing convenience for the patient. This will increase commercial interest in LFS, especially since reformulation would allow for further exclusivity periods of approved drugs. Finally, the area of LFS-assisted vaccination has seen relatively little investigation. LFS has the benefit of acting as an adjuvant, recruiting immune cells to the site of antigen administration. This could eliminate the need for toxic chemical adjuvants. The types of immune responses that can be induced also has not been investigated. The ability of LFS to induce cytotoxic T cells in cancer immunotherapy, for example, would be quite interesting.

MNs have received considerable interest because of advances in manufacturing and their overall ease of use. However, they are not useful for all applications. The relative area of permeabilization is small, being localized to the sites of needle insertion. Use of MNs (or any other permeabilization method) as a pretreatment followed by the application of drug or a patch only complicates use, decreasing patient compliance. This effectively limits the application of MNs to those where the therapeutic may be delivered simultaneously with the MNs. This is why vaccination is an attractive application. Patches can be mass-produced and coated or loaded with minimal amounts of antigen. Further, patients could self-administer the patch, a feature that, so far, is exclusive to MNs. This is especially important for use in developing countries. The need for adjuvants, while associated with a myriad of side effects, will be acceptable, particularly if MNs can induce responses against viruses and cancers that currently lack a vaccine.

Electrically-based delivery methods will likely see more limited use. Like LFS, they require electrical input, making them more expensive and difficult to implement than MNs. Additionally, only electroporation induces permeability, albeit to a lesser extent than LFS. Finally, both electroporation and iontophoresis can elicit pain and discomfort from the start of treatment. Iontophoresis in particular is quite limited on its own. While uncharged species can experience some enhancement, it is significantly reduced compared to charged species. Additionally, delivery typically takes hours, which may not make the kinetics of delivery clinically relevant. Until these devices are further miniaturized, combination with another permeabilization method will only complicate treatment regimens, reducing compliance.

However, like MNs, vaccination is an interesting application for electrically-based methods, specifically electroporation. Currently injected vaccines against viruses must use live vectors that themselves can elicit immune responses, limiting the ability to continually boost. The use of DNA-based vaccines are now feasible because of electroporation allowing efficient cellular uptake of the material. This could potentially enable immunization against cancers and viruses. Whether electrical methods or MNs are more widely used for vaccination will most likely depend on which technology is approved first. However, MNs would seem to have the advantage due to their simplicity.

TDD is an important route of administration due to its benefits over oral and needle-based methods. There is significant room for development and implementation of these methods but work must be tailored to the individual advantages afforded by each method. Studies addressing unmet clinical needs that would benefit specifically from the advantages offered

by a particular method will drive innovation. By simultaneously considering effectiveness and convenience, these methods have the potential to positively impact patients and physicians alike.

Acknowledgments

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Abbreviations Used

CPE	Chemical Penetration Enhancer
LC	Langerhans Cell
LFS	Low-Frequency Sonophoresis
LTR	Localized Transport Region
MN	Microneedle
SC	Stratum Corneum
TDD	Transdermal Drug Delivery
US	Ultrasound

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Article Highlights Box

- A variety of methods exist for non-invasive and reversible skin permeabilization for the purpose of drug and vaccine delivery, as well as for the non-invasive sensing of analytes.
- Low-frequency sonophoresis can efficiently permeabilize a relatively large area of the skin.
- Microneedles are poised to make a clinical impact in the area of vaccination.
- Electroporation facilitates the use of DNA-based vaccines that previously could not elicit meaningful immunological responses.
- Iontophoretic technology is being miniaturized to enable wearable patch devices.
- Further studies surrounding these methods are necessary to maximize their clinical relevance and impact.

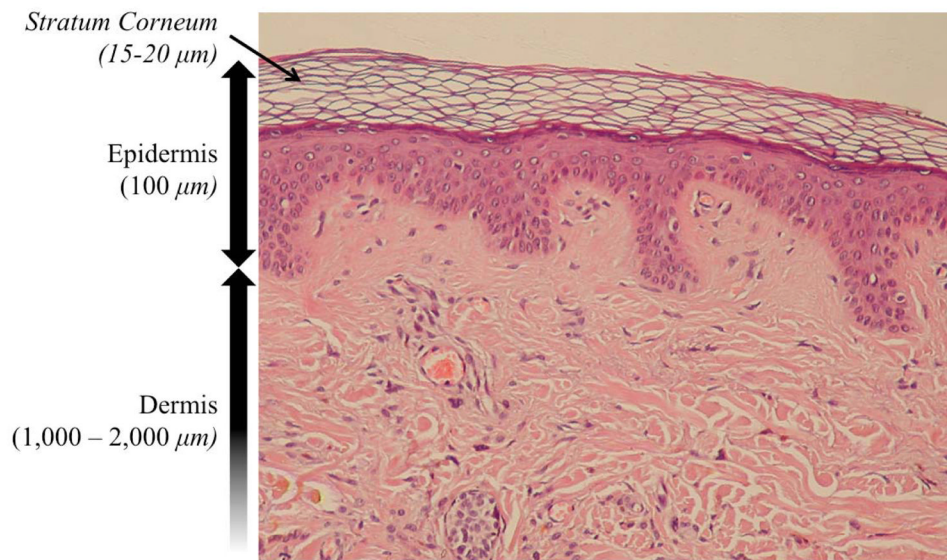


Figure 1. Histological cross-section of the skin. The outermost layer of the epidermis, the SC, is composed of dead corneocytes locked in a lipid matrix. Below the SC lies the viable epidermis, comprised of keratinocytes. Below this region is the dermis. SC: *Stratum corneum*.

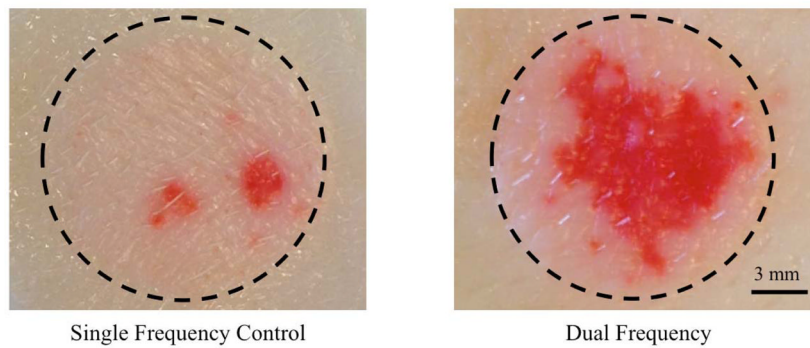


Figure 2. LTR formation by allura red staining in representative images of skin treated with LFS alone (left) or with dual-frequency US (right). The dotted line indicates the area of the skin exposed to US.
LTR: Localized transport region. LFS: Low-frequency sonophoresis. US: Ultrasound.

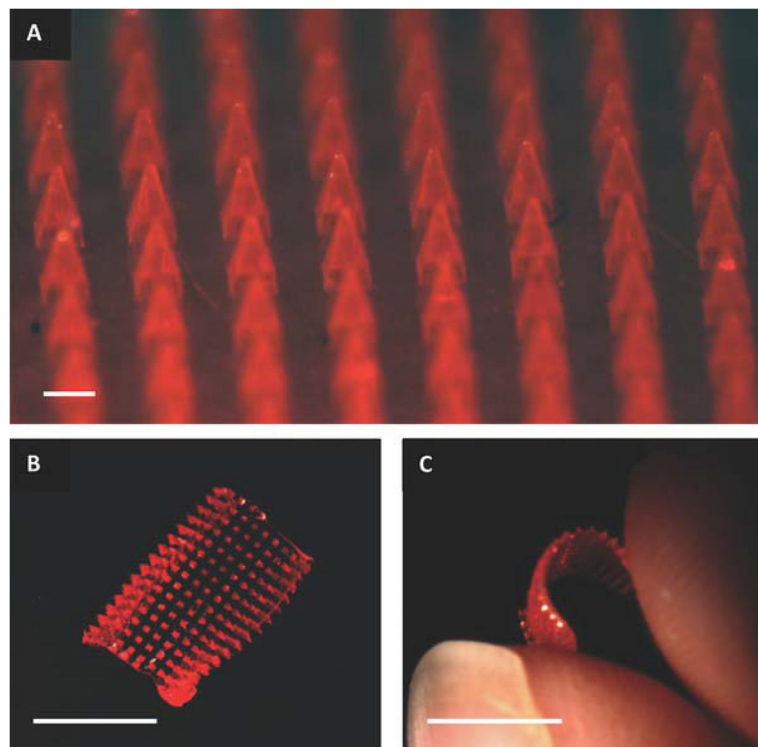


Figure 3. Images of a flexible MN patch. (A) The MN morphology is highly reproducible (scale bar: 200 μm). The flexibility of the patch is demonstrated in (B) and (C) (scale bar: 1 cm). Reprinted with permission from [41]. MN: Microneedle.

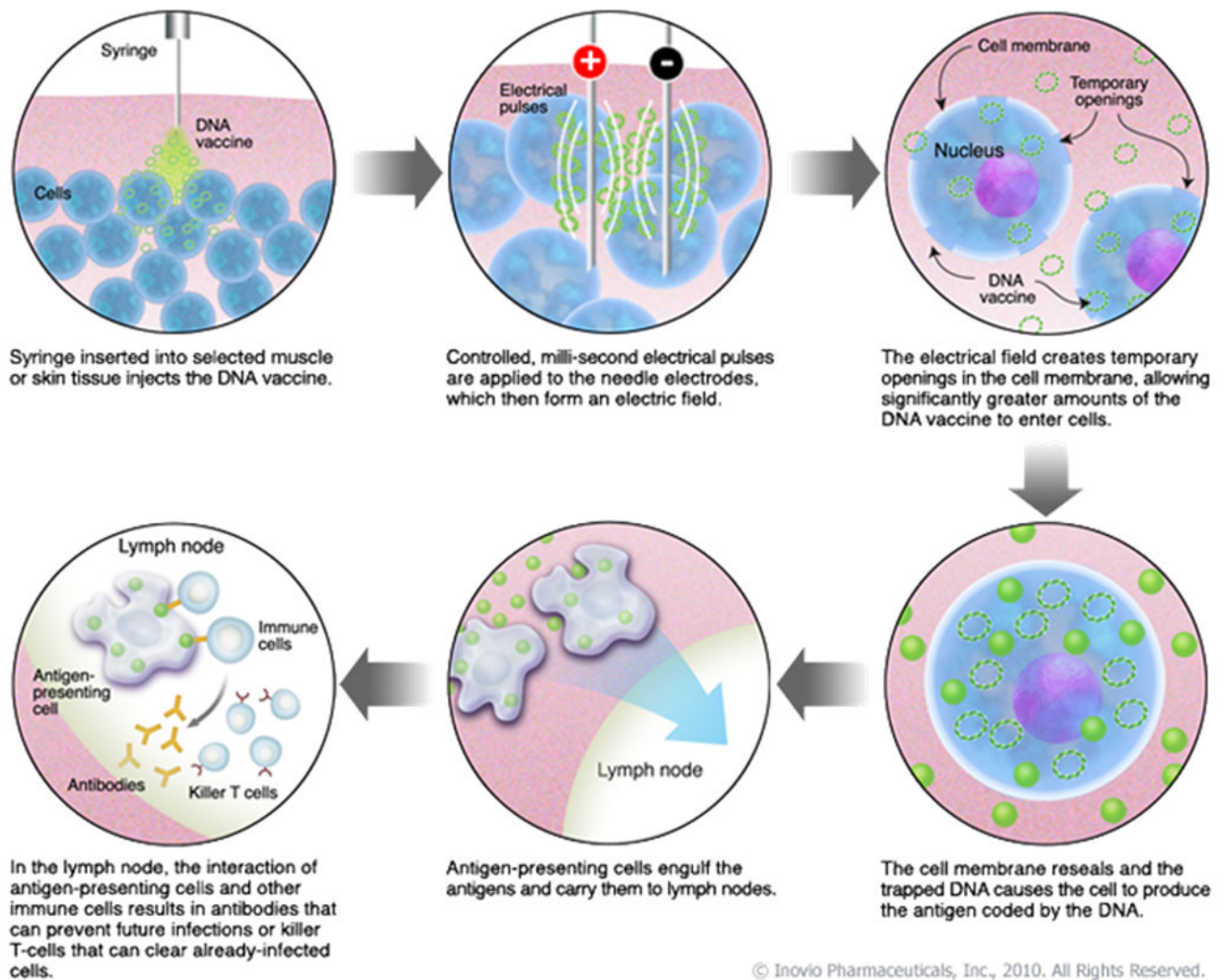


Figure 4. Mechanistic overview of electroporation-facilitated DNA-based vaccine delivery. The DNA material is first injected into the skin (upper left) followed by placement of electrodes. The short electric pulses create transient pores in the cell membrane, facilitating cellular uptake of the DNA (upper right). The cell then begins to produce the antigen encoded for by the DNA, and antigen-presenting cells engulf these antigens and present them to other immune cells to elicit protection. Image courtesy of, and reproduced with permission from, Inovio Pharmaceuticals, Inc.

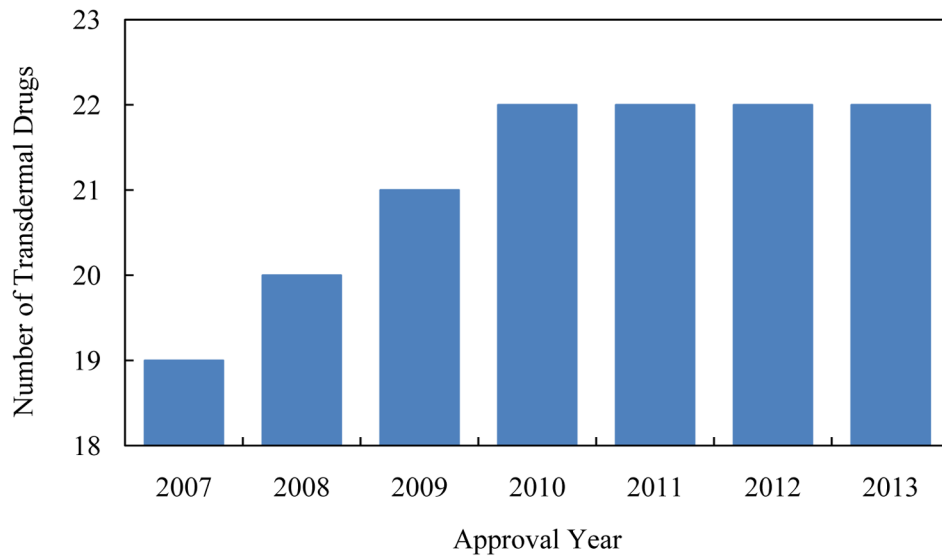


Figure 5. The cumulative number of drugs approved by the FDA for transdermal administration since 2007. The value for year 2007 was taken from Reference 2. Data for subsequent years was taken from the FDA Orange Book (Food and Drug Administration, Approved Drug Products with Therapeutic Equivalence Evaluations, 33rd Edition).

Table 1

Drugs approved by the FDA for transdermal administration

Drug	Company	Indication	Approval Year
Rivastigmine	Novartis	Dementia	2007
Rotigotine	UCB, Inc.	Parkinson's Disease	2007
Granisetron	ProStrakan, Inc.	Chemotherapy-Induced Nausea and Vomiting	2008
Oxybutynin Chloride	Watson Pharmaceuticals, Inc. (Now Actavis PLC)	Urinary Incontinence	2009
Buprenorphine	Purdue Pharma L.P.	Pain and Opioid Dependence	2010

Table 2

Companies developing and commercializing skin permeabilization technologies

Technology	Company	Headquarters	Product	Mechanism	Status	Company Value
LFS/Sensing	Echo Therapeutics, Inc.	Philadelphia, PA	Symphony® Continuous Glucose Monitor	Glucose monitoring by interstitial fluid after skin permeabilization	Currently carrying out clinical testing in support of a CE Mark for approval in Europe	\$26M
	Becton Dickinson/Sanofi Pasteur	Franklin Lakes, NJ/Lyon Cedex, France	Intanza®	Prefillable injection system utilizing a microneedle for dermal delivery of vaccine against seasonal influenza	Received marketing authorization from the European commission in February 2009, won FDA approval in May 2011 (Fluzone®)	N/A
Microneedles	Corium International, Inc.	Menlo Park, CA	MicroCor®	Dissolvable microneedle device	Completed Phase I study, further clinical testing of parathyroid hormone delivery underway	Private, secured loan for \$35M in December 2012
	NanoPass Technologies, LTD.	Nes Ziona, Israel	MicronJet™	Hollow microneedle device	Granted commercial licenses to Janssen Pharmaceuticals, Inc. for vaccine delivery and Circassia LTD. for allergy and autoimmune disorders	Private, secured Series B funding in July 2012
	Seventh Sense Biosystems, Inc.	Cambridge, MA	TAP 20C™	Microneedle penetration of the skin followed by application of vacuum for blood extraction	Initiated clinical testing to compare analyte concentrations in blood collected by the TAP 20C™ or conventional fingerstick	Private, secured \$29.5M debt and equity funding to date
	TheraJect	Fremont, CA	TheraJect™ Patch	Dissolvable microneedle device	Pre-clinical <i>in vivo</i> studies of vaccine delivery, received research grant from the National Science Foundation for Sumatriptan delivery	Undisclosed
	Valeritas, Inc.	Bridgewater, NJ	Micro-Trans™	Microneedle device	Undisclosed	Private, secured a \$100M structured debt financing in June 2013
	Vaxxas, Inc.	Cambridge, MA	The Nanopatch™	Vaccine-coated microneedle device	Initiated a research collaboration with, and granted an exclusive	Private, secured \$16.46M series A funding in 2011

Technology	Company	Headquarters	Product	Mechanism	Status	Company Value
Electrical Techniques					license to Merck for the Nanopatch™	
	Zosano Pharma	Fremont, CA	ZP Patch	Drug-coated microneedle patch	Entering Phase 3 Pivotal trial for the treatment of severe osteoporosis	Private, secured a \$30M financing round in July 2009
	Ichor Medical Systems	San Diego, CA	TriGrid™ Delivery System	Electroporation platform for vaccination	Received a contract from the US Defense Threat Reduction Agency to assess the immunogenicity of DNA vaccines against equine encephalitis virus using the TriGrid™ Delivery System	Private, does not disclose financial info
	Inovio Pharmaceuticals, Inc.	Blue Bell, PA	Cellectra®	Electroporation platform for vaccination	Hepatitis C vaccine Phase I clinical trial started in Korea. US studies will begin in 2014	\$374M
	NuPathe Inc.	Malvern, PA	Zecuity®	Iontophoresis-facilitated delivery of Sumatriptan for acute treatment of migraines	FDA approved January 2013	\$51.2M
	OncoSec Medical, Inc.	San Diego, CA	OncoSec Medical System™	Electroporation platform for vaccination	Multiple Phase 2 clinical trials for vaccination against various cancers	\$50.7M
	NB Therapeutics	Bristol, PA	Iontophoresis Platform	Iontophoresis-facilitated delivery of terbinafine HCl for the treatment of toenail fungus	Phase 2 clinical trial completed in 2012	Private, raised \$1M through convertible debt offering in 2012

Table 3

Active clinical trials utilizing skin-permeabilizing technologies

Technology	Treatment	Indication	Sponsor	Phase	Method		Status			
					Randomized	Masking				
Microneedles	Drug candidate BA058 coated onto microneedles	Osteoporosis	Radius Health, Inc./Nordic Bioscience A/S	2	Yes	Double	Ongoing			
	Microneedle pretreatment followed by lidocaine cream	Pain	University of Oklahoma	2/3	Yes	Double	Not Yet Recruiting			
Electroporation	TAP 20C™ Microneedle-facilitated blood extraction	N/A	Seventh Sense Biosystems, Inc.	N/A	No	Open-Label	Not Yet Recruiting			
	HIV vaccine with TriGrid™ electroporation	HIV vaccine	National Institute of Allergy and Infectious Diseases/Profectus Biosciences, Inc.	1	Yes	Double	Recruiting			
	VGX 3100 DNA vaccine candidate with Clectra® electroporation	Cervical intraepithelial neoplasia	Inovio Pharmaceuticals, Inc.	2	Yes	Double	Ongoing			
	PENNVAX-G DNA vaccine candidate with Clectra® electroporation	HIV vaccine								
	Interleukin-12 plasmid followed by electroporation	SCIB1 with TriGrid™ electroporation	Merkel Cell Carcinoma	OncoSec Medical, Inc.	2	No	Open-Label	Recruiting		
			Cutaneous T Cell Lymphomas					2	No	Open-Label
Melanoma			2					No	Open-Label	Recruiting
Melanoma	Melanoma	Scancell LTD.	1/2	No	Open-Label	Recruiting				