INTRODUCTION

Transdermal drug delivery is an attractive method of administration drug (1,2). Delivery across the skin circumvents the enzymatic degradation, poor intestinal absorption, and first-pass liver effect associated with oral delivery. It also avoids the pain and inconvenience of injections. In addition, drug delivery across the skin readily lends itself to sustained or modulated delivery from a passive or active patch. For these reasons, transdermal drug delivery represents a multi-billion dollar market, which has significant impact on medical practice.

Despite these advantages, transdermal delivery is currently limited to a small group of drugs that share a narrow set of common characteristics. Successful transdermal drugs have low molecular weight (<500 Da), have an octanol–water partition coefficient much greater than one, require low doses, and cause little or no skin irritation. Very few drugs can cross skin at useful rates because the stratum corneum, which is the outer 10–20 µm of the skin, is an excellent barrier.

To overcome the stratum corneum barrier, a variety of chemical and physical techniques have been developed to create nanometer-scale
disruptions to stratum corneum structural organization and thereby increase skin permeability. Chemical approaches, involving solvents, surfactants and other compounds, have had varied success, where increased skin permeability has often been associated with increased skin irritation and has often been applicable only to small molecules (3). Physical approaches, such as iontophoresis, electroporation, and ultrasound, have perturbed stratum corneum structure and have provided electrophoretic and possibly convective driving forces (4). These methods have been more effective to increase skin permeability to macromolecules and can be designed to avoid irritation.

These chemical and physical methods to disrupt stratum corneum on the nanometer scale, however, have had limited clinical impact. This is, in large part, because the increases in transdermal transport are still not sufficient for many drugs under clinically acceptable conditions. Moreover, the physical methods typically require a device with a power supply, which adds cost and complexity.

Recent research suggests that the approach to disrupting skin on the nanometer scale may be too mild. Micron-scale skin disruption should make skin much more permeable, yet still be safe and well tolerated by patients. Given that almost all conventional drugs, proteins, DNA and vaccines are sub-micron in size, creating holes of microns dimensions in the stratum corneum should permit delivery of a broad range of compounds. Yet, micron-scale disruption is unlikely to have significant safety or cosmetic concerns. The skin barrier is routinely breached during common experiences of minor abrasion, shaving, dry skin, and hypodermic injection, yet infection rarely occurs. This is because the skin is designed to prevent entry of pathogens, even in the presence of minor stratum corneum defects.

This observation leads to the hypothesis that micron-scale disruption of stratum corneum can dramatically increase skin permeability to a broad variety of compounds without significant safety concerns. Prompted by this idea, a number of methods to disrupt stratum corneum on the micron scale have been developed, including thermal ablation, microdermabrasion, and microneedles. Microneedles represent an especially attractive approach, because microneedle devices can be very simple and need not involve a power supply or other costly components. Microneedles are typically solid or hollow needles measuring microns in size that painlessly pierce across the stratum corneum to transport drugs into the skin for local or systemic delivery.

Although microneedles were already described in a 1976 patent (5), the first paper to demonstrate microneedles for transdermal delivery was not published until 1998 (6). This delay was in large part due to the unavailability of microfabrication methods to make needles of such small dimensions. However, the microelectronics revolution provided a workshop of instrumentation designed specifically to make micron, and sub-micron,
structures, which have now been adapted to make microneedles. For this reason, the first microneedles were etched from silicon wafers, although more recent efforts have emphasized FDA-approved metals and polymers (7,8).

The first uses of microneedles involved piercing the skin with an array of solid microneedles to make the stratum corneum more permeable, after which a drug formulation or patch was applied to the treated site, i.e., the “poke-and-patch” method. Solid microneedles have also been coated with drug formulations that dissolve off within the skin after microneedle insertion, i.e., “coat-and-poke.” The “poke-and-release” approach involves making microneedles out of dissolving or degrading polymers that encapsulate drug, which then release drug within the skin with predetermined kinetics. Finally, fabrication of hollow microneedles facilitates micro-infusion of a liquid drug formulation by the “poke-and-flow” method, which is more akin to a minimally invasive injection than a transdermal patch. Some of these microneedle scenarios have been the subject of previous reviews (7–17).

As discussed in the following sections, a variety of different microneedle designs and delivery scenarios have been studied and shown to deliver a breadth of different compounds in vitro and in vivo. This review seeks to provide a comprehensive review of the peer-reviewed literature on microneedles studied for transdermal delivery. We have not, however, included work presented only in conference proceedings or other non-peer-reviewed literature and have also not included the relatively large literature that primarily addresses fabrication issues in the absence of directly assessing drug delivery capabilities.

THE POKE-AND-PATCH APPROACH

Solid microneedles have been fabricated by a number of methods to pierce the skin and thereby increase skin permeability. The first needles were prepared by plasma etching tapered spikes into silicon wafers to produce needles measuring tens to hundreds of microns in length, tens of microns in base radius, and approximately 1 μm in tip radius (Figs. 1A,1B) (6,18,19). More recently, metal and polymer microneedles have been fabricated with similar dimensions, although tip radius has tended to be less sharp (e.g., 1–10 μm) (20–22).

In vitro studies have demonstrated that skin permeability can be increased by up to 10,000-fold after microneedle treatment for delivery of compounds including calcein, insulin, bovine serum albumin, and latex nanoparticles as big as 100 nm in diameter (Figs. 1A–1D) (6,18,19,22). Residual holes in the skin were measured to be 6 μm in radius, which is smaller than microneedle base dimensions, probably due to partial skin recoil after microneedle removal. Theoretical analysis indicated that
Figure 1  Representative microneedles investigated for the poke-and-patch approach. Silicon microneedle array containing 400 microneedles, each 150 μm in

(Caption continued on facing page)
increased skin permeability could be fully explained by diffusion through the water-filled holes created by microneedle penetration (18). Additional studies have demonstrated increased skin permeability after piercing with small hypodermic needles as a model for microneedles (23,24).

In addition to the delivery of inert model compounds, DNA delivery has been studied using living skin obtained from human surgical procedures and maintained by tissue culture techniques (Fig. 1E). Using these ex vivo methods, plasmid DNA was shown to be delivered and to express a reporter gene (i.e., β-galactosidase) at the sites of needle penetration (25–27).

In vivo delivery has been studied in the context of insulin delivery to diabetic, hairless rats (Fig. 1F) (21). After piercing the skin with an array of microneedles, insulin was delivered across the skin for a 4-hour period. Blood insulin levels increased and glucose levels decreased by as much as 80%, indicating delivery of bioactive protein. Increased delivery of an antisense oligonucleotide was also demonstrated after piercing hairless guinea pig skin with microneedles in vivo (13).

Using a related approach, liquid formulations of DNA, protein, and live, attenuated virus vaccines against hepatitis B, anthrax, and Japanese encephalitis, respectively, were applied to the skin of animal models. Then, blunt-tipped microneedles were scraped across the skin (Fig. 1G) (28–30). The antigen formulations were deposited in the micron-scale troughs created in the skin and induced strong immune responses that were, in most cases, at least as good as hypodermic injection. In a separate study, a tattoo machine was used to pierce the skin and drive a liquid DNA formulation into the skin for transfection (31).

Using a combined approach with iontophoresis, hairless guinea pigs in vivo were first pierced with microneedles and then an antisense oligonucleotide formulation was applied to the skin in the presence of an electric field (20). Pretreatment with microneedles before iontophoresis increased transdermal delivery by 100-fold compared to iontophoresis alone. Similar results were found for delivery of human growth hormone to hairless guinea pigs in vivo (13) and dextrans across hairless rat skin in vitro (32).

**Figure 1 (continued)** length on an area < 0.1 cm² shown (A) at low magnification resting on a forefinger and (B) at high magnification. (C) Columnar, silicon microneedles measuring 150 μm in length with a base diameter of 80 μm. (D) Polymer microneedles made of polyglycolic acid measuring 600 μm in length with a base diameter of 100 μm and a tip diameter of 10 μm. (E) Silicon microneedles measuring 150 μm in length with a base diameter of about 50 μm. (F) Stainless steel microneedles measuring 1000 μm in length with a base width of 200 μm and a thickness of 50 μm. (G) Silicon “microenhancer” array containing flat-tipped, pyramidal microstructures measuring 150 μm in length used to scrape the skin. *Source: From Refs. 6, 19, 21, 22, 25, 28, 66.*
Increased skin permeability due to microneedles has also been exploited as a means to extract interstitial fluid from the skin to assay glucose levels as a non-invasive monitoring method. Data from hairless rats, as well as a small number of human subjects, indicated that up to a few microliters could be extracted within a few minutes and that glucose levels measured in the extracts correlated well with blood glucose levels (33,34).

A similar poke-and-patch approach has also been employed to pierce microneedles into the vascular wall using a modified balloon catheter to increase drug penetration in vitro (35). Microneedles have also been employed to transflect nematodes with plasmid DNA (36). Although microneedle dimensions varied for these applications and the anatomy of the transport barrier was quite different, microneedle-based delivery was nonetheless highly effective.

**THE COAT-AND-POKE APPROACH**

In addition to using microneedles to increase skin permeability, microneedles have also been used to carry drugs into the skin through the holes they create. To accomplish this, metal microneedles have been coated with drug formulations using dip coating methods. Water-based, room-temperature processes that use only FDA-approved excipients have been shown to coat microneedles with a broad range of compounds, including small molecules, proteins, plasmid DNA, virus particles, and polymer nanoparticles (Fig. 2A) (37). Coating formulations have been optimized (i) to form uniform coatings by adding surfactants that lower surface tension to facilitate microneedle wetting with coating solution and (ii) to form thick coatings by adding thickening agents that increase viscosity to cause a larger volume of coating solution to adhere to microneedles during drying (38). Coatings with up to 1 mg of drug per array can be achieved.

Another coating method involves the use of chitosan to coat the base substrate to which the microneedles are attached and thereby provide a drug reservoir for release across the skin using a variation on the poke-and-patch method (Fig. 2J) (39). Using a different approach, acupuncture needle tips were coated with microporous calcium phosphate, after which the micropores were filled with trehalose that could serve to stabilize proteins (40).

Coated microneedles have been used to deliver drugs and vaccines in vivo. Desmopressin was delivered to hairless guinea pigs and found to have up to 85% bioavailability (Figs. 2K–2L) (41). Ovalbumin administered as a model antigen to hairless guinea pigs developed strong antibody responses that were up to 50-fold greater than intramuscular or subcutaneous delivery of the same dose (42,43). This can be explained by the highly immunogenic dendritic cells found in the skin (44). More recently, coated
Figure 2  Representative microneedles investigated for the coat-and-poke approach. Individual stainless steel microneedles each about 700 μm in length, 160 μm in width and 50 μm in thickness coated with solid films containing: (A) calcein, (B) vitamin B₂, (C) bovine serum albumin conjugated with Texas Red, (D) plasmid DNA conjugated with YOYO-1, (E) modified vaccinia virus–Ankara conjugated with YOYO-1, (F) 1-μm diameter barium sulfate particles, (G) 10-μm diameter latex particles, and (H) liquid formulations containing red, yellow or green dye each filled exclusively into the three circular pockets of the microneedles. (I) Prototype microneedle device containing a stainless steel microneedle array of 50 microneedles with an integrated adhesive film to stick to the skin. (J) Silicon microneedles measuring 130 μm in length with a base diameter of 80 μm and a tip radius of <1 μm that were coated with chitosan films containing calcein and bovine serum albumin. (K, L) Titanium microneedles measuring 200 μm in length with 170 μm width at the arrow head and a thickness of 35 μm that were coated with desmopressin. Source: From Refs. 37, 38, 39, 41.
microneedles have been used for ocular delivery, demonstrating delivery of pilocarpine and other model compounds into the rabbit eye in vivo (45).

THE POKE-AND-RELEASE APPROACH

As a novel alternative to coated microneedles, drugs have been encapsulated within microneedles made of polymers and polysaccharides for rapid or sustained release in the skin. In this way, after insertion into the skin, microneedles can dissolve or degrade, which thereby releases encapsulated drug in a controlled manner. Because the needles eventually disappear, there is no biohazardous sharp waste, which facilitates safe disposal. Microneedles have been made in this way by filling micro-molds with molten matrix material along with dissolved or suspended drug particles, which solidify after cooling.

Figure 3  Representative microneedles investigated for the poke-and-release approach. (A) Microneedles made of maltose, measuring 500 μm in length, and encapsulating 10 wt% calcein as a model drug. (B) Bevel-tip, polymer microneedles made of PLGA, measuring 600 μm in length, and encapsulating calcein within their tips. (C) A complete 20 × 10 array of polymer microneedles made of PLGA. Source: From Refs. 46, 49.
One design for rapidly dissolving microneedles involves a maltose-based matrix that encapsulated sodium salicylate and ascorbate-2-glicoside (Fig. 3A) (46). Molten maltose was solidified by a process related to that used to make hard candies. Maltose microneedles were inserted into the skin of human subjects in vivo and found to dissolve within 5 minutes with no adverse reactions. Using a different fabrication process to model microneedles, millimeter-scale needles were made out of dextrin that encapsulated erythropoietin and insulin and shown to effectively deliver these compounds to mice in vivo (47,48).

Degradable microneedles can also be used for controlled release delivery over time. Toward this end, microneedles have been fabricated out of polylactic-co-glycolic acid (PLGA) to encapsulate a model small molecule and protein (Fig. 3B and 3C) (49). Controlled release was demonstrated in vitro for times ranging from hours to months, depending on the encapsulation formulation.

THE POKE-AND-FLOW APPROACH

In contrast to solid microneedles, hollow microneedles provide additional capabilities as well as complications. Hollow microneedles enable delivery of liquid formulations (that may already be approved for injection), which can be administered much more rapidly and with modulated flow rates, if needed. However, hollow microneedles are more complex to fabricate, given their inherently weaker and more sophisticated geometry, and have posed difficulties to achieve large flow rates.

Hollow microneedles have been fabricated by a number of etching and molding methods and shown to deliver insulin and diclofenac to rats in vivo, which established the feasibility of this approach (Fig. 4A) (18,50). Another study demonstrated microneedle injection of methyl nicotinate in human subjects (Fig. 4B) (51). Additional research has shown that delivery using microneedles can be precisely targeted to the epidermis or to specified depths in the dermis, which is of interest for dermatological, vaccine, and research purposes (Fig. 4C) (52).

Despite these successes, only microliter volumes of fluid were delivered into the skin in these studies. In fact, one study reported an inability to deliver any detectable amounts of fluid into skin using microneedles (19). To address this problem, fluid flow through microneedles was measured experimentally and modeled theoretically, which showed that fluid delivery using microneedles is not primarily limited by flow through the microneedles themselves, but is limited primarily by the resistance to flow out of the microneedles and into skin (53). Partially retracting microneedles after insertion into the skin was found to increase flow rates by up to an order of magnitude (54). Retraction allowed the skin, which was deformed during
microneedle insertion, to recoil back toward its original position and thereby relieve skin compaction and increase local flow conductivity (55).

Manufacturing and delivery using microneedles can also be facilitated by using larger needles. Small hypodermic needles measuring 1–3 mm in length have been used to vaccinate mice and rabbits against anthrax (29).

Figure 4  Representative microneedles investigated for poke-and-flow approach. (A) Hollow, silicon microneedles measuring 350 μm in length and containing a 70 μm-wide bore. (B) Hollow, silicon microneedles measuring 200 μm in length and having a lumen diameter of 40 μm. (C) Hollow, glass microneedle with a tip opening effective radius of 30 μm. (D) Hollow, metal microneedles measuring 500 μm in length shown next to a 27-gauge hypodermic needle. Hollow, metal microneedle measuring 1.5 mm in length (E) incorporated into an intradermal injection device and (F) pierced across swine skin. Source: From Refs. 50, 51, 54, 56, 67.
and nonhuman primates against Japanese encephalitis (Figs. 4E and 4F) (30). Another simplification is the use of hollow microneedles for passive delivery (i.e., without pressure-driven flow), which has been shown to deliver insulin to diabetic rats (Fig. 4D) (56).

Hollow microneedles have also been developed for delivery to cells and tissues other than skin. In combination with electrical stimulation using microneedles, neuro-active chemicals have been administered to neural tissue (57). Brain slice cultures have been perfused using microfluidic microneedle devices (58). Hollow microneedle arrays have been used to deliver genetic materials into animal and plants cells (59).

For diagnostic applications, hollow microneedles have been used to withdraw blood (60) and to extract interstitial fluid for glucose monitoring (61). Microneedles have been modified to perform microdialysis for continuous medical monitoring (62).

OTHER CONSIDERATIONS

Successful drug delivery also requires that microneedles be strong enough to insert into the skin without breaking. An experimental and theoretical study indicated that the force to insert microneedles into the skin of human subjects depended primarily on needle tip sharpness, whereas microneedle strength depended on multiple geometric and materials properties (63). Optimized solid and hollow microneedles were found to have insertion forces many fold smaller than failure forces, which provides a large margin of safety. Insertion forces, as well as skin deformation during microneedle insertion, have been reduced by insertion at high velocity (64), insertion with a drilling motion (52), and insertion with vibration (65).

Microneedles have generally been described as painless and non-irritating. One study found that microneedles caused essentially no pain other than the sensation associated with pressing against the skin during insertion (66). Another study similarly reported little or no pain, as well as little or no skin irritation, associated with microneedles (28).

CONCLUSIONS

In conclusion, a number of different microneedle delivery approaches have been investigated in vitro, in animals, and in humans. Poke-and-patch provides a simple method to enable sustained delivery of hydrophilic drugs and macromolecules from a transdermal patch. Coat-and-poke provides bolus delivery of sub-milligram doses in a patient-friendly manner that may replace some injections. Poke-and-release offers bolus or sustained delivery from microneedles that dissolve or degrade in the skin and then disappear. Poke-and-flow enables rapid and modulated delivery using a more sophisticated microneedle system. To achieve these goals, microneedles can be
designed not to break and not to cause pain or irritation. Overall, microneedles hold promise to be a widely useful method for minimally invasive delivery of drugs, proteins, and vaccines.

REFERENCES
