Clinical microneedle injection of methyl nicotinate: stratum corneum penetration

Raja K. Sivamani1,2, Boris Stoeber3, Gabriel C. Wu1,2, Hongbo Zhai2, Dorian Liepmann1, Howard Maibach2

1Department of Bioengineering, University of California, Berkeley, CA, USA, 2Department of Dermatology, School of Medicine, University of California, San Francisco, CA, USA and 3Department of Chemical Engineering, University of California, Berkeley, CA, USA

Background/purpose: In recent years, microneedles were proposed as a method to painlessly deliver drugs past the stratum corneum. Microneedles have been fabricated in several designs, but limited studies have tested microneedle injections in humans. In this work, we compare microneedle injections with topical application (TA) to investigate if microneedles enhance in vivo drug delivery past the stratum corneum.

Method: In vitro tests were used to measure microneedle pressures and injection volumes. In vivo microneedle injections were performed on the volar forearm of 11 healthy volunteers. Two sets of microneedles, pointed and symmetric, were used to develop microneedle/syringe apparatuses that were used to inject approximately 1 μL of 0.1 M methyl nicotinate, and were compared against TA. A Laser Doppler Perfusion Monitor was used to record maximum blood flow and the time to maximum blood flow at the treatment sites.

Results: Pointed and symmetric microneedle-injected sites showed a significantly faster time to maximum blood flow than TA. The pointed microneedle injections also resulted in a higher maximum blood flux. Volunteers reported feeling pressure but no pain from the microneedles during the injections.

Conclusion: The microneedles aid in bypassing the stratum corneum and enhance drug delivery through it. The design of the microneedle influences its delivery capabilities, because the pointed microneedles seem to be less susceptible to clogging during the injection.

Key words: microneedle – skin – in vivo – nicotinate – clinical

A MAJOR BARRIER to transdermal drug delivery has been the stratum corneum, the 10–15 μm-thick outer layer of skin. Traditionally, hypodermic needles have been used to pierce through this barrier and deliver various types of drugs, vaccines, and treatments. Although effective for drug delivery, a major drawback of hypodermic needles is that they cause pain and many patients have needle phobia (1, 2). However, recent applications of semiconductor fabrication techniques led to the development of microneedles.

Microneedles are designed to pierce and successfully deliver injections past the stratum corneum, but are too short to stimulate the pain nerves (3, 4). Microneedles exist in two basic designs: in-plane and out-of-plane (Fig. 1). In-plane microneedles have been integrated with circuitry, pumps, and sensors (5–7) and have been used in clinical tests to measure blood glucose levels (8). Reed and Lye (9) provide an extensive review of in-plane microneedle fabrication methods. In-plane microneedles are more easily integrated with electronic processes, but a disadvantage is that fabrication is restricted to one-dimensional arrays (9).

Out-of-plane microneedles have been fabricated in several designs. Solid microneedles have been shown to increase skin permeability (3). Hollow microneedles were subsequently developed so that fluid could be injected through them (10–13). Although most microneedles have been fabricated out of silicon, others have been fabricated from glass (10), metal (10), and polymers (10, 14). Stoeber and Liepmann (10) fabricated a microsyringe backing along with the microneedle array and measured in vitro dye injection depths of 75–100 μm. Some in vivo injection studies have been performed with out-of-plane microneedles but few human studies have been carried out. Kaushik et al. (4) carried out the
first human study to show that microneedles were painless upon insertion. Prausnitz (15) provides an overview of solid microneedle in vivo studies that investigate insulin permeability and vaccine delivery. McAllister et al. (11) used a single glass microneedle to inject insulin into diabetic rats and reduce their blood glucose by 70%. Gardeniers et al. (12) infused insulin into diabetic rats through a microneedle array and showed that it was comparable to subcutaneous insulin injection. Matsuda and Mizutani (14) implanted polymer microneedles for a month in rats to study the polymer degradation characteristics.

In this study, we performed a clinical injection study with hollow microneedles to investigate if they enhance percutaneous drug penetration.

Materials and Methods

Microneedles

Pointed and symmetric silicon microneedle arrays were fabricated by Stoeber and Liepmann (10) (Fig. 1A and B). Microneedles were glued to a syringe with Steel Hardner (J-B Weld, Sulphur Springs, TX, USA) (Fig. 2). Arrays consisted of eight evenly spaced microneedles.

In vitro

Microneedle injections were carried out in 200 μm-thick sections of dermatomed cadaver skin that was free of dermatological disease. The skin was stretched during the injection to reflect the in vivo tensile state of skin. Injections were carried out over 30 s at 6–10 psi to deliver about 1 μL.

In vivo

Volunteers

Eleven non-smoking volunteers with no pre-existing skin health conditions between the ages of...
18 and 39 (mean = 29 ± 7) years participated in the study. The study was approved by the University of California San Francisco (UCSF) Committee on Human Research and all volunteers gave their informed consent. Prior to testing, all volunteers were asked to rest for 20 min to allow them to acclimate to the testing conditions. The room temperature was 75°F and the relative humidity 50–70%.

**Experimental procedure**

Injections and topical applications (TA) of 0.1 M methyl nicotinate (Sigma, St Louis, MO, USA) were administered to the volar forearms. Four treatments were carried out with each patient: TA, pointed microneedle injection (PMn), symmetric microneedle (SMn), and microneedle control (MnC). The MnC refers to pressing an empty syringe/microneedle device on the arm. Each treatment was applied for 30 s over 1 cm².

Methyl nicotinate is a vasodilator that has been used in many studies of percutaneous penetration (16–21). Methyl nicotinate-induced dilation of surface capillaries has been shown to follow circadian rhythms (22) with the maximum being at about noon and the minimum being at about midnight. Because of this, the experiments were carried out at the same times each day to make the results comparable to one another. Measurement of the blood flow through the skin capillaries was measured with a Laser Doppler Perfusion Monitor DRT-4 (Moor Instrument, Devon, UK). Baseline blood flows of each volunteer were measured before application of the methyl nicotinate. In this way, each volunteer served as their own control.

**Results**

In vitro

Symmetric and PMn’s were carried out in cadaver skin. Pressure measurements were made near the microneedle exit orifices within the syringe. As the force on the plunger was increased, a resulting pressure built up inside syringe (Fig. 3). When the force was maintained constant, the pressure slowly declined (Fig. 3D) and was measured to be 0.001 suggesting that liquid was slowly exiting through the microneedles. However, once these microneedles were exposed to open air, the pressure no longer stayed constant under constant force and responded in a spiked response (Fig. 3). This suggests that the skin responds with a pressure that opposes the pressure placed on the microneedle syringe. This was seen especially in injections where the pressure was increased rapidly than when the pressure was gradually increased (data not shown).

**Discussion**

We have shown that hollow microneedles can bypass the stratum corneum during injections and deliver drugs to the skin capillaries.
Traditional hypodermic needle injections will continue to be advantageous in treatments requiring deeper penetration past the epidermis, like subdermal, muscular, or intravenous injections. However, hollow microneedles offer distinct advantages: they are painless and can reduce needle phobia in patients, are simpler to use than traditional injections, and can be integrated into devices for controlled, continuous drug delivery. Another advantage for hollow microneedles among transdermal drug delivery systems is the potential to inject large-sized protein formulations, such as sustained-release insulin formulations that range from 2 to 30 μm (24). The lumens of the hollow microneedle are 35–300 μm (10–13) and could successfully inject these formulations. Microneedles will be beneficial from a public health perspective too, because more people may be willing to receive vaccinations because of increased convenience and comfort of microneedle injection.

Pointed and symmetric microneedles

Pointed and symmetric microneedles differ in the placement of the needle lumen (Fig. 1A and B). The piercing and entry zone of the symmetric microneedle coincides with its lumen; in pointed microneedles, the lumen is offset from its piercing and entry zone. As a result, the symmetric microneedles may be more susceptible to clogging and have an increased resistance to fluid flow. Also, the pointed microneedle has a larger area for delivery and thereby lower resistance to fluid flow because of the elliptical structure of its lumen. This may explain why the symmetric microneedle-induced maximal blood flux was lower than the pointed microneedles (Fig. 5). Both are able to bypass the stratum corneum as reflected in the decreased time to maximum blood flux (Fig. 4), but the pointed needle may deliver more methyl nicotinate into the skin over the 30 s injection period. This suggests that hollow microneedles should be designed with a lumen different from the pierce and entry zone to maximize efficacy in drug injection.

Conclusions

Our results demonstrate that hollow microneedles are clinically effective in bypassing the stratum corneum for drug injections. The design of the microneedle can impact how quickly drugs can be introduced through the needles. Microneedles can serve as a painless alternative to hypodermic vaccine injection and to administer drugs that may normally be administered in a topical manner. Revolutionary continuous and controlled drug therapies can be made possible when pumps and sensors are mounted onto microneedle arrays.

Methyl nicotinate (MolWt = 137 g/mol; Log Poctonal/water = 0.81) is a convenient and widely used indicator in percutaneous penetration studies but other indicators should be explored in future studies. Methyl nicotinate induces vasodilation through several steps involving the release of prostaglandin D2 (25, 26). Future studies could employ other vasodilators that directly affect the capillary vessels, like histamine. Fluorescent substances could be injected to visualize distribution...
of the injection. Future investigations will help expand the knowledge of clinical microneedle injection characteristics and efficacy.

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References


Address:
Prof H. Maibach
Department of Dermatology
School of Medicine
University of California San Francisco
Box 0989, Surge 110
San Francisco, CA 94143-0989
USA

Tel: +1 415 476 2468
Fax: +1 415 753 5304
e-mail: himjlm@itsa.ucsf.edu