Evaluation of Microneedles in Human Subjects

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20.1 Introduction

Microneedles are micron-sized structures which create microscopic holes in the upper layers of the skin, thereby enhancing topical and transdermal delivery of therapeutic moieties. Since microneedles are minimally invasive, they usually do not stimulate nerve endings in the dermis, thereby offering a pain-free delivery system capable of administering small-molecule drugs, macromolecules, and vaccines. Based on their structural design, microneedles can be broadly categorized into solid and hollow microneedles, and they can be fabricated from a wide range of materials. As indicated by the literature on microneedles, a significant amount of data has been generated for a variety of microneedles from in vitro and in vivo animal studies pertaining to fabrication, characterization of microneedles, and drug/vaccine delivery (Kim et al. 2012; Pettis and Harvey 2012; van der Maaden et al. 2012; Donnelly et al. 2010; Sachdeva et al. 2011). However, certain aspects of microneedle technology such as pain associated with microneedles, patient and provider perceptions of this technology, safety concerns, and the efficacy of this technology in humans cannot be determined from animal studies. In recent years, several human studies and clinical trials have been performed to address these concerns and assess the efficacy of microneedles for drug and vaccine delivery in humans; these studies are discussed in this chapter.

20.2 Human Studies to Validate Microneedle Performance and Safety

20.2.1 Microneedle Insertion into the Skin

The most important function of microneedles is to overcome the barrier imposed by the skin's outermost layer, stratum corneum, thereby facilitating the delivery of drugs into the body. This requires that microneedles puncture across the stratum corneum and into the skin. Many studies have shown that successful skin penetration by microneedles depends on several factors including microneedle geometry, material, applied force, and insertion strategy (Davis et al. 2004; Bal et al. 2008; Coulman et al. 2011; Haq et al. 2009). For example, microneedle geometry is an important factor that determines the force required for insertion without needle breakage. To achieve safe and reliable microneedle insertion, the force inducing mechanical failure of microneedles should be much higher than the insertion force of microneedles, which mainly depends on the tip radius, tip angle, and ratio between needle height and base width (Davis et al. 2004; Bal et al. 2008). Davis et al. demonstrated successful insertion of metal hollow microneedles in human subjects and indicated that the margin of safety (ratio between the fracture and insertion force) could be maximized with hollow metal microneedles having a small tip radius and a large wall thickness (Davis et al. 2004). Also, the work done by Bal et al. suggested that longer and sharp-tipped microneedles could make a deeper insertion (Bal et al. 2008), and similar results were found in other human studies as well (Coulman et al. 2011; Haq et al. 2009). In general, safe and reliable insertion can be achieved by microneedles bearing a small tip radius, acute tip angle, and high aspect ratio.

Skin deformation is also a barrier to successful microneedle insertion. Most incomplete insertions due to skin deformation occur when the aspect ratio of the microneedles is small or the microneedle length is short (Coulman et al. 2011). Typically, skin deformation during needle insertion could be overcome by increasing the needle length, applying higher force/speed during insertion, or utilizing especially designed applicators that provide constant force and minimize skin deformation (Bal et al. 2008; Coulman et al. 2011; Haq et al. 2009; Daddona et al. 2011). Since the degree of deformation depends on the location on the body, it may be desirable to consider different needle designs and application strategies depending on the needle insertion site.

The assessment of skin penetration by microneedles can be performed by several methods. Histological analysis has been widely used

for in vitro and *in vivo* animal studies, but it is hard to be applied to human subjects because it requires skin excision. Also, it was reported that the dimensions of microchannels created by microneedles may be overestimated when visualized by histological techniques (Coulman et al. 2011).

Dye staining and electrical resistance measurements can indicate the disruption of the stratum corneum *in vivo* (Davis et al. 2004; Haq et al. 2009; Wermeling et al. 2008). In dye-staining studies, when the site porated with microneedles is stained with a dye such as methylene blue or gentian violet, only the disrupted areas of the stratum corneum are stained, thereby aiding in visualization of the created microchannels. Upon poration, the electrical resistance of the skin drops rapidly, and therefore these measurements can also be used to confirm successful barrier disruption. These methods, however, do not provide information about the three-dimensional penetration profile created by microneedles.

Transepidermal water loss (TEWL) measurements have also been used to evaluate the level of skin disruption (Bal et al. 2008; Haq et al. 2009). This method has been used in the cosmetics and dermatology industry to determine changes in skin barrier properties. Although TEWL measurement only provides the degree of skin damage in a qualitative manner, it is a useful tool to investigate the effect of microneedle geometries on skin disruption and resealing. Studies on human subjects have demonstrated that TEWL values dramatically increase upon microneedle insertion, and the highest TEWL values were obtained with longer needles and multiple treatments, indicating greater barrier disruption (Bal et al. 2008; Haq et al. 2009).

Recently, optical coherence tomography (OCT) has become an attractive way to investigate structural and biomechanical features of the skin. OCT is a noninvasive interferometric technique utilizing local optical backscatter for imaging and has been widely used in ophthalmology. Microchannels created by microneedles were successfully visualized using OCT, and the penetration depths ranged between 15 and 65 % of the full needle length depending on the needle geometry, needle arrangement and location, and insertion site (Coulman et al. 2011; Enfield et al. 2010), indicating that the biomechanical properties of a treatment site should be considered for effective skin disruption. It was also found that the width of the microchannel in the stratum corneum layer was approximately 50 times smaller than the width of the microneedle, thus implying that microneedle insertions are less invasive than predicted by histology results (Coulman et al. 2011).

20.2.2 Liquid Infusion Via Hollow Microneedles

Compared to drug delivery using solid microneedles, successful delivery of liquids via hollow microneedles is challenging because success relies not only on insertion of microneedles but also on issues associated with liquid infusion such as flow rate, volume, and leakage. Among various factors affecting infusion of liquids, management of leakage is of critical importance for successful liquid infusion using microneedles. To minimize leakage during infusion, microneedles should be long enough to ensure complete and secure insertion. However, the length of the needle should be short enough for minimizing pain associated with needle penetration.

Researchers have demonstrated successful liquid delivery into human subjects with microneedles varying in lengths from 0.5 to 3 mm (Laurent et al. 2007, 2010; van Damme et al. 2009; Gupta et al. 2011a, b, c). Laurent et al. reported that following infusion with a 1.5-mm-long, 30-gauge microneedles attached to a glass syringe which allows perpendicular insertion into the skin, more than 90 % of the injected liquid was deposited in dermal tissue, and a mean fluid leakage volume of 2–3 μL was observed (Laurent et al. 2007). Van Damme et al. demonstrated intradermal influenza vaccination using a silicon hollow microneedle array (450-μm tall, 1 x 4 array) (van Damme et al. 2009), and their immunogenicity data suggested that the leakage during injection was negligible, although they

did not describe the amount of vaccine left on the skin surface after injection.

Another source of leakage is the skin itself since it provides significant resistance to fluid flow. The dermal layer has a limited capacity for accommodating fluid, which causes an increase of pressure in the dermis during injection. Therefore, it is important to understand the relationship between the accumulated pressure at the injection site and injection parameters such as flow rate and volume. Gupta et al. demonstrated that infusion pressure increased as more saline volume was delivered into the dermal layer, and the infusion pressure was independent of the insertion depth (Gupta et al. 2011a, b, c). They performed the experiments with glass hollow microneedles of three different lengths (0.5, 0.75, and 1 mm), and the results indicate that the leakage due to backflow could be managed by adjusting the flow rate, as long as the microneedles are securely inserted into the skin. Interestingly, there was a point where the infusion pressure was stabilized at low flow rate (0.1 mL/min). This suggests that there exists a steady state where the incoming flow rate is equal to the outgoing flow rate from the injection site into the body and, further, encourages the possibility of microneedle-assisted delivery of large liquid volumes over a period of time without increasing infusion pressure.

This study also demonstrated that infusion pressure could be lowered by partial microneedle retraction using a custom rotary device, which required an infusion pressure less than half of that required when using nonretracted microneedles. The pressure did not increase significantly for the retracted microneedles at larger infusion volumes (>0.6 mL). In addition, the delivery of a large volume of liquid via microneedles could be facilitated by the use of hyaluronidase, which is an enzyme that degrades hyaluronic acid in the extracellular matrix of the skin, thereby allowing the accommodation of additional fluid in the dermis. For small volumes (<0.3 mL), however, the pressure required to infuse into the skin was not significantly affected by microneedle length, flow rate, retraction, and the use of hyaluronidase, implying that a secure microneedle insertion is the most important factor for successful liquid infusion using hollow microneedles in human subjects.

20.2.3 Pain

One of the core advantages of microneedles over traditional injections is reduced pain and thereby increased patient compliance. Studies by various research groups have helped quantify this pain reduction and describe the key design factors affecting pain.

20.2.3.1 Solid Microneedles

The first studies on pain after microneedle applications were done with solid microneedles. Kaushik et al. compared 150-μm-long microneedles in a 20 × 20 array to a 2-mm, 26-gauge hypodermic needle (Kaushik et al. 2001). Twelve participants were blinded for manual needle insertions, and pain scores were recorded. Microneedles resulted in significantly less pain, with a median pain score of 0/100 compared to a score of 23/100 for hypodermic needle. Similar findings were observed by Haq et al., where two types of silicon microneedle arrays, each containing 36 needles measuring 180- or 280-μm long, were compared to a 25-gauge hypodermic needle in 13 participants (Haq et al. 2009). Both microneedle designs were less painful than the hypodermic needle. The 280-μm-long microneedles were considered less painful than the 180-μm-long microneedles, but the investigators intentionally applied more force when administering the shorter microneedles in order to ensure complete insertion.

Pain associated with various microneedle designs was investigated by Bal et al. in 18 participants (Bal et al. 2008). Microneedle patches with 16 microneedles, varying in length from 200 to 550 μm, were compared; no hypodermic needle was used for comparison. There was no significant difference in pain between the different microneedle designs, although the 550-μm-long microneedles had the highest median and maximum pain scores.

In a more detailed study, Gill et al. investigated various microneedle designs to determine

which design factors affected pain (Gill et al. 2008). In this double-blinded study, 10 participants rated the pain of insertion associated with microneedles with lengths from 480 to 1450 μm, widths from 160 to 465 μm, thicknesses from 30 to 100 μm, tip angles from 20° to 90°, and array sizes from 1 to 50 microneedles. All microneedle designs tested were significantly less painful than a 26-gauge hypodermic needle, and microneedle length had the most significant effect on pain, followed by needle density, while width, thickness, and tip angle did not affect pain significantly over the range of parameters studied.

The primary takeaway message from these studies is that solid microneedle insertions universally result in less pain compared to hypodermic needles. Microneedle length is the primary factor affecting microneedle pain, but significant differences in pain among the various designs investigated did not appear until microneedle length approached 1 mm.

20.2.3.2 Hollow Microneedles

The use of hollow microneedles is mainly dependent on microneedle design and fluid flow parameters, which in turn affect pain associated with this mode of application. Gupta et al. investigated the effect of microneedle length, infusion volume, flow rate, needle retraction, and additional use of hyaluronidase on pain levels during infusion of saline in ten participants (Gupta et al. 2011a). Microneedle insertions were significantly less painful than hypodermic needle insertions regardless of length. Microneedle lengths from 500 to 1000 μm did not have a significant effect on infusion-related pain, except at a large infusion volume of 1.0 mL, where longer needles were reported to be more painful. Increasing the infusion volume also increased pain, while the flow rate did not affect pain significantly except at an infusion volume of 1.0 mL for which a sharp piercing pain was sometimes reported. Partial retraction of the microneedle prior to infusion was investigated to reduce infusion pressure by relieving tissue compaction caused by microneedle insertion. Partial retraction significantly reduced infusion pressure, but also significantly increased infusion pain, possibly related to increased fluid-mechanical micro-damage to the tissue due to increased fluid flow. Incorporation of hyaluronidase into the injection formulation significantly reduced pain for an infusion volume of 1.0 mL. Overall, when compared to intradermal infusion with a 26-gauge needle, microneedle infusions generally required greater pressure but caused less pain.

In another study with 645 participants, Laurent e t al. compared the pain associated with needle insertion and saline infusion for microneedle application (1.5-mm microneedle applied to the deltoid) versus the standard Mantoux technique for intradermal delivery (26-gauge, 3/8″ needle) (Laurent et al. 2007). Microneedle insertion was reported as pain-free for all participants; however, a faint burning-like sensation was sometimes reported during saline infusion. At least three additional studies have been reported that indicate hollow microneedle insertions as significantly less painful than hypodermic needle insertions (van Damme et al. 2009; Gupta et al. 2011a, c). For infusion of drugs through hollow microneedles, some studies have reported equal or greater VAS pain scores with the hollow microneedles compared to hypodermic needles (van Damme et al. 2009; Gupta et al. 2011a; Pettis et al. 2011a, b; Durando et al. 2012), while others reported significantly less infusion pain (Gupta et al. 2012; Prymula et al. 2012; Dhont et al. 2012), which probably depends on the degree of tissue deformation and micro-damage caused by different needle geometries and injection protocols.

Overall, insertion of solid and hollow microneedles can be done painlessly, but pain associated with infusion through hollow microneedles is variable and remains a potential source of discomfort for patients.

20.2.4 Safety

Microneedles have been shown to enhance the delivery of a wide range of molecules into the skin, including small molecules, peptides, vaccines, and plasmid DNA (Donnelly et al. 2010). However, in order to be clinically feasible

as a drug delivery technique, it is important that microneedle treatment is both well tolerated by patients and safe with regard to any potential local skin irritation and systemic effects.

Skin irritation is defined as a nonimmunological local inflammatory reaction that is usually reversible and can lead to erythema and edema (Bal et al. 2008). Cytokines from epidermal cells play an important role in skin inflammatory processes, and keratinocytes, which comprise 95 % of the epidermal cells, produce a variety of cytokines in response to barrier disruption (Williams et al. 1996). Therefore, physical barrier disruption by microneedles may also potentially induce an inflammatory reaction. The degree of skin irritation can be assessed by various noninvasive biophysical techniques including visual inspection of skin color (Gill et al. 2008; Van Damme et al. 2010) and scoring methods, such as chromameter (Noh et al. 2010), laser Doppler imaging (Ansaldi et al. 2012; Corsini et al. 2000), reflectance spectroscopy (Noh et al. 2010), and visual scoring (Van Damme et al. 2010). There are several factors that may potentially affect the safety of microneedles, which include the type of microneedles (solid or hollow), microneedle dimensions (length, width, thickness, tip angle, number of needles in an array), and materials the microneedles are fabricated from (metal, silicon, glass, or biodegradable polymers).

In 2001, Kaushik et al. carried out the first human study with 150-μm-long silicon microneedles in 12 male and female healthy volunteers aged 18–40 years (Kaushik et al. 2001). The areas of the skin treated with microneedles were visually inspected post insertions, and neither redness nor swelling was observed in all cases, suggesting that the microneedle treatment did not cause significant tissue damage or irritation. On the other hand, hypodermic needle insertions always led to appearance of blood at the insertion site.

In another study, skin irritation associated with application of solid and hollow metal microneedle arrays of various lengths (200, 300, 400, and 550 μm) was investigated by Bal et al. in 18 healthy volunteers aged 21–30 years using chromameter and laser Doppler imaging methods (Bal et al. 2008). The hollow microneedles used in that study resulted in less skin irritation compared to the solid microneedles, and the shape and length of the microneedles affected the degree of irritation. A higher degree of erythema and blood flow was observed for 400-μm-long microneedles compared to 200-μm-long microneedles. However, in all cases, the irritation was minimal and lasted less than 2 h.

Gill et al. investigated the safety of longer solid metal microneedles with lengths of 480, 700, 960, and 1450 μm in human volunteers (Gill et al. 2008). Redness was observed for all microneedle insertions, but the erythema decreased in 2 h. A tiny droplet of blood was observed at the insertion site after some insertions with the 1450-μm-long microneedles, while the shorter microneedles did not result in any bleeding. There were no signs of edema after all microneedle insertions.

The clinical safety of even longer, hollow, metal microneedles (1–3-mm long) was also evaluated in 66 healthy adult volunteers with ages ranging from 18 to 45 years by a visual scoring method (Laurent et al. 2010). No serious adverse events were reported in the intramuscular injection and intradermal injection (microneedles; BD Soluvia™ Microinjection System) groups. Local pain at the injection sites was frequently reported in the intramuscular group but never in the intradermal group.

The safety of microneedles fabricated from metal (Bal et al. 2008; Gill et al. 2008), silicon (van Damme et al. 2009), and glass (Gupta et al. 2009, 2011a, 2012) has also been reported. For metal microneedles with heights lower than 960 μm, no or minimal local irritation was observed, which lasted less than 2 h (Bal et al. 2008; Gill et al. 2008). Van Damme et al. reported that for injection using hollow, silicon microneedles with a height of 450 μm, local reactions at the insertion site were frequent among recipients, but these reactions were mild and invariably transient (van Damme et al. 2009). Similar findings were observed for injection using hollow, glass microneedles (500–900-μm long) as well, where very mild erythema or edema was observed in the skin, but this irritation did not appear to be associated with an inflammatory response (Gupta

et al. 2009, 2011a, 2012). In contrast, hypodermic needle insertions led to the presence of a drop of blood at the insertion site which was not observed for microneedle insertions.

Overall, compared with hypodermic needles, the use of microneedles is considered safe, owing to their small size and lack of significant damage to skin tissue and blood vessels, which means negligible pain, local irritation, or systemic reactions.

20.2.5 Skin Resealing

Following microneedle insertion, the time over which the created microchannels remain open is critical for optimal drug delivery. Ideally, the microchannels should remain open for the entire time when the patch or drug formulation is applied on the skin and should close soon thereafter to minimize risk of infection.

Skin resealing kinetics in humans was first extensively investigated by Gupta et al. using skin impedance measurements (Gupta et al. 2011b). Metal microneedles were inserted into the skin, and impedance measurements were monitored until they reached the baseline values indicating complete pore closure. Microchannels closed within 2 h of microneedle insertion when the treated site was left open to the environment. However, when the treated site was occluded, pore closure was delayed up to 3–40 h, depending on the microneedle geometry. Similar findings were observed by Wermeling et al. where, under occluded conditions, pore closure was reported around 30 h post insertion, as indicated by impedance measurements (Wermeling et al. 2008).

The effects of different microneedle lengths (500–1500 μm), dimensions (75- vs. 125-μm thickness; 200- vs. 500-μm width), and microneedle numbers (10 vs. 50) on pore closure were also investigated by Gupta et al. (2011b). The time for complete pore closure depended on the length of the microneedles, the number of microneedles, and the area of poration, which in turn characterizes the depth of poration and the degree of injury to the skin.

The lifetime of the pores and its effect on drug delivery was studied by Wermeling et al. (2008). In this study, six subjects were pretreated with 400 metal microneedles (620-μm long) followed by application of a naltrexone hydrochloride gel patch. Drug levels in the plasma indicated that pores were open for at least 48 h, and for 72 h in two subjects. Pharmacologically active drug levels were found in the plasma even at 72 h post patch placement. However, when skin resealing was investigated in another set of ten subjects who were treated with microneedles only, skin electrical measurements indicated that the pores remained open for up to 30 h. Therefore pores were open for at least 30 h, and the prolonged delivery of naltrexone up to 72 h could, for that reason, be attributed to a drug depot formation in the skin. This study shows the direct effect of pore lifetime on drug delivery.

Pore closure kinetics vary depending on the age of the subject. Kelchen et al. reported the micropore closure kinetics in 16 elderly subjects compared to control group. Data indicate longer time frames are required to restore skin barrier function, suggesting a longer window of opportunity for drug delivery in the elderly population (Kelchen et al. 2016).

In summary, following poration, pores close within a relatively short period of time when left open to the environment, thereby reducing risk of infection or other side effects. Pore closure can further be delayed by introducing occlusive conditions which may be beneficial for delivering drugs over extended periods of time. The time required for skin resealing is dependent on the dimensions, geometry, and number of microneedles applied; the age of the subject; and the degree of injury appears to determine the time required for complete skin resealing.

20.3 Human Studies of Drug and Vaccine Delivery

Drug delivery using microneedles can be achieved via different application modes. In the poke-and-patch approach, drug-free, solid microneedles are inserted into the skin creating microchannels, followed by application of a drug patch or drug formulation on the porated skin

site, which then allows diffusion of the drug from the patch or formulation into the skin. In the second approach, solid microneedles can be coated with the drug formulation; once these needles are inserted into the skin, the interstitial fluid in the skin dissolves the coating, thereby depositing the drug directly in the skin. In the third approach, the drug can be encapsulated into a biodegradable matrix of dissolving microneedles. Upon insertion, these microneedles dissolve, depositing the drug in the skin. Finally, liquid formulations can be infused into the skin using hollow microneedles. The physicochemical properties of the drug moiety, duration of delivery (bolus or extended periods of time), dosage, and dosing regimen are some of the factors that may determine the best mode of microneedle application.

20.3.1 Small Molecules

20.3.1.1 Naltrexone

Wermeling et al. used the microneedle pretreatment approach to investigate systemic delivery of naltrexone in a first-in-human proof-of-concept study for delivering skin-impermeable hydrophilic compounds (Wermeling et al. 2008). In this study, six healthy volunteers were treated with microneedles on the upper arm with 400 solid metal microneedles with a length of 620 μm and a base width of 160 μm. Following microneedle insertion, a patch containing naltrexone hydrochloride formulation was applied, and blood samples were taken at predetermined time points to monitor blood plasma levels of the drug. In the control group (n=3), where subjects were not pretreated with microneedles, delivery from the naltrexone patch over a period of 3 days yielded undetectable drug plasma levels, while pretreatment of the skin with microneedles resulted in steady-state plasma concentrations within 2 h of patch application, and the levels were maintained for at least 48 h. Transient erythema was observed in all cases, which was reported to be an effect of the drug formulation itself and not microneedle treatment. This study demonstrates the possibility of systemic delivery of hydrophilic compounds using the microneedle pretreatment approach.

20.3.1.2 Methyl Nicotinate

Sivamani et al. studied the clinical efficacy of hollow microneedles for drug delivery using methyl nicotinate, which induces vasodilation, as a model drug (Sivamani et al. 2005). Hollow metal microneedles with a length of 200 μm and a lumen diameter of 40 μm, with asymmetrically pointed or symmetric geometries, were inserted into the volar arms of 11 healthy volunteers, and 1 μL of 0.1-M methyl nicotinate was injected into the skin. Efficacy of microneedle-mediated delivery in comparison with topical application of the drug was measured by the change in blood flow using laser Doppler imaging. For both microneedle geometries, microneedle-mediated delivery resulted in a significantly faster increase in blood flow than for topical application, indicating that microneedles delivered the drug more efficiently to cause vasodilation. The pointed microneedles resulted in a higher maximum blood flux as compared to the symmetric microneedles, suggesting that the geometry of the needles can play a role in drug delivery. Microneedle treatment was well tolerated by the subjects, who reported a feeling of pressure during infusion but no pain.

20.3.1.3 Lidocaine and Dyclonine

Microneedle-mediated delivery of lidocaine, a local anesthetic, was investigated by Gupta et al. (2012). In this randomized, single-blind, within-subject study, lidocaine was infused into the forearms and dorsal hand sites of 15 healthy volunteers using 500-μm-long glass hollow microneedles. As a control, subjects also received lidocaine via the Mantoux injection method using a 26-gauge hypodermic needle. The pain associated with administration of microneedles and a hypodermic needle and the area and depth of numbness induced at different time points post insertions were measured by visual analog scale scoring. Microneedle treatments were reported as significantly less painful compared to hypodermic injections at both sites, with 77 % of subjects preferring microneedle treatment. Also, 80 % of the subjects indicated that microneedle treatment was not painful, suggesting better patient compliance with this approach. Both treatment methods resulted in a rapid onset of drug action and a similar

area and depth of numbness at the different time points tested, indicating the efficacy of microneedle-mediated delivery of lidocaine.

Li et al. reported successful delivery of dyclonine, another topical anesthetic agent, using a microarray consisting of 400 solid microneedles with a length of 70 μm (Li et al. 2010). In this randomized, double-blind study, 25 healthy volunteers were treated with the microarray on one forearm and a needle-free sham device on the other (negative control). A 1 % dyclonine cream was applied on the treated sites. The pain associated with an external stimulus applied at 5-min intervals over a period of 1 h was measured by visual analog scale scoring. Microneedle pretreatment resulted in a faster onset of action, thereby reducing the time for pain reduction, and it also resulted in a greater degree of pain reduction compared to the sham control.

20.3.1.4 Aminolevulinic Acid and Methyl Aminolevulinate

Mikolajewska et al. studied the combination of microneedles and photodynamic therapy for topical delivery of 5-aminolevulinic acid and methyl aminolevulinate, which have been shown to be effective for treating superficial basal cell carcinoma, actinic keratosis, Bowen's disease, and other dermatoses (Mikolajewska et al. 2010). In this study, 14 healthy volunteers were pretreated with 600-μm-long polymer microneedles, after which 5-aminolevulinic acid and methyl aminolevulinate creams with different concentrations of the actives were applied to the site for 4–24 h. Microneedle treatment increased the 5-aminolevulinic acid- and methyl aminolevulinate-induced protoporphyrin IX production, as indicated by increased fluorescence when exposed to red light. It was also reported that microneedle pretreatment did not affect pain during light exposure or erythema levels.

20.3.2 Peptides and Proteins

20.3.2.1 Parathyroid Hormone

Parathyroid hormone is a polypeptide that acts when calcium levels in blood are low. It increases blood calcium levels by bone resorption, increases absorption of calcium in the intestine and reabsorption of calcium in the kidneys, and has an anabolic effect on bone mineral density and new bone formation. Current parathyroid hormone therapy consists of daily subcutaneous injections to ensure bioavailability of parathyroid hormone. This frequent dosage regimen can be inconvenient for patients, and therefore a microneedle patch design consisting of solid microneedles coated with parathyroid hormone was explored as a more efficient and patient-friendly administration system.

Cosman et al. (2010) compared parathyroid hormone delivery of coated microneedles with subcutaneous injections. A cohort of 165 postmenopausal women subjects aged 50–81 years were administered parathyroid hormone daily for 6 months. Treatment with parathyroid hormone-coated microneedles resulted in a faster time to peak drug concentration and a shorter half-life compared to subcutaneous administration. The microneedle approach also resulted in higher patient compliance and no prolonged hypercalcemia. Daddona et al. (2011) also reported similar findings where the T_{max} (0.14 h) and mean terminal half-life (0.5 h) were shorter with microneedles than subcutaneous injection (0.4 h and 0.8 h for T_{max} and half-life, respectively), indicating that the absorption from the subcutaneous space is rate limiting and therefore determines the terminal decline in plasma concentration. The daily treatment of 20 μg of parathyroid hormone with coated microneedles for 6 months increased lumbar spine and hip bone mineral density comparably to subcutaneous injections with the same dose. Increasing the dose to 40 μg of parathyroid hormone using coated microneedles increased total hip bone mineral density by 1.3 % over subcutaneous injection and placebo.

Several clinical studies (Frolik et al. 2003; Hock et al. 1992; Kitazawa et al. 1991) suggested that the rapid onset and offset of parathyroid hormone level may favor anabolic effect rather than catabolic effect of parathyroid hormone, indicating that parathyroid hormone pharmacokinetic profile with these features can be critical in modulating biological effect of parathyroid hormone.

A future study is warranted to determine if the plasma profile created by a microneedle patch can induce greater effect of other anabolic pharmaceuticals compared to conventional injection.

20.3.2.2 Insulin

Diabetic patients with type I or type II diabetes mellitus on insulin therapy need to self-administer insulin on a daily basis by means of subcutaneous injections, insulin pens, or catheters connected to insulin pumps in order to maintain appropriate blood glucose levels. This mode of administration is associated with poor patient compliance due to fear of needles, pain associated with injections, and inconvenience, which often leads to poor diabetes management. Therefore, insulin delivery via other administration routes and enhancement techniques, including microneedles, has been investigated by several groups.

Gupta et al. (2009) investigated microneedle-mediated intradermal injection of Humalog® (insulin lispro, Eli Lilly, USA) in two type I diabetes subjects, as a first-in-human study. Hollow glass microneedles were inserted into the skin at various depths ranging from 1 to 5 mm to study the effect of insertion depth on insulin delivery. Optimal results were observed at an insertion depth of 1 mm, as reflected by rapid insulin absorption and reduction in glucose levels. This data indicates that uptake by the dermal (or possibly lymphatic) capillaries present in this heavily vascularized region results in faster pharmacokinetic and pharmacodynamic profiles compared to other microneedle insertion depths. Microneedle-mediated bolus insulin delivery (at 1-mm insertion depth) resulted in a significantly higher delivery compared to administration via a subcutaneous catheter (at 9-mm depth), suggesting better management of postprandial glucose levels with the microneedle approach. Similar results were observed in a follow-up study by the same group (Gupta et al. 2011c), where five type I diabetes subjects were administered bolus doses of lispro insulin using a 0.9-mm-long hollow glass microneedle and a 9-mm-long subcutaneous catheter. Microneedle delivery resulted in a faster onset of action and a more rapid achievement of euglycemia with similar relative bioavailability of lispro in the two delivery routes.

Pettis et al. (2011b) also investigated the dependence of insulin lispro absorption on the insertion depth using hollow metal microneedles. A rapid onset of action and a faster offset was achieved at insertion depths of 1.25 and 1.5 mm, which correspond to the papillary dermal region, which is rich in capillary and lymphatic network. The relative insulin bioavailability was not significantly different between intradermal and subcutaneous routes, although microneedles resulted in more rapid effects. The pharmacokinetic and pharmacodynamic profiles of microneedles can be altered by varying the microneedle length. In another study, Pettis et al. (2011a) compared fast-acting lispro and regular human insulin using intradermal (1.5 mm) and subcutaneous route (8 mm) in 29 type I diabetes subjects aged 18–55 years. The best postprandial glucose control was found with lispro insulin injected by intradermal or subcutaneous routes at 2 min before meal consumption. Regular human insulin (Humulin®, Eli Lilly, USA) administered by the subcutaneous route had the slowest and most extended absorption profile compared to all the other dosing schemes. Therefore, microneedle-mediated intradermal delivery of lispro and regular human insulin can confer a potential clinical advantage over subcutaneous administration with respect to rapid postprandial metabolic control.

Overall, microneedle-mediated intradermal injection of insulin and its analogs can provide rapid absorption kinetics compared to the subcutaneous route. This approach has potential in replacing the subcutaneous route of administration, which is the current standard for insulin therapy, thereby improving patient compliance and diabetes management.

20.3.3 Vaccines

The intradermal route for vaccination has been explored since the early 1940s with various antigens including influenza, smallpox, diphtheria, typhoid, rabies, and hepatitis B, demonstrating

effective immunizations. However, due to the lack of an appropriate delivery technique, it has been difficult to deliver vaccines efficiently and in a controlled manner into the intradermal space. With the advent of minimally invasive delivery systems such as microneedles in recent years, intradermal vaccination has been gaining interest, as this route enables delivery of the vaccine directly to the dermal and epidermal dendritic cells present in the skin, which might result in an even better immune response.

20.3.3.1 Influenza

Influenza is one of the leading causes of death worldwide, with 500,000 deaths occurring every year and most deaths occurring among the elderly (Thompson et al. 2003). Influenza can be effectively prevented by vaccination. The traditional approach to influenza vaccination is an intramuscular injection. This mode of administration has several disadvantages, such as the need for skilled healthcare practitioners to administer the vaccine, pain associated with injections, poor patient compliance, and risk associated with the need for a person to go to a healthcare facility/pharmacy to get the vaccine during an epidemic. To overcome these disadvantages, microneedle-mediated influenza vaccination is being actively investigated. The BD Soluvia® Microinjection System (Becton Dickinson, USA) was the first microneedle product to be approved in the European Union for intradermal influenza vaccination in 2009 and has since been approved in other parts of the world as well.

Holland et al. investigated the efficacy of microneedle-mediated intradermal injection of influenza vaccine compared to the intramuscular route in the elderly (older than 60 years) (Holland et al. 2008). Subjects were administered 15 or 21 μg of hemagglutinin (HA) per strain using the BD Microinjection System for the intradermal route and 15 μg of antigen per strain for the intramuscular route. Intradermal delivery resulted in a superior immune response compared to the intramuscular vaccination. This study indicated that the elderly, the population at highest risk and therefore with the greatest need for protection, can be effectively vaccinated against influenza using microneedles without the need for adjuvants.

Arnou et al. also studied intradermal vaccination in the elderly in a 3-year study with 3707 subjects aged 60–95 years (Arnou et al. 2009). Subjects were vaccinated with 15 μg of HA per strain via intradermal or intramuscular route, and four dosing schemes were tested: ID-ID-ID, IM-ID-ID, IM-IM-ID, and IM-IM-IM, over three consecutive years (ID, intradermal; IM, intramuscular). In year 1, intradermal vaccination induced significantly higher antibody responses and seroconversion rates for all three strains compared to intramuscular vaccination. In years 2 and 3, seroprotection rates were consistently higher for intradermal vaccination compared to intramuscular vaccination. The delivery route used for the first vaccination did not influence reactogenicity to intradermal vaccination in the subsequent year.

Several clinical studies have also indicated that intradermal vaccination with a hollow microneedle can result in superior immunogenicity compared to the intramuscular route with a lower dose, suggesting dose-sparing effects. In a study by Leroux-Roels et al., the seroprotection, seroconversion, and geometric mean antibody titers were all higher for the intradermal group vaccinated with 9 μg of antigen compared to 15 μg dosing with the conventional intramuscular vaccination route (Leroux-Roels et al. 2008). Van Damme et al. found similar results when comparing the immunogenicity profiles for vaccinations with 3 and 6 μg of antigen by the intradermal route with that of 15 μg of antigen by the intramuscular route (van Damme et al. 2009). Intradermal vaccinations were carried out using hollow, silicon microneedles, and the immune responses at both the lower doses were similar to that of the full-dose (15 μg) intramuscular vaccination, suggesting dose-sparing effects. In another study, Beran et al. found that intradermal vaccination with 3 and 6 μg of antigen in 1150 healthy volunteers aged 18–57 years was less immunogenic than the intramuscular route at 15 μg (Beran et al. 2009). However, an intradermal dose of 9 μg was comparable to the intramuscular route

(15 μg). Similar dose-sparing results were reported by Arnou et al. and Hung et al. as well (Arnou et al. 2010; Hung et al. 2012).

To investigate the superiority of the intradermal route of vaccination, Morelon et al. studied the efficacy of intradermal vaccination in renal transplant recipients who have shown a poor antibody response to prior intramuscular influenza vaccination (Morelon et al. 2010). They found that the patients vaccinated with 15 μg of antigen per strain via the intradermal route (n=31) had higher immune responses against all three strains than the patients vaccinated via intramuscular route (n=31) with the same dose, suggesting that the intradermal mode of vaccination enabled by microneedles may have added benefits for immunizing renal transplant patients.

Ansaldi et al. investigated the efficacy of intradermal vaccination using a hollow microneedle against circulating heterologous H3N2 influenza strains in subjects aged 60 and above (Ansaldi et al. 2012). After administration of influenza vaccine to 50 adults, the intradermal route of administration induced higher antibody titers than the intramuscular route and conferred a broader immunity with a higher cross-reactive response.

Overall, influenza vaccination with hollow microneedles has shown better efficacy in some clinical studies with additional benefits such as dose-sparing effects and increased patient acceptance, suggesting that the enhanced immunogenicity by intradermal vaccination may be the preferred route of administration for the elderly and patients with less immunity.

Most recently, a human study was carried out using a dissolving microneedle patch to administer trivalent influenza vaccine in comparison to subcutaneous injection of two vaccine doses administered 21 days apart (Hirobe et al. 2015). Local reactogenicity in the skin was seen in the form of transient erythema, purpura, and pigmentation, but there were no remarkable adverse systemic effects. Immunogenicity was similar in microneedle patch and subcutaneous injection groups. A phase 1 clinical trial of a different trivalent influenza vaccine administered by dissolving microneedle patch is also under way (Prausnitz et al. 2016), but results have not yet been reported.

20.4 Skin Needling Studies

Microneedles have also been used for cosmetic needling applications, which have been reported to improve skin texture, induce collagen production, treat acne-related scars, and hyperpigmentation. Needling is expected to trigger the release of growth factors that stimulate formation of collagen and elastin in the dermis, which helps in collagen induction therapy and healing scars (Aust et al. 2008).

Dermaroller (Dermaroller, Fresenheim, France) is a widely used microneedle device for microneedling cosmetic applications, although other companies make similar products. It is a handheld device with a roller head consisting of solid metal microneedles embedded on its surface. Devices with smaller microneedles are available for use at home, while the longer microneedles require a visit to a doctor's clinic. This device is indicated for collagen induction therapy and scar treatment.

In a recent study, 36 subjects with atrophic facial scarring were subjected to multiple microneedling sessions with a Dermaroller consisting of 1.5-mm-long microneedles (Majid 2009). Following treatment, the majority of the subjects observed a reduction in the severity of scarring, with 80 % of the subjects assessing their treatment as "excellent." Fabbrocini et al. found similar results in a study with 32 patients with rolling acne scars (Fabbrocini et al. 2009). Subjects were treated with Acnomega 100 (Merck, Switzerland), a topical product containing alpha and omega hydroxy acids, enoxolone, and zinc, for 3 weeks followed by treatment with a Dermaroller consisting of 1.5-mm-long microneedles. The Dermaroller was passed on the skin four times in four different directions to ensure an even pricking pattern. After only two microneedling sessions, the severity of the scars reduced greatly in all subjects, and the overall aesthetic improved. While immediately after

each microneedling session, the treated areas had redness and swelling which disappeared within 2–3 days; none of the subjects had any treatment-related side effects such as visible signs of the procedure or hyperpigmentation.

20.5 Patient and Provider Preference

20.5.1 General Opinion of Microneedles

To study the patient and provider opinions of microneedles, Birchall et al. conducted a focus group study with 27 patients and 31 healthcare providers (Birchall et al. 2011). The participants received a 5-min objective introduction to microneedle technology, and the ensuing discussions were assessed. The concept of microneedle technology was well accepted, with 100 % of patients and 75 % of providers indicating an overall positive impression of microneedles. The most frequently identified advantages of microneedles by the participants were reduced pain, reduced tissue damage, and benefit to patients who must take frequent injections.

Several other studies have also reported the general approval of microneedle-based influenza vaccine shots after use. Some were limited to participants who already chose microneedle administration over intramuscular injection and therefore provide limited information on patient approval (Dhont et al. 2012; Eizenberg et al. 2011). One controlled study showed similar preference among the elderly in Italy for microneedle-based intradermal injection versus intramuscular injection (Durando et al. 2012). Another study in adults in Europe showed significantly higher preference for microneedles in the elderly population, but significantly lower acceptance in the non-elderly adults (Reygrobellet et al. 2010). Among healthcare providers, 69–88 % expressed a preference for the microneedle injection for their patients (Durando et al. 2012; Dhont et al. 2012; Eizenberg et al. 2011; Reygrobellet et al. 2010; Arnou et al. 2011).

20.5.2 Willingness to Vaccinate with Microneedles

Studies on willingness to get vaccinated with hollow microneedle injections have been conducted with naïve participants and randomized controlled trials comparing microneedles and intramuscular immunizations. For naïve users, 60–74 % of those who normally prefer not to get vaccinated indicated that they would choose to be vaccinated if a microneedle option were available and recommended to them (Arnou et al. 2011). However, two large controlled studies with 6,500 participants showed that there is no significant change in willingness to get revaccinated between the microneedles and intramuscular groups (Reygrobellet et al. 2010). Thus, the naïve users strongly preferred the microneedle patch, but the subjects in the randomized controlled trials were ambivalent. The difference in these results may be explained by the effect of experience in the randomized controlled trial or the inclusion of normally unvaccinated participants in the naïve user study. It is unknown at this stage if microneedle-based vaccines significantly improve vaccination coverage among the general population.

20.5.3 Willingness to Self-Administer

In a focus group study, where groups of 6–14 physicians or members of the general public had an investigator-led discussion on microneedles, participants identified that microneedle patches may be easier to self-administer than intramuscular injections (Birchall et al. 2011). However, they expressed concerns about how one can confirm complete drug delivery after self-administration. One group examined immune response and patient preferences for self-administered microneedle-based influenza vaccines versus healthcare worker-administered vaccines (Coleman et al. 2012). The data indicate that 93 % of users could correctly self-administer the device on the first attempt, and both the experimental groups had similar immune responses. Members of the group that experienced self-administration of microneedles (BD Soluvia® System) were significantly more likely to accept

self-administration for future vaccinations than members of the group that experienced administration of the same device by a nurse.

This suggests that there may be a paradox for microneedle developers: patients may ultimately accept self-administration in large numbers, but they may be reluctant to try self-administration without experiencing it first.

20.5.4 Patient and Provider Concerns About Microneedles

The focus group study provided the most open-ended responses for potential concerns about microneedle use. Participants' concerns included efficacy of microneedles compared to standard modes of treatments, delayed onset, increased cost, reliable dosing, and narcotic and nefarious misuse of microneedles (Birchall et al. 2011). In other studies, side effects associated with microneedle administration were assessed. Patients reported a greater "bother" or physical discomfort at the injection site after influenza vaccination with a microneedle injection compared to an intramuscular injection, which is believed to be associated with the local immune response in the skin (Durando et al. 2012; Reygrobellet et al. 2010). These factors may affect patient perceptions and preferences.

Overall, patients and providers have a favorable opinion of microneedles. Microneedles may help improve vaccination coverage by encouraging more people to get vaccinated, and patients may be willing and able to self-administer microneedle devices. Participants are concerned about efficacy, increased cost, safe use within the community, and injection site reactions. The next few years will reveal if the preference pattern for microneedles seen in controlled studies will translate to acceptability in real use scenarios.

Conclusion

The study of microneedle technology has progressed from *in vitro* and animal studies into a growing set of human studies and clinical trials. Through proper design, microneedles have been shown to be inserted reliably into the skin, allow liquid infusion into skin, avoid pain, permit skin resealing after removal, and otherwise have a promising safety profile. Drug delivery studies have shown the ability of solid and hollow microneedles to administer a range of small-molecule drugs, as well as parathyroid hormone, insulin, and influenza vaccines. Patients and providers have generally viewed microneedles positively and expressed a willingness to self-administer medications using microneedles. Overall, human studies have built on preclinical findings to show that microneedles have great promise to improve efficacy, safety, and/or compliance with pharmaceutical therapies in human medical practice.

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