Clinical administration of microneedles: skin puncture, pain and sensation

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Abstract Injections using hypodermic needles cause pain, discomfort, localised trauma and apprehension. Additionally, careful use and disposal of needles is required to avoid transmission of blood-borne pathogens. As an alternative, microneedles can facilitate drug delivery without significantly impacting on pain receptors or blood vessels that reside beneath the skin outer layers. In this study we aim to determine the pain and sensory response to the application of wet-etch silicon microneedles, when used in such a way as to reliably penetrate skin, and provide a preliminary indication of how skin responds to microneedle injury with time. Twelve subjects received single-blinded insertions of a 25-G hypodermic needle and two microneedle arrays (36 needles of 180 and 280 μm height). The optimal method for microneedle application was determined in a pilot study. Pain intensity was scored using a visual analogue scale (VAS) and sensory perception determined using an adapted McGill Pain Questionnaire Short Form. Skin penetration was determined by external staining and measurement of trans-epidermal water loss (TEWL). Mean VAS scores, verbal descriptions and questionnaire responses showed that the 180 and 280 μm microneedles caused significantly less pain and discomfiting sensation in participants than the hypodermic needle. Methylene blue staining and TEWL analysis confirmed that microchannels were formed in the skin following microneedle application. Evidence of microchannel repair and resealing was apparent at 8–24 h post-application. In summary, this study shows that pyramidal wet-etch microneedles can penetrate human skin with minimal pain and sensory discomfort, creating transient pathways for potential drug, vaccine and DNA delivery.

Keywords Microneedle · Pain · Sensation · Visual analogue scale · Clinical trial

1 Introduction

Over 15 billion injections are given worldwide each year, with at least 40 million curative or therapeutic injections being administered globally each day (WHO 1999, 2004). With only 5% of injections being used in prophylactic immunisations, the vast majority are prescribed for therapeutic medical interventions. Whilst injections using hypodermic needles are reliable and effective, they cause pain and discomfort and a considerable degree of apprehension in young and vulnerable patient groups. Indeed, patients suffering from needle-phobia, or “belonephobia”, commonly avoid seeking medical and dental assistance due to their fear of injections (Kleinman 1994; Fredrikson et al. 1996; Marks 1988; Ost 1992; Milgrom et al. 1997; Nir et al. 2003). Needle use also carries the inherent risk of transmission of blood-borne pathogens from patient to patient where needles are re-used, or from patient to health care worker. In some countries the likelihood of cross infection with needles is further exaggerated where insuf-
icient resources are available for consistent and effective disposal and where needle re-use may be unavoidable due to economic or supply constraints or necessity. In particular, the use of needles and syringes for delivering vaccines creates key challenges for immunisation programmes in developing countries including stability, transport and storage of the vaccine in liquid form, the requirement for trained clinicians to administer injections and sharps disposal. Other potential disadvantages of injections include hypersensitivity, lipohypertrophy, bruising and bleeding at the site of administration. It is also debatable whether localised injections are refined enough to deliver the medicament or antigen consistently to the most appropriate site to exert maximum effect. Clearly, conventional injection practices, whilst effective, are crude and invasive.

Transdermal drug delivery (TDD) is a proven patient choice, providing the potential for improved user convenience and compliance and controlled release of medicament for enhanced duration of action and reduced side effects (Hutin et al. 2003; Barry 2001). As the skin plays a definitive role as a protective barrier however, only a very limited number of drug candidates are able to penetrate through the skin and therefore TDD is currently not an option for most applications. To this end, various strategies for facilitating the transcutaneous delivery of a larger range of therapeutics have been developed. These include electro- poration, ultrasound, thermal ablation, iontophoresis and skin bombardment (Barry 2001; Coulman et al. 2006a; Asbill et al. 2000; Denet et al. 2004; Dreher et al. 2005).

Whilst demonstrating proof-of-concept, these methods all require complex and relatively expensive equipment and have yet to make a significant clinical impact.

The last 10 years has seen the development of minimally invasive needle devices, microneedles, that provide a new method for delivering medications and vaccines into and through skin (Coulman et al. 2006a; Henry et al. 1998; McAllister et al. 2000; Prausnitz 2001; Birchall et al. 2006). Microneedles are designed specifically to penetrate the outermost skin barrier layer, the stratum corneum (SC), creating transient pathways for transcutaneous delivery. It is purported that microneedles can facilitate drug delivery through SC interruption without necessarily stimulating the pain receptors or blood vessels that reside beneath the skin outer layers (Henry et al. 1998; McAllister et al. 2000; Kaushik et al. 2001). Kaushik et al. (2001) measured pain response to an array of 400 microneedles of approximately 150 µm length (Kaushik et al. 2001). The authors showed that pain response following microneedle application was statistically indistinguishable from application of a smooth surface and statistically inferior to pain response following insertion of a 26-gauge hypodermic needle. In this study pain was measured using a visual analogue scale, which is an efficient, reliable and validated tool for scoring subject pain, but provides limited information on sensation or overall assessment of the human perception of microneedle application. Importantly, this study did not attempt to simultaneously demonstrate both microneedle functionality and pain response as there was no in vivo assessment during the study to verify skin puncture due to microneedles.

Sivamani et al. (2005) also assessed pain when comparing in vivo human injections of 1 µl methyl nicotinate using 200 µm length hollow microneedle arrays to topical application. The data revealed increased blood flux post application of microneedles whilst comments from the volunteers describe the application of microneedles as a feeling of “pressure but no pain” (Sivamani et al. 2005). Shirkhanzadeh (2005) reported that microneedles coated with porous calcium phosphate would appear to be well tolerated in human subjects although pain scoring was not performed (Shirkhanzadeh 2005). Miyano et al. (2005) reported that detachable, biodegradable microneedles of 500 µm length manufactured from maltose do not cause any pain on skin insertion (Miyano et al. 2005). Further reports suggest that microneedles do not cause any significant pain when used for extraction of interstitial fluid or blood for glucose monitoring (Smart and Subramanian 2000; Wang et al. 2005).

Pain is subjective and has several dimensions and therefore, is hard to clinically measure or quantify. Any measurements of pain are dependent on how a person views and communicates the pain they are feeling and this is individual to them. A reliable device for assessing pain will be able to measure pain consistently without being affected by minor changes in environment, administration or circumstances, yet would identify if the experienced pain was to change. One such device is the McGill Pain Questionnaire (MPQ) which contains a visual analogue scale (VAS) to measure pain intensity, certain key descriptor words and an evaluative index using key words such as mild, discomforting and excruciating (Melzack 1975). The MPQ measures dimensions of pain quality by including a set of descriptor adjectives, an intensity scale and pain drawing. The MPQ is one of the most widely used of all pain measurement tools, it has been shown to be highly reliable, is able to measure multidimensional aspects of pain and provides both quantitative and qualitative data (Kahl 2005). It is also important however, not to use a measurement tool that is overly time consuming to administer, making it impractical to employ in a clinical setting. One of the disadvantages of the MPQ is that it takes at least ten minutes to administer, which in some studies is not viable. To overcome this, Melzack developed a short-form version (MPQ-SF), which uses key adjectives from the longer MPQ (Melzack 1987; Collins et al. 1997). As shown by Melzack and Katz (1994), the
MPQ-SF provides a reliable and valid method for assessing the qualitative nature of an individual’s pain experience (Melzack and Katz 1994).

In this study we aim to utilise these tools to determine whether the wet-etch silicon microneedles we have previously used in our ex vivo studies (Pearson et al. 2008; Birchall et al. 2005; Coulman et al. 2006b) elicit pain on application to human volunteers. Uniquely, we will simultaneously confirm that the solid microneedles have been applied appropriately for clinical use, i.e. that they have been used in such a way as to reliably penetrate the stratum corneum. Our results also provide distinctive data for sensory perception on microneedle application and a primary indication of how skin responds to microneedle injury with time. We include details of a preliminary study that was performed to confirm that the data obtained in the clinical study relates to pain, sensation and injury arising from the microneedles themselves rather than the microneedle applicator.

2 Materials and methods

2.1 Materials

Methylene blue dye was from Fisher Scientific UK, Loughborough, UK. Dulbecco’s modified Eagle’s medium (DMEM; in 25 mmol l⁻¹ HEPES) and fetal bovine serum were from Invitrogen Ltd, Paisley, UK.

The microneedle arrays used in this study were provided by The Tyndall National Institute, Cork, Ireland. These platinum-coated "wet-etch" manufactured silicon microneedles have been shown to be of appropriate dimensions to create microchannels, approximately 50 μm in diameter, extending through the stratum corneum (SC) and viable epidermis (Wilke et al. 2005). Wet etching using potassium hydroxide (KOH) provides a method of mass production with lower fabrication costs than dry etching (McAllister et al. 2000; Birchall et al. 2005; Wilke et al. 2005). The process starts with a standard silicon wafer which is coated with silicon nitrids on a silicon oxide layer. Square shape patterns are transferred into the masking double layer by standard photolithography. After lithography, the patterned silicon wafer is etched using a 29% KOH solution at a temperature of 79°C. The needle formation is based on convex-corner undercut. The silicon wafers are subsequently coated with a thin (0.3 μm) layer of platinum. In this study the microneedle arrays comprised 36 pyramidal shaped microneedles of either 280 or 180 μm length with a base diameter of approximately 180 μm. The needle tips can be less than 1 μm wide. The microneedle arrays were characterised and checked for damage prior to use in any procedure using scanning electron microscopy (SEM; Fig. 1).

Aluminium applicator rods used in preliminary studies were supplied by Professor David Barrow at the Cardiff School of Engineering.

3 Methods

3.1 Preliminary study on microneedle application method

3.1.1 Design of microneedle applicators

Seven very simple and basic microneedle applicator designs were considered. The applicator designs were:

(a) Inverted 2 ml plastic syringe with the plunger surface heated and smoothed to remove any protrusions

![Fig. 1](image1.png) Each of the 36 pyramidal 180 and 280 μm length microneedle arrays were characterized by SEM. Each array was inspected for damage (left) and each needle clearly observed for defects in its structure (right). The left image shows a complete 280 μm microneedle array, and the right image is a single magnified 180 μm microneedle.
3.1.2 Ethical and consent approval

For the collection and use of excised human skin ethical approval was obtained from the South East Wales Local Research Ethics Committee. Skin samples were obtained from redundant skin in mastectomy or breast reduction specimens from women who had given their informed consented.

3.1.3 Study design for pain assessment

Participants (n=13) were recruited after obtaining written informed consent to have different applicators applied to the inside of their forearms. Initially, as a control, participants received a single application of unmodified cylindrical and square aluminium rods. Subsequently, the modified applicators were applied single-blind and randomised to either left or right forearm. The pressure of application was kept consistent for each applicator. After the application a simple pain questionnaire was administered. Recovery time of at least 1 h was provided between applications, in case previous applications had increased the sensitivity of the forearm. Verbal descriptions of the application from the volunteers were recorded during the application process and transcribed verbatim.

3.1.4 Ex vivo testing of applicators

Full-thickness human breast skin was obtained from mastectomy or breast reduction. Skin was collected from a variety of donors ranging from 45 to 65 years of age. The skin was frozen at −20°C and defrosted for 2 h before use. The two least painful applicators, as determined from
subject pain questionnaire, were tested ex vivo for skin puncturing efficiency. Araldite adhesive was applied to the top of the applicators and a microneedle array (in this case 49 microneedles of 250 μm length) bonded in a central position. Three different skin application techniques were tested:

1. Applying the applicator in a single rolling motion, whereby the applicator is placed at an angle of approximately 45° to the skin surface and rotated firmly forward through an angle of 90°, finishing at approximately 135°
2. Pushing the applicator vertically onto the skin, gently rotating the applicator for 10 s before removal
3. Punching the applicator vertically onto the skin with force, holding down for 10 s and then lifting off

Following application skin samples were treated topically with 10% methylene blue dye solution for 5 min before the excess dye was wiped from the skin surface. The samples were viewed under a light microscope.

3.2 Clinical study on microneedle pain, perception and skin puncturing

3.2.1 Ethical and consent approval

Ethical approval for this study was obtained from the South East Wales Local Research Ethics Committee. Written informed consent was obtained from healthy volunteers in adherence with the principles of the Declaration of Helsinki.

3.2.2 Study design

Participants (n=12) received single-blind applications of two types of microneedle array (36 pyramidal microneedles of either 180 or 280 μm height) and a 25-gauge subcutaneous hypodermic needle, injected at an angle appropriate for sub-cutaneous injection. Figure 3 shows the hypodermic needle in scale with one of the microneedle arrays. Each of these three applications were given to each buttock. The buttock was used as 6 mm skin biopsies were subsequently taken for further study. Applications of needle devices on one buttock were used to assess pain, perception and skin puncturing whereby the assessor was also blinded to the application device being administered. The second application was used to assess transepidermal water loss (TEWL) from the skin.

Following application to the human subjects each microneedle array was carefully examined by SEM for morphological changes. We observed no structural damage to any of the arrays or to any individual microneedles. The puncture sites on each patient were also carefully examined by dermatoscopy post-application and no microneedle or microneedle array artefacts were visible.

3.2.3 VAS and sensation questionnaire

The pain intensity rating was taken immediately after application of each needle using an electronic sliding VAS whereby the participant moved a slider along a 10 cm slide where one end represented “No Pain=0 cm” and the other end the “Worst Pain Imaginable=10 cm”. The slider was set to 0 cm at prior to each reading. The device does not disclose to the participant their rating, although a digital display viewed only by the assessor shows the rating for recording.

The perception questionnaire is based on the McGill Pain Questionnaire Short Form (MPQ-SF). It contains the four main assessment points of the MPQ-SF and three additional words (pressing, pricking and cold) taken from the long form MPQ, which unpublished research within our
Table 1 Summary of oral comments recorded during and immediately post-application of prototype applicator designs

<table>
<thead>
<tr>
<th>Applicator</th>
<th>Summary of transcribed comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Does not feel sharp on the skin. Pressure can be felt but no pain. Could offer advantage over both original applicators</td>
</tr>
<tr>
<td>B</td>
<td>Causes less sharpness than the square applicator but feels very similar to the round applicator. Therefore, although it offers advantage over the square applicator it is probably not significantly better than the round applicator</td>
</tr>
<tr>
<td>C</td>
<td>Similar to applicator B, causes less sharpness than the square applicator but feels very similar to the round applicator. Therefore, although it offers advantage over the square applicator it is probably not significantly better than the round applicator</td>
</tr>
<tr>
<td>D</td>
<td>Feels the same as the round applicator therefore would offer no advantage over it</td>
</tr>
<tr>
<td>E</td>
<td>Feels soft on the skin. Causes no pain or does not feel sharp. Could offer advantage over both original applicators</td>
</tr>
<tr>
<td>F</td>
<td>Cannot really feel the applicator on the skin. Causes no pain. Could offer advantage over both original applicators</td>
</tr>
<tr>
<td>G</td>
<td>Similar to applicator F, causes no pain and could offer advantage over both original applicators</td>
</tr>
</tbody>
</table>

group have shown to be of specific relevance in this study. The formatting and presentation of the MPQ-SF was designed to ensure reliability and validity of the data collected. To prevent leading of the participants the “Pain Rating Index” of descriptor words was relabelled the “Sensation Rating Index” as this provided a truer context of what was felt during device application.

3.2.4 Transepidermal water loss (TEWL)

TEWL was measured to determine the level of disruption to skin barrier function following application of the hypodermic needle and microneedle arrays. A Tewameter TM 210 (Courage and Khazaka Electronic GmbH, Köln, Germany) was used to measure TEWL at a control site as well as the three sites pre- and post-application of needle devices. The TEWL probe is a delicate measuring device, and was protected from shock, dirt, manual contact and liquids, using a cap when not in use. Before TEWL measurement each participant rested for 15 min to acclimatise to the ambient room temperature and relative humidity, which were maintained at 22°C and 45±5%, respectively. TEWL measurements were taken by carefully resting the TEWL probe horizontally on the application site, with the probe head vertical and perpendicular to the skin. The probe was held in place using a clamp stand to prevent any interferences arising from hand movement or heating of the probe by the assessor. Once the participant was comfortable, a reading was taken over 3 mins. The presented values represent the mean TEWL reading for the 20 s prior to stopping the measurement. If there were any uncharacteristic spikes during this period a more representative 20 s reading was used. TEWL readings were taken 1, 4, 8 and 24 h after application of the needles 20 µl of methylene blue stain was applied to the needle treated skin surface. The stain was left in place for 10 min before excess stain was removed using ethanol wipes. The sites were then visually assessed through the dermatoscope and a photographic image recorded (Canon IXUS 500 digital camera; Canon, UK).

3.2.6 Statistical analysis of results

Non-parametric Wilcoxon signed rank test was performed using Prism GraphPad. Statistical significance was shown when \( p < 0.05 \) and applied to both the VAS and TEWL data.

4 Results

4.1 Preliminary study on microneedle application method

The method of application of microneedles will be key to efficient and painless penetration through the skin barrier.

Fig. 4 Methylene blue staining of ex vivo human skin. A 49 microneedle array of 250 µm microneedle length was mounted onto the inverted syringe applicator and applied to human skin. The presence of blue staining confirms successful interruption of the stratum corneum barrier.
layer. A preliminary study assessed pain response and puncturing efficacy of different applicator designs prior to the clinical study. Seven potential basic applicator designs (Fig. 2) were investigated. A summary of oral comments post-application of the applicators are presented in Table 1.

As shown in Table 1, some of the applicator designs were less painful on administration than the applicators commonly used in laboratory studies, i.e. cylindrical and square-end metal rods. Figure 4 confirms that one of these applicator designs, i.e. the inverted syringe (A), was able to efficiently penetrate human skin when mounted with a microneedle array. Similar results were obtained with the foam applicator (E). Whilst Table 1 suggests that designs F and G are also suitable from a pain perspective, the ex vivo skin tests revealed that these applicators were not as efficient as penetrating human skin due to the cushioning effect of the rubber and/or foam mounting. Ex vivo testing also showed that the first method of application was shown to be optimal for applying the microneedles, i.e. applying the applicator in a single rolling motion.

Based on this simple pilot study the foam applicator and the syringe applicator were shown to be most appropriate for use in the clinical study. As the syringe applicators are easier and less costly to obtain and are supplied sterile, these applicators were selected for application of microneedles to human volunteers (Fig. 5).

4.2 Clinical study on microneedle pain, sensation and skin puncturing

The 12 subject clinical study firstly aimed to test the pain response against single-blinded application of microneedle arrays of two different microneedle heights (mounted on the reverse of a syringe barrel) and a hypodermic needle. The mean VAS pain scores following application of each ‘needle’ treatment show that the 180 and 280 μm microneedles caused significantly less pain ($p=0.027$ and $p=0.0005$ respectively) in participants than the hypodermic needle [Fig. 6(a)]. The 280 μm microneedles were perceived to be significantly less painful than the 180 μm microneedles ($p=0.039$). Individualised data in Fig. 6(b) further show that the hypodermic needle was always more painful than either microneedle device whilst 11 of the 12 participants found the 180 μm microneedles to be more painful than the 280 μm microneedles.

![Image](image-url)
Table 2  Ranking of reports of pain and discomfort following device applications

<table>
<thead>
<tr>
<th>Participant (order of application)</th>
<th>Least Pain/discomfort</th>
<th>Middle Pain/discomfort</th>
<th>Most Pain/discomfort</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (280/hypo/180)</td>
<td>180</td>
<td>280</td>
<td>Hypo</td>
</tr>
<tr>
<td>2 (hypo/280/180)</td>
<td>180</td>
<td>280</td>
<td>Hypo</td>
</tr>
<tr>
<td>3 (hypo/180/280)</td>
<td>180/280</td>
<td>–</td>
<td>Hypo</td>
</tr>
<tr>
<td>4 (280/hypo/180)</td>
<td>180</td>
<td>280</td>
<td>Hypo</td>
</tr>
<tr>
<td>5 (180/hypo/280)</td>
<td>180</td>
<td>280</td>
<td>Hypo</td>
</tr>
<tr>
<td>6 (280/hypo/180)</td>
<td>180</td>
<td>280</td>
<td>Hypo</td>
</tr>
<tr>
<td>7 (180/280/hypo)</td>
<td>180/280</td>
<td>–</td>
<td>Hypo</td>
</tr>
<tr>
<td>8 (280/180/hypo)</td>
<td>180</td>
<td>280</td>
<td>Hypo</td>
</tr>
<tr>
<td>9 (180/280/hypo)</td>
<td>180/280</td>
<td>–</td>
<td>Hypo</td>
</tr>
<tr>
<td>10 (180/hypo/280)</td>
<td>180</td>
<td>280</td>
<td>Hypo</td>
</tr>
<tr>
<td>11 (hypo/180/280)</td>
<td>180/280</td>
<td>–</td>
<td>Hypo</td>
</tr>
<tr>
<td>12 (280/180/hypo)</td>
<td>180/280</td>
<td>–</td>
<td>Hypo</td>
</tr>
</tbody>
</table>

Ranking deduced from audio-recorded comments made by participants both during and after the application of each device.

Oral comments confirmed that every participant found the hypodermic to be the most painful and uncomfortable of the three applications. Five of the 12 participants felt that treatment with the 180 μm microneedle array was slightly more discomforting than the application of the 280 μm microneedle array as clearly explained by participant 6: “Third one (180) felt as if it was being pressed down harder”, “The first one (280) was like someone holding onto your arm”. The hypodermic needle was immediately highlighted as the most painful and uncomfortable, as explained by participant 5: “definitely the most painful”. Table 2 summarises the level of pain/discomfort felt by

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![Fig. 7 Sensory evaluation following needle application. Each participant was asked to complete an adapted SF-MPQ to rate the sensations experienced during application of the 180 μm length and 280 μm length microneedle arrays and 25-G hypodermic needle. These data show the percentage of participants who rated each sensation occurring as mild (empty square) or moderate (filled square)](image-url)
each participant following each needle application. The order of needle application, from the randomisation schedule, did not affect the participants’ description of differences in pain and discomfort.

In addition to perceived pain this study further explored the sensation following microneedle and hypodermic needle treatment. Collated responses to the sensation questionnaire are shown in Fig. 7. Overall a greater number, variety and severity of sensations were experienced when participants were treated with the hypodermic needle when compared with either microneedle array. Participants felt greater ‘sharp’ and ‘stabbing’ sensations when the hypodermic needle was applied and more ‘pressing’ and ‘heavy’ sensations when the microneedles were applied.

Allied to pain and sensory response an important arm of this study was to demonstrate that the microneedle arrays had been applied in such a way as to penetrate the skin. This allows for meaningful comparison of pain response using an application procedure that is relevant, that is, using an application method and pressure that is shown to work in human skin. Monitoring the presence of skin punctures also provides a preliminary indication of skin healing following microneedle insult. The application sites of the two different microneedle array designs and the hypodermic needle used in pain and sensory measurements were subsequently stained using an external application of methylene blue dye. The stain was applied to the skin surface at 1 hr, 4 hrs, 8 hrs or 24 hrs after initial ‘needle’ application. Methylene blue staining clearly showed that microchannels are formed in the skin following application of the microneedles (Fig. 8). A larger puncture mark is observed when a 25-G hypodermic needle is applied and in this case, unlike microneedle treated skin, bleeding and erythema is also apparent. Whereas methylene blue staining plainly shows that the hypodermic injury is still amenable to staining at 24 h, evidence of microchannel repair and rescaling following microneedle application is apparent at 8–24 h post-application. Generally the 280 μm length microneedle array was more effective at skin penetration than the 180 μm length microneedle array with a mean of

Fig. 8 Skin puncture marks for each needle device as observed with time following methylene blue staining. The blue dye was externally applied to the sites of puncture at 1, 4, 8 and 24 h after needle use. (180) 180 μm microneedle array, (280) 280 μm microneedle array, (Hypodermic) 25-G hypodermic needle.
96% of the individual pyramidal needles puncturing the skin in participants (shown at 1 h staining) compared to a mean of 50% for the 180 μm length microneedle array.

Water loss from the skin is restricted by the SC layer. Transepidermal water loss (TEWL) measurement is a standardised method of determining changes in skin barrier properties, being frequently used in the cosmetics and dermatology industry (Treffel et al. 1994; Rosado et al. 2005; Aramaki et al. 2002; Schwindt et al. 1998). High TEWL values correspond to damaged skin whilst low TEWL correlate to healthy undamaged skin. The measurement of TEWL in this study was used to provide information concerning the compromised integrity of skin epidermis following application of the microneedle and hypodermic needle treatments.

Following skin puncture for pain, sensory and staining analysis, each participant received a second application of each of the 180 and 280 μm microneedle arrays and the 25-G hypodermic needle. TEWL was measured immediately after application and at three further intervals over a period of 24 h. In accordance with the external staining data, TEWL measurements further demonstrated perturbation of the SC barrier following needle treatments with water loss increasing post-application of each device (Fig. 9). Mean TEWL increased significantly (P<0.05) immediately post-application for all applications: from 5.1 (SD=3.8) to 8.8 (SD=5.4) g H₂O m⁻² h⁻¹ for the hypodermic needle; from 5.9 (SD=4.0) to 7.9 (SD=2.8) for 180 μm microneedles; and from 5.7 (SD=3.4) to 10.3 (SD=13.0) for the 280 μm microneedle array. In each case skin water loss recovered to baseline within 24 h with their being no significance (P>0.05) for any device when compared to control.

Figure 10 allows comparison of the percentage of puncture marks observed via methylene blue staining in microneedle treated skin with the TEWL readings at various timepoints. It is evident that as the number of distinct puncture marks reduces over the 24 h period the TEWL values normalise accordingly.

5 Discussion

In total seven designs of applicators were pre-tested, without any microneedles mounted, to ensure that the applicator itself would not bias the pain response data in the subsequent clinical study. Further, laboratory tests were performed to ensure that selected designs, once mounted with microneedle arrays, were able to puncture excised human skin. Our results showed that the simplest applicator design, i.e. the reverse end of a 2 ml plastic syringe, was the most appropriate for use in the clinical study from pain, accessibility, ease of use and skin penetration perspectives. Ex vivo testing also showed that the pyramidal micro-

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Fig. 9 Mean values (n=12) for trans-epidermal water loss (TEWL) at 1, 8 and 24 h post-application of (a) 25-G hypodermic needle (triangle), (b) 180 μm microneedle array (cross) and (c) 280 μm microneedle array (circle), each compared to control (square).

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needles penetrated skin more effectively and reproducibly if applied in a single rolling motion rather than by downward vertical pressure. This indicates that these arrays pierce skin more efficiently when the force is distributed over one row at a time, rather than over the whole array. This is not surprising given the likely ‘bed-of-nails’ effect resulting from vertical application and the fact that similarly dimensioned microneedles mounted onto a cylindrical drum have been shown to effectively penetrate outer skin layers for cosmetic and medical applications (Dermaroller SARL, France).

A 12 subject human clinical study was used to explore whether clinically untested wet-etch silicon microneedles elicit pain when used in such a way as to reliably penetrate the stratum corneum, and compare microneedle pain response with that following insertion of a 25-G small hypodermic needle. VAS scores showed the hypodermic needle to be significantly more painful on insertion than either 180 or 280 μm microneedle arrays. This was further substantiated by descriptive comments and a sensation assessment from each participant. The fact that the participants scored and described the 180 μm microneedles to be more painful than the 280 μm microneedles may initially appear to be contradictory. This result is however perfectly understandable when you consider how the microneedles were used in this study. Uniquely, this study focuses on the experienced pain response to microneedle use when they are applied in such a way as to ensure sufficient penetration of the skin outer layer to facilitate drug delivery. The amount of application force required to provide reliable skin penetration is analogous with the force used to massage an aching muscle. Prior to applying the microneedle designs to human volunteers we have performed a large number of ex vivo studies using human skin, obtained from surgical procedures under ethical approval and informed patient consent. These studies have clearly shown that smaller microneedles (i.e. 180 μm in height) need to be more firmly applied to skin than larger microneedles (280 μm in height) to ensure comparable skin penetration. The clinician in our clinical study was therefore trained to apply the microneedle arrays to human skin, using more force with the 180 μm microneedle array. The oral commentaries from participants support the VAS data by highlighting key words and analogies given by the participants relating to pain response.

The sensory questionnaire further probled the perception of the participants to microneedle and hypodermic needle treatment. The microneedle applications were commonly perceived as being ‘pressing’ and ‘heavy’ with the hypodermic needle application perceived as more ‘sharp’ and ‘pricking’. These data relate to surface area and ease of needle puncture. Microneedles spread over a larger surface area may require more force of penetration than an individual sharp hypodermic needle. Sensory responses suggest that further developments in microneedle array design, microneedle applicator morphology and clinical application technique that reduce the force required to ensure penetration of the SC would be beneficial. Importantly however, this study confirms that the pain and sensation felt from application of the wet-etch pyramidal microneedle devices were relatively similar, and in each case significantly lower than for the hypodermic needle. The verbal comments from the participants correlate with the pain questionnaire results with participants stating that application of the hypodermic was sharper and more pricking than either of the microneedle designs.

As previously mentioned, we thought it important to demonstrate that the pain and sensory response data would be relevant to clinical application of the microneedles. Therefore, the functionality of the microneedles, at least

Fig. 10 TEWL measurements for 180 μm (cross) and 280 μm (circle) microneedle arrays compared against the percentage of puncture marks observed by external staining (out of 36) following application of the 180 μm (white bars) and 280 μm (black bars) microneedle arrays.
from a skin penetration perspective if not a drug delivery viewpoint, was monitored. Following application of the microneedle arrays, skin puncture marks could be identified by staining with a topically applied solution of methylene blue dye. The intensity of puncture staining reduced with time after microneedle application indicating temporal scaling of the transient skin microchannels over the 24 h study period.

Trans-epidermal water loss (TEWL) measurements were used as an additional indicator of skin barrier disruption. It is acknowledged that TEWL is an extremely sensitive measurement of skin permeability and as the microneedle punctures are less than 100 μm at their widest and 280 μm at their theoretical deepest, it is likely that TEWL is affected by the participant’s movements, diet and atmosphere over the 24 h study period, despite taking rigorous precautions at the time of measurement. Nevertheless, TEWL data correlated with the skin staining experiments in that an increase in TEWL is observed immediately after microneedle puncture and this increase diminishes to baseline over the 24 h study period. The TEWL values also reflect the relative efficiency of skin puncture with larger differences in TEWL observed when using 280 μm microneedles compared with 180 μm microneedles.

6 Conclusions

We have shown that pyramidal microneedles fabricated using low-cost wet-etching processes can penetrate human skin, providing transient microchannels for transcutaneous drug delivery, with minimal pain and discomfort. Our data suggest that the size of the microneedle must be optimised to ensure consistent skin penetration without excessive application force. The transient nature of skin disruption following application of microneedles was demonstrated with preliminary indications of microchannel healing and repair within the 24 h study period. More extensive studies investigating these skin repair responses at a cellular level are underway.

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References


J. Birchall, S. Coulman, A. Anstey et al., Cutaneous gene expression of plasmid DNA in excised human skin following delivery via microchannels created by radio frequency ablation Int. J. Pharm 312, 15–23 (2006)


