Characterisation of mechanical insertion of commercial microneedles

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Abstract

The protection provided by the human skin is mostly attributed to the stratum corneum. However, this barrier also limits the range of molecules that can be delivered into and across the skin. One of the methods to overcome this physiological barrier and improve the delivery of molecules into and across the skin is via the use of microneedles. This work evaluates the mechanical insertion of two different commercially available microneedle systems, Dermapen® and Dermastamp™. The influence of biaxial skin strain on the penetration of the two microneedle systems was evaluated ex vivo using a biaxial stretch rig. From the skin insertion study, it was apparent that for all levels of biaxial strain investigated, the Dermapen® required less force than the Dermastamp™ to puncture the skin. In addition, it was observed that the oscillating microneedle system, the Dermapen®, resulted in deeper skin insertion ex vivo in comparison to the Dermastamp™. The use of this new biaxial stretch rig and the ex vivo skin insertion depth study highlights that the oscillating Dermapen® required less force to perforate the skin at varying biaxial strain whilst resulting in deeper skin penetration ex vivo in comparison to the Dermastamp™. Although the Dermapen® punctured the skin deeper than the Dermastamp™, such difference in penetration did not influence the permeation profile of the model drug, imiquimod across the skin as evidenced from a 24-hour ex vivo permeation study.
1. Introduction

The human skin is the largest organ in the human body and is comprised of three layers; the epidermis, dermis and hypodermis. The epidermis is a multilayer compartment of the skin that comprises of the stratum corneum (SC), stratum granulosum, stratum spinosum and stratum basale [1]. The outermost layer of the epidermis, the stratum corneum, is avascular and has evolved to provide protection against physical and chemical attack. The protection provided by the stratum corneum has also resulted in a barrier to the delivery of compounds across the skin either for therapeutic or cosmetic purposes [2].

Microneedles are one of the strategies explored to improve the delivery of compounds across the skin. These are minimally-invasive needles with lengths that ranging between 250-1000 µm capable of perforating the stratum corneum in order to promote the delivery of compound across the skin [3]. Due to their size, microneedles offer painless skin insertion as they are unlikely to stimulate the dermal pain receptors upon application [4]. Some of the microneedle products available on the market, licensed for cosmetic use, include the Dermastamp™ and Dermapen®.

The Dermastamp™ consists of a stamp with an array of microneedles arranged at the base of the device. The microneedles are inserted into the skin in one vertical motion, creating micron sized channels in the skin. The Dermapen® is a motor driven microneedling device that inserts its needles into the skin in a continuous oscillating motion at one of five programmed frequency levels. The use of a motor helps circumvent the issue of varying insertion force between users. It also features an adjustable dial to control the needle’s depth of penetration during use. However, little research exists evaluating the effectiveness of such a motor driven device or the associated advantages or disadvantages in its use in comparison to the Dermastamp™ and its single stamping motion.
In order to effectively generate microneedle channels, the skin's topology must also be considered. The human skin features a roughened surface due to the variation in structure of the keratinocytes on the stratum corneum \[5,6\]. This surface is undulating in nature, being up to 150 microns from peak to trough for those aged over 60 \[6\], thus clearly demonstrating the need to smooth the skin as far as possible to maximise depth of penetration by the needles. To achieve smoothing, the skin must be stretched. It is understood that when skin is uniaxially stretched, the skin acts in a compressive fashion in the perpendicular direction to maintain the area of the surface, causing micro-furrows to develop \[7\]. This highlights the need for biaxial stretching to mitigate against this and ensure microneedle insertion into the skin. Biaxial skin stretching has been performed in several studies \[8–10\] with a non-linear stiffening of skin being found as a function of strain. This relationship has been supported by a simulation study by Flynn and Rubin \[11\] however little other data appears to exist regarding how increase in strain affects the penetration of microneedle into the skin.

In this work, we compare the insertion force profiles of two commercially available microneedle systems; Dermastamp™ and Dermapen®. This study evaluates the influence of biaxial skin strain on the insertion force of two different microneedling systems into the skin. Besides that, the influence of microneedle oscillation during microneedle application was evaluated using an in vitro and an ex vivo set up.

2. Materials

Dermapen® (ZJChao, China) and Dermastamp™ (Teoxy Beauty, Wuhan, China). The Dermapen® is an oscillating microneedling pen featuring a 36-needle removable array, with tip radius of 44-68 µm and conical geometry. In order to mimic how the Dermapen® would be use by a patient in a real-world setting, the plastic ring around the microneedle cartridge was not removed for all skin insertion and permeation study. The Dermastamp™ is a non-oscillating microneedle stamp featuring a 42-needle
array of 1mm length, tip radius of 21-25 µm and curved conical geometry. The geometry of the microneedles from respective devices are visualised using Leica DM4000B (Leica Microsystem, Germany). The geometry of the microneedles is shown in Figure 1.

Porcine skin was used to study the insertion force profile of commercial microneedles instead of ex-vivo human skin due to its limited availability and the ethical difficulties associated with its use. Various studies have highlighted that porcine skin is a suitable alternative to human [12]. Porcine flank skin samples from six-month old animals were obtained from a local abattoir, reared specifically for food. Skin were collected prior to any steam cleaning, and then prepared. The skin was washed with distilled water and dried using tissue. Full thickness skin was used to avoid altering the skin’s biomechanical properties, which may lead to over-penetration of microneedles into the dermal tissue [13]. After that, the skin samples were stored at -20 °C and used within six months. Gentian violet solution 1% w/v (De La Cruz products, USA) was used as a dye to highlight the microneedle channels created in porcine skin. Parafilm M® (Brand Bermis, Wertheim, Germany) of 127 µm thickness was used as a skin simulant in the in-vitro insertion study. Imiquimod was purchased from Cayman Chemicals, USA. 5% w/w imiquimod cream (Aldara™), MEDA Company, Sweden was purchased from Manor pharmacy, UK. Sodium acetate was purchased from Sigma-Aldrich, UK. Acetonitrile (HPLC grade) and glacial acetic acid were obtained from Fisher Scientific, UK. Teepol solution (Multipurpose detergent) was ordered from Scientific Laboratory Supplies, UK. D-Squame standard sampling discs (adhesive discs) were purchased from Cuderm corporation, USA. Deionised water was obtained from an ELGA reservoir, PURELAB™ Ultra, ELGA, UK. All reagents were of analytical grade, unless otherwise stated.
3. Methods

3.1. Biaxial Stretch Rig Development

Following two designs presented in literature [9,10], a low cost biaxial skin stretching rig was produced. The rig consists of four manual linear stages arranged as shown in Figure 2 on an 8 mm laser cut acrylic base. Further laser cut components permit clamping to be achieved using M4 Cap Head Bolts and 5mm acrylic plates. Friction between the clamping plates was improved using 40 grit emery cloth, double sided taped to the plates. The centre of the rig, over which the microneedle array is inserted, consists of an acrylic block topped with a 6mm layer of natural cork to simulate the stiffness of skeletal muscle [14]. Aluminium foil was overlaid on the cork with a thin covering of detergent. This was performed to reduce the friction experienced by the skin on the cork mat during stretching thus aiding the amount of strain that could be achieved within the skin. In addition, a laser cut jig for locating the biaxial stretch rig on the bed of a Texture Analyser (TA), (Stable Micro Systems, Surrey, UK) was also prepared to ensure consistency of the location of insertion of the microneedle array. Zero strain was assumed for each piece of skin when initially clamped.

3.2. Biaxial strain on microneedle skin insertion force

In order to investigate the effect of biaxial stretching on microneedle puncture performance of the Dermapen® and Dermastamp™, an insertion experiment was performed. The prepared porcine skin was inserted into the biaxial stretch rig and clamped, Figure 3 (a). The skin samples were then subjected to five levels of biaxial strain; 1.00, 1.0625, 1.125, 1.875 and 1.25 (i.e. a biaxial stretch of 0mm, 2.5mm, 5mm, 7.5mm and 10mm of a 40x40 mm grid). The level of biaxial strain was measured using a 40x40 mm grid of 5 mm squares ink-stamped onto the skin samples, Figure 3(a). The skin sample was biaxially stretched and a pair of Vernier callipers used to measure the level of stretch i.e. 0mm, 2.5mm, 5mm, 7.5mm and 10mm. Strain in each direction was calculated using Equation 1.
Equation 1 - Equation for strain where $\varepsilon$ is strain, $l$ is length, and $\Delta l$ is the change in length of skin. $\varepsilon$ strain, has no unit as the units from $\Delta l$ and $l$ cancel each other out.

The skin-loaded rig was then placed under the probe of the TA, using a laser cut jig to align a quadrant with the probe’s central position. A microneedle-loaded probe, see Figure 3(b), was then attached to the TA. The following parameters were used for the TA program; 5kg Load Cell, Pre-Test Speed: 0.5mm/sec, Test Speed: 0.5mm/sec, Post-Test Speed: 10mm/Sec, Trigger Force: 0.01N. The microneedles were inserted into the skin sample by the TA and the force-displacement profile recorded. Following their removal, the Gentian Violet dye was applied to the skin, Figure 3(c) to visualise the number of microneedle channels generated. The number of microchannels generated were counted to measure the percentage of successful microneedle insertion. The Dermastamp™ was housed in a custom mount that consist of a turned aluminium with a roll pin used to hold the microneedle array in place. An M6 grub screw was used in the rear of the mount as an attachment to the TA. For the Dermapen®, a 3D printed (Fused Deposition Modelling) jacket was designed to house the device within an aluminium tube and stub assembly via a tapered interference fit. The assembly was then attached to the TA again by an M6 grub screw. The Dermapen®’s adjustable needle length was set to 1000 µm, the same length of the Dermastamp™ needles.

3.3. In vitro skin simulant insertion study

As an alternative method to determine the microneedle penetration depth, a polymeric film (Parafilm M®) was used as a skin simulant. This insertion study was adopted from Larrañeta et al. 2014 [15]. In brief, eight layers of Parafilm M® were stacked onto each other on a cork layer. Both microneedle systems were applied onto the Parafilm M® stacks. Six replicates were performed, and the pores
The percentage of holes created per layer for respective microneedle length was calculated using following Equation 2:

\[ \text{% hole generated: } \left( \frac{N \text{ microneedle channels observed}}{N \text{ microneedles per array}} \right) \times 100 \]

Equation 2 - Equation to establish the percentage of holes produced by the microneedle devices. Where \( N \) represents ‘number of’.

### 3.4. Ex vivo skin insertion study

In order to evaluate the penetration depth of the Dermapen\(^*\) and Dermastamp\(^{TM}\) needles into the skin, an ex vivo penetration study using porcine skin was conducted. The porcine flank skin was defrosted at room temperature for an hour prior to the experiment. Using scissors, excess hair was carefully trimmed from the skin. A 36-microneedle array tip was used, and the vibration speed was set to level 1 (412 cycles/min) [16]. The microneedle skin pre-treatment was applied by gently stretching the skin and placing the Dermapen\(^*\) vertically upon the skin for 10 seconds. A microneedle length of 1000 \( \mu \)m was used in this study. After treating the skin with the microneedle pen, 10 \( \mu \)l of 1 % Gentian Violet Dye was applied to the surface of the skin and left at room temperature for 60 minutes. Excess dye was removed and the skin was then visually inspected to identify microneedle pores. The skin samples were then cryo-sectioned (Leica CM3050 S Research Cryostat, UK) and the depth of microneedle penetration was measured under an optical microscope (Zeta Profilometer, KLA-Tencor, US). The same procedure was repeated to evaluate the depth of Dermastamp\(^{TM}\) penetration into the skin.

### 3.5. Skin permeation study

In order to investigate the influence of the different microneedle system on skin permeation, an ex vivo skin permeation study using a Franz-type diffusion cell was conducted using a model compound, imiquimod. Imiquimod was selected as a model compound as the molecule displayed poor...
permeation across the skin [2]. The application of microneedle system to skin is hypothesised to improve the permeation of imiquimod into the skin. Prior to the permeation study, skin samples were defrosted for at least an hour at room temperature. The skin was trimmed into small pieces according to the surface area of the donor chamber of the Franz diffusion cell (Soham Scientific, Cambridgeshire, UK). The trimmed skin samples were equilibrated by placing them above the receptor compartment for 15 minutes prior to skin treatment. The porcine skins were subjected to the following treatment modalities: i) application of 5% w/w of imiquimod cream alone as a control ii) application of 1000 µm microneedles to the skin as a pre-treatment using Dermapen® followed by 5% w/w of imiquimod cream iii) application of 1000 µm microneedles to the skin as a pre-treatment using Dermastamp™ followed by 5% w/w of imiquimod cream. Next, the porcine skins were placed on top of the receptor compartment filled with 3 ml of degassed 100 mM acetate buffer pH 3.7. This buffer was selected as the receptor phase in order to maintain a sink condition throughout the permeation study. This is due to the insolubility of imiquimod at neutral or basic pH values. Various groups have reported the use of acetate buffer pH 3.7 as the receptor phase in imiquimod permeation studies [17–19]. The skin was then secured between the donor and receptor compartment of the diffusion cell using a metal clamp, with the stratum corneum side facing the donor compartment. Upon assembling the Franz diffusion cell, the permeation experiment was conducted over a period of 24 hours in a thermostatically controlled water bath set at 36.5 °C.

After a 24-hour permeation experiment, the excess cream was removed and collected from the skin surface by careful application of sponges soaked with 3% v/v Teepol® solution. The sponges were pooled for imiquimod HPLC analysis as a total skin wash. Any formulation which might have spread to the donor chamber was collected by the sponges and stored for imiquimod analysis by HPLC as a donor chamber wash. Upon removing excess formulation from the skin surface, 15 sequential tape strips were collected from the skin. The amount of imiquimod from the different Franz cell elements (skin wash, donor chamber wash, pooled tape strips and remaining skin after tape stripping) were
extracted by the addition of 5, 5, 10 and 5 mL of methanol extraction mixture (Methanol 70%: Acetate Buffer pH 3.7 100 mM 30%) respectively using a previously reported method [20]. Samples were then vortexed for 1 minute and sonicated for 30 minutes before being left overnight. Subsequently, samples were vortexed again and sonicated for a further 30 minutes. 1 ml of the extracts were collected and spiked with 100 µl of 100 µg/ml propranolol as an internal standard. The samples were then filtered through 0.22 µm membrane. For the receptor fluid, 1 ml of the solution from each Franz cells were collected and spiked with 100 µl of 100 µg/ml propranolol as an internal standard before being filtered through 0.22 µm membrane. HPLC analysis was carried out using an Agilent 1100 series instrument (Agilent Technologies, Germany) equipped with degasser, quaternary pump, column thermostat, autosampler and UV detector. System control and data acquisition were performed using Chemostation software. The details of the HPLC chromatographic conditions are as follow: column C18 (150 x 4.6 mm) ACE3/ACE-HPLC Hichrom Limited, UK. The mobile phase composition for analysis of extracts from skin wash, donor chamber wash, pooled tape strips and remaining skin consists of 10 mM acetate buffer: acetonitrile (79:21). Whilst, the mobile phase composition for analysis of receptor fluid consists of 10 mM acetate buffer: acetonitrile (70:30). The system operated at a flow rate of 1.0 mL/minute, UV detection at λ max=226 nm, injection volume of 40 µL and column temperature of 25 °C.

Statistical analysis

All results were reported as the mean with standard error of mean (SEM) (n≥5). Statistical calculations were performed in Prism (IBM, USA), a software package. The Student’s t-test and One-Way ANOVA followed by a Tukey HSD post-hoc test was applied to compare the results of different groups. Statistically, a significant difference was denoted by p value < 0.05.
4. Results and Discussion

4.1. Influence of biaxial strain on commercial microneedle perforation.

A biaxial skin stretching experiment was conducted in order to investigate the effect of skin strain on the insertion of two commercial microneedle systems. From Figure 4 it can be seen that the force needed by the Dermapen® to perforate the skin was significantly lower than the Dermastamp™ for the range of strain rates investigated. It was also found that an increase in force was needed for the Dermastamp™ to puncture the skin as the strain increased, however this force plateaued at a biaxial strain of circa 1.1. In contrast, a linear relationship is presented for the Dermapen® suggesting that insertion force increases with a higher strain rate.

The relationships shown in Figure 4, an increase in force with increases in biaxial strain, align with Lanir & Fung’s work that showed skin as a non-linear material that exponentially stiffens when biaxially stretched [10]. As stiffness is defined as the resistance to bending or deformation, it is proposed that as skin exponentially stiffens with an increase in strain. This results in the force needed to deform the skin and insert the needles will increase significantly with biaxial stretching.

Following microneedle insertion, the formed puncture sites were visualised by application of Gentian Violet Dye. The percentage of successful microneedle insertions is shown in Figure 5 for the two microneedle systems. It is evident that as the biaxial strain of the skin sample increases, an increase in the number of successful microneedle insertions was observed for the Dermapen®, which then plateaus as the skin was subjected to further biaxial strain. For the Dermastamp™, as the biaxial strain of the skin increases, we observed a rise in the percentage of successful microneedle insertion. However, as biaxial strain of the skin was increased further, the percentage of successful microneedle insertion into the skin decreased.
Previous work by Maiti et al. has shown that subjecting the skin to strain may help smooth its surface [21]. Such topographical change in skin structure may help mitigate the presence of micro-furrows on the skin which fold around the microneedles and can present resistance to microneedle insertion [22]. One of the ways to achieve skin smoothening is via subjecting the skin to strain or stretching [23]. However, the current work suggests that smoothening the skin by subjecting the skin to biaxial strain may help improve microneedle insertion up to an optimum strain (1.0625 and 1.125) as shown with the Dermastamp™ in Figure 5. Beyond this optimum strain, the percentage of successful microneedle penetration decreases due to increased skin stiffness with increasing strain as shown by previous investigators [10].

For the Dermapen®, the increase in the percentage of successful microneedle insertions with increasing strain is attributed to the observation that the skin smooths upon stretching [23]. Subjecting the skin to biaxial strain results in flattening of the micro-furrows and permits an increased probability of the needles puncturing through the stratum corneum. This is due to the linear motor, that oscillates the microneedle array, providing a secondary force to assist with insertion into the skin, irrespective of the rise in skin stiffness with the increasing strain. These results demonstrate that the Dermapen® is more effective than the Dermastamp™ in generating microneedle channels across the skin.

Unlike the Dermastamp™, the presence of plastic shoulders at the tip of microneedle cartridge of the Dermapen® imposes an additional surface tension to the skin during microneedle application. This helps to further mitigate the propensity of the skin to fold around the needles while mitigating the variability in puncture force. This is evidenced by the smaller standard deviation error bar for Dermapen® relative to Dermastamp™ for the level of skin strain investigated shown in Figure 4. The combination of these physical factors mimics the insertion mechanism of a mosquito’s proboscis. The shoulder of the cartridge of the Dermapen® plays a similar role to that of the mosquito labium which
applies lateral strain to the skin prior to puncture. This ultimately focusses the force at the tip of the
Dermapen® permitting a more effective insertion [24]. The microneedles in this case are equivalent
to the mosquito’s labrum which insert itself at defined frequency in a stamping manner allowing
deeper insertion with repeated insertion.

4.2. In vitro skin simulant insertion depth study of commercial microneedle

An in-vitro skin simulant study, using Parafilm M®, was performed to compare the percentage of
successful microneedle channels against depth for the two commercial microneedle systems being
considered; the Dermapen® and Dermastamp™.

The insertion profiles of the commercial microneedle systems were established using a methodology
developed and validated by Larraneta et al. [15]. It involves the insertion of the microneedle devices
into a stack of eight Parafilm M® layers, followed by the separation of the layers and their visualisation
under an optical microscope to evaluate the number of microneedle channels formed, leading to the
insertion profiles in Figure 6.

The two microneedle systems typically pierce the first five layers, with approximately 100% of the
needles piercing the first three layers before the percentage of microchannels generated begins to
decrease. The generated channels displayed uniform geometry as shown in Figure 6 (a) and (b).
However, less than 50% of the microneedles successfully pierced the fifth and sixth layer.

Hutton et al showed that microneedle patches fabricated from a copolymer of methyl vinyl ether and
maleic acid were capable of penetrating the Parafilm M® layers to a depth of approximately 60% of
the microneedle height [25]. Vora et al also showed that microneedles fabricated from poly(vinyl
pyrrolidone) (PVP) loaded with nano- and microparticles were capable of penetrating the Parafilm
layers up to 60% of the microneedle length [26]. This work aligns with our findings that the commercial
microneedle systems were capable of penetrating Parafilm M® layers up to circa 60% of the microneedle length. Furthermore, the results in Figure 6 (c) suggest that for an in vitro skin simulant model, the insertion profiles are similar for both microneedle systems. In a follow up study, Larraneta et al discovered that the insertion profile of microneedle arrays was more dependent on needle density and design rather than the material used [27]. Such observations may explain the similar insertion profiles of the two commercial microneedles systems, as both microneedles are made from the same material; stainless steel.

4.3. Ex vivo skin insertion study

An ex vivo penetration study was conducted to ascertain the microneedle penetration depth of the two different commercially available microneedle systems in actual skin tissue. Figure 7 shows that successful penetration of microneedles into ex vivo porcine flank skin evidenced from the visualisation of microneedle channels from cryo-sectioned skin samples. From Figure 7 it was apparent that the region surrounding the microneedle pore retained a normal structure with intact stratum corneum. However, the microneedle channels displayed a deep indentation with disrupted stratum corneum.

In the context of drug delivery, it has been shown by Andrews et al that drug entry into and across the skin is not just limited by the outermost layer of the skin, the stratum corneum, but the penetration of molecules is limited by the overall epidermis itself [28]. This would suggest that both microneedle systems were capable of perforating the skin to generate microneedle channels which could be used by drug molecules to enter deeper layers of the skin.

It was evident that the microneedle penetration depth by the Dermapen® was significantly deeper in comparison to the Dermastamp™. Such observation may be attributed to the oscillating motion of the device during skin application which has been suggested to improve skin penetration [29]. Previous work by Izumi et al investigated the influence of vibration on the penetration of microneedles into an
in vitro silicone skin model. The group observed that the application of vibrating microneedles at 30 Hz during skin application resulted in a reduction in the force needed to penetrate the skin [30]. This reduction in puncture force is attributed to the reduction in effective frictional forces experienced by microneedles under vibration [31]. The rapid vibration of the microneedles also mitigates the likelihood of viscoelastic materials such as skin from attaching to the microneedle during the insertion step. This reduction of effective frictional forces experienced by oscillating microneedles may also serve as an explanation as to why the Dermapen® displayed lower peak insertion force in comparison to the Dermastamp™, shown in Figure 4.

Another factor that may influence microneedle insertion into the skin is the different organization of the microneedles on the Dermastamp and Dermapen systems. From Figure 7 (a) and (b) along with microscopy image from Figure 6 (a) and (b) it is evident that the 36 microneedles on the Dermapen® are organised in rows whereas the 42 microneedles on the Dermastamp™ are organised in concentric circles. The needles on Dermapen® are closely distributed to one another in comparison to the needles on the Dermastamp™. Previous work by Olatunji et al highlighted that insertion force increases with when microneedle interspacing decreases [32]. In contrast to the finding by Olatunji et al, we observed that although the needle interspacing on the Dermapen® are closer than the Dermastamp™, the Dermapen® still required less insertion than the Dermastamp™. By comparing our findings to that of Olatunji et al, it can be postulated that the method (oscillating vs non-oscillating) in which the microneedle is applied to the skin overrides the influence of microneedle interspacing on insertion force and penetration depth.

By comparing the penetration data for both microneedle systems from Figure 6 and Figure 7, it is evident that the insertion of microneedles into in vitro skin simulant, Parafilm M® stacks, were significantly deeper than that of ex vivo skin tissue. Such disparity in results suggest that the in vitro test developed by Larraneta et al may have some limitations when the insertion data is translated to
ex vivo tissues and potentially in vivo. Both Parafilm M® and skin are inherently viscoelastic materials which display both elastic and viscous properties under deformation. Unlike skin, which is an elastic biological tissue that returns to its normal state after mild stretching or compression [33], Parafilm M® exhibits irreversible plastic deformation when stretched or compressed [34].

4.4. Skin permeation study

A permeation study was conducted to investigate the effect of different commercial microneedle systems on the permeation of a model drug, imiquimod that displayed poor cutaneous permeation [2]. One of the strategies to overcome the limited permeation of imiquimod is to employ permeation enhancing strategy such as microneedle. Upon microneedle application, transient microchannels are generated within the skin that promote the delivery of the drug across the skin. The amount of imiquimod (µg) recovered from the various Franz cell components following the 24-hour permeation study is displayed in Figure 8.

For all treatment modalities, we observed no statistical difference in the amount of imiquimod recovered from different Franz cell components (donor wash, skin wash, tape strips and remaining skin) except for the receptor fluid. With regards to receptor fluid, it was seen that when the skin was pre-treated with either microneedle systems, we observed enhanced delivery of imiquimod across the skin relative to the cream only control. However, it was worth noting we observed no statistical difference in the permeation of imiquimod into the receptor fluid between Dermapen® and Dermastamp™.

One possibility for the similarity permeation profile for the two microneedle systems is attributed the fact that both systems successfully breached the stratum corneum, epidermis and down to superficial dermis as highlighted in Figure 7 (c) and (d). It has been reported that thickness porcine epidermal layer varies between 30-140 µm [35] and it was shown that both microneedle system penetrated into
the porcine skin to a depth of at least 200 µm, reaching the dermal layer of the skin. This layer of the skin is viscoelastic due to the presence of a dense network of collagen and elastin [36–38]. Although the Dermapen® may puncture the skin deeper than the Dermastamp™, immediately upon microneedle removal the channels generated in the dermal layer of the skin immediately recoils and reseals conferring similar resistance in permeation for imiquimod across the dermis for both microneedle systems. A limitation which is frequently highlighted when a patch-and-poke strategy is adopted for solid microneedles systems [39].

5.0 Conclusion

In conclusion, this work expands our knowledge on the mechanical insertion of microneedles into the skin. Applying biaxial strain on the skin indeed influences the penetration of microneedles into the skin. It was apparent that the two commercially available microneedle systems, Dermapen® and Dermastamp™ have very different insertion force profiles with increasing strain. For all the skin strain levels investigated, it was evident that the Dermapen® required less insertion force than the Dermastamp™. Interestingly, the percentage of successful insertion continues to increase before plateauing with increasing skin strain for the oscillating Dermapen®. In contrast for the Dermastamp™, the percentage of successful microneedle insertions increases with strain before decreasing at higher strain rate. In terms of insertion depth, it was apparent that the penetration of the Dermapen® was much deeper than that of the Dermastamp™. Such a difference was not detected when the microneedle systems were evaluated using the commonly used Parafilm M® stack insertion study but only became apparent when the devices were evaluated ex vivo. The lower insertion force and deeper penetration provided by the Dermapen® was attributed to the oscillating feature of the microneedle system which mitigates the effective frictional force experienced by the needle during skin insertion. Lastly, although the Dermapen® may puncture the skin deeper than the Dermastamp™, such difference in penetration did not affect the permeation profile of the model drug, imiquimod across the skin as shown in the ex vivo permeation study.
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Declaration of Competing Interest:
All the authors have no conflict of interest

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Figures and legends

Figure 1 Close up microscopy image showing the geometry of microneedles from Dermapen® and Dermastamp™
Figure 2 - A schematic of the conceived manual biaxial stretch rig
Figure 3 - Schematic detailing the setup to investigate effect of biaxial strain on microneedle skin insertion. A 40x40 mm grid of 5 mm squares ink was stamped onto the skin samples in **Step (a)** in order to measure the level of biaxial strain on the skin. Using a texture analyser (TA), respective microneedle systems were attached to the probe of the instrument to allow insertion into the skin as shown in **Step (b)**. Visualisation of microneedle channels using Gentian Violet dye as depicted in **Step (c)**.
Figure 4 Biaxial skin strain and insertion force relationship for commercial microneedle systems Dermapen® and Dermastamp™. Data expressed as means ± SD, n=5.

Figure 5 Averaged percentage of maximum number of dyed microneedle insertion holes generated for each level of stretch. Data expressed as means ± SD, n=5.
Figure 6 Microscopic images of first layer of Parafilm M® stack punctured by stainless steel microneedles by (a) Dermapen®, (b) Dermastamp™ Scale bar: 300 µm (c) Insertion profile of different commercial microneedle systems, Dermapen® and Dermastamp™ into Parafilm M® layers, data expressed as means ± SD, n=6.
Figure 7 Visual image of porcine flank skin surface after gentian staining following (a) Dermapen® and (b) Dermastamp™ application. Optical microscopy images of porcine flank skin cross sections after application of (c) Dermapen and (d) Dermastamp. The skin was stained with 1% gentian violet solution to allow visualisation of microneedle channels formed after microneedle treatment. n=10, data is expressed as mean ± SEM.
Figure 8 HPLC analysis of the mean amount of imiquimod recovered from the different Franz cell components (donor chamber wash, skin wash, tape strips, remaining skin and receptor fluid) post-permeation study. Data is presented as the mean ± SEM (n = 6). Differences were calculated using one-way ANOVA, followed by Tukey’s post hoc test, and deemed significant at p<0.05. n.s = not statistically significant at p>0.05.