Effect of Microneedle Geometry and Supporting Substrate on Microneedle Array Penetration into Skin

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ABSTRACT: Microneedles are being fast recognized as a useful alternative to injections in delivering drugs, vaccines, and cosmetics transdermally. Owing to skin's inherent elastic properties, microneedles require an optimal geometry for skin penetration. *In vitro* studies, using rat skin to characterize microneedle penetration *in vivo*, require substrates with suitable mechanical properties to mimic human skin's subcutaneous tissues. We tested the effect of these two parameters on microneedle penetration. Geometry in terms of center-to-center spacing of needles was investigated for its effect on skin penetration, when placed on substrates of different hardness. Both hard (clay) and soft (polydimethylsiloxane, PDMS) substrates underneath rat skin and full-thickness pig skin were used as animal models and human skins were used as references. It was observed that there was an increase in percentage penetration with an increase in needle spacing. Microneedle penetration with PDMS as a support under stretched rat skin correlated better with that on full-thickness human skin, while penetration observed was higher when clay was used as a substrate. We showed optimal geometries for efficient penetration together with recommendation for a substrate that could better mimic the mechanical properties of human subcutaneous tissues, when using microneedles fabricated from poly(ethylene glycol)-based materials. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

Keywords: microneedles; microneedle geometry; skin substrate stiffness; polydimethylsiloxane

INTRODUCTION

Transdermal drug delivery systems offer numerous benefits over the delivery of drugs using parenteral or oral route, as they are painless and obviate the first-pass effects and possible enzymatic degradation through the oral route.[1,2] Microneedles have been used as an effective means of enhancing skin permeation with over 350 published studies highlighting their utility in delivering different low molecular weight drugs, biotherapeutics and vaccines, including published human studies with a number of small molecules, protein drugs, and vaccines.[3] Microneedles have been fabricated from a range of materials, using different techniques,[3,4] forming arrays of different dimensions, variable shapes, and geometries. This diversity in microneedle design has called for an investigation of the effect of microneedle design on their ability to enhance transdermal permeation. A number of studies have reported the effect of microneedle shape,[5] tip diameter,[6] length,[7] force of insertion,[8] velocity of insertion,[9] and density of microneedle arrays,[9] etc. These factors, together with materials used for microneedle fabrication and force applied to an array will determine microneedle insertion and fracture properties. Another important parameter to be considered is the skin's inherent elasticity, which would pose a challenge to microneedle penetration. Previous studies have reported folding of skin around microneedle arrays, either preventing penetration or allowing for suboptimal penetration, especially for microneedles of lengths shorter than 300 μm.[10,11] The depth of microneedle penetration has been correlated to the force of application,[12] which in turn counters skin's elasticity.

Because microneedles are usually fabricated as arrays, it is of particular interest to study how force is distributed across a microneedle array, with density of microneedles influencing the penetration. A number of simulation studies have been reported in the literature, modeling breaching of soft tissues on needle application.[13,14] Modeling studies on skin tissues have also been carried out to better mimic microneedle insertion properties. Olatunji et al. recently published their findings on the effect of interspacing between microneedles and application force, reporting that the force required for insertion was dependant on the interspacing between microneedles, irrespective of microneedles on an array.[15] The study included simulation experiments to characterize microneedle bending force, buckling force, and insertion force against the tip interspacing of microneedles. While they demonstrated that there should be an optimal number of microneedles on an array to enhance drug delivery, too many needles will form a "bed of nails", reducing penetration and efficiency of microneedle penetration. Apart from this, a few other simulation studies have also been conducted for analyzing the effect of microneedle geometry on skin penetration.[16–18] However, a comprehensive *in vitro* study on skin models, coanalyzing various aspects of microneedle geometry, including base diameter, tip diameter, center-to-center spacing, and insertion force is still lacking in literature.

On the other hand, *in vitro* experiments using microneedles have been carried out using a variety of animal skin models. To mimic the mechanical properties of human skin's inherent subcutaneous fatty tissues, researchers have used

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clay,[19] layers of Kimwipes,[20] dental wax,[15] soft sponge,[7] and filter paper soaked with phosphate-buffered saline (PBS) layered above a corkboard.[21] While these materials widely vary in hardness, none of them has been demonstrated to possess mechanical properties similar to human subcutaneous tissues and hence, the extent of microneedle penetration observed may vary greatly when different substrates are used. This requires the careful calibration of penetration properties of microneedles under substrates of varying stiffness and comparing them to full-thickness skin models from humans to derive better correlation.

In this study, we address the two lacunas highlighetd earlier. Microneedle arrays of various base and tip diameters and varying spacings were fabricated and tested for their extent of penetration on excised rat skin model on two different substrates, a hard (clay) and a soft (polydimethylsiloxane, PDMS) substrate were used to study the influence of substrate stiffness on microneedle penetration. These substrates were then compared to microneedle penetration on full-thickness pig and human skin samples. To our knowledge, this is the first study elucidating the effects of microneedle geometry as well as substrate stiffness on the extent of microneedle penetration through the skin, and hence could serve as a platform for the design of optimized microneedle array based products as well as testing platforms.

EXPERIMENTAL

Materials

Poly(ethylene glycol) diacrylate, PEGDA (M_n = 258), 2-hydroxy-2-methyl-propiophenone (HMP), lidocaine hydrochloride (LH), and trypan blue were purchased from Sigma–Aldrich (St Louis, MO, USA). All other materials were of the reagent grade and were used as received. PDMS (Sylgard 184 Silicone Elastomer Kit) was obtained from Dow Corning (Midland, MI, USA).

Fabrication of Microneedles

Microneedles were fabricated using a photolithography-based method involving UV light based photopolymerization, previously developed in our lab.[19] Briefly, prepolymer solution (a mixture of PEGDA with 0.5% v/v HMP) was filled into a cavity formed by supporting glass coverslips on a base glass slide. This prepolymer solution is then exposed to high power UV light source (EXFO OmniCure® S200-XL, Canada) with a UV filter (320–500 nm). This resulted in the fabrication of a thin film termed as "microneedle backing layer". Subsequently, this film was placed on a similar substrate and exposed to UV light through a patterned photomask, forming "microneedle shafts". Photomasks (Infinite Graphics, Singapore) of different patterns were prepared to govern the geometry of microneedles with base diameter of 200, 300, and 400 μm and a center-to-center spacing varying between 2× and 5× base diameter. Fabricated microneedles were washed with purified water from Millipore Direct-Q® (Molsheim, France) to remove the excess residual prepolymer solution and let to air dry before being removed from the coverslip and used for experiments. Microneedles were imaged using Nikon SMZ 1500 stereomicroscope (Nikon, Japan), to quantify the microneedle geometric characteristics. The base-to-tip ratio was defined as base diameter to tip diameter ratio.

Microneedle Penetration in Rat Skin Using a Hard Substrate (Clay)

Rat abdominal skins were obtained from rat cadavers from the National University of Singapore (NUS) Animal Center. Hair on the skin was first removed using a pair of scissors. To obtain a uniform flat piece of skin for penetration studies, subcutaneous fat was evenly excised with scissors and scalpel and thereafter fixed on a stable platform prior to penetration experiments. Two different substrates were used. As a model for hard substrate, paper clay (SESCO Art and Craft, Singapore) was dried overnight and used as a tissue-like mechanical support underneath the stretched and defatted rat skin. The clay support measured 7 mm in thickness. Fabricated microneedles were placed on the skin surface and force was applied with a force gauge (Dillon GL-500, Fairmont, MN, USA) for a minute to determine the force required for microneedle insertion. The point of penetration was identified as a sudden drop in the force applied (and therefore skin resistance) and was recorded digitally on the force gauge; the force at the point of penetration was recorded for calculation of force per needle at the point of penetration. Force per needle was calculated by dividing the force at penetration by the total number of needles on an array. Because microneedle arrays of different needle spacing were fabricated and array area was kept consistent at 1.44 cm^2, force per needle varied between different arrays.

The area of insertion was then immediately stained with a hydrophobic dye, trypan blue (0.4% solution) for 5–10 min. Trypan blue specifically stains sites of *stratum corneum* perforation. The excess stain was washed away with purified water followed by 70% ethanol solution, leaving blue circles on the skin demonstrating *stratum corneum* perforation. Excess hair on the skin was removed with hair removal cream Veet (Reckitt Benckiser, Poland) or a manual razor. The premise behind using the hair removal cream after trypan blue staining was to potentially avoid the damage to the skin and exposure of hydrophobic layers, which are then stained by trypan blue. Subsequently, areas stained with the dye were viewed using Image Soft hand-held microscope (Eikona, People's Republic of China) and the number of perforations was quantified. Spots on the skin which appear in the shape of the microneedle array relative to other spots were counted as penetration spots. The same procedure was repeated for needles of all base diameters and spacing. Penetration into rat skin was also confirmed by histological sectioning of liquid nitrogen frozen skin samples followed by haematoxylin and eosin staining.[19] All animal experiments were approved by the Institutional Animal Care and Use Committee, NUS.

Microneedle Penetration in Rat Skin Using a Soft Substrate (PDMS)

A PDMS substrate was fabricated by casting a silicone elastomer base and a silicone elastomer curing agent in the ratio of 9:1 as per the manufacturer's protocol. This mixture was then poured in the cover of a 100 × 20 mm petri dish (Greiner, Germany) and degassed in a vacuum chamber for 30 min followed by heat curing at 70°C for 2 h. The fabricated solid film measuring 6 mm in thickness was easily withdrawn from the petri dish cover and used as a skin substrate. Rat skin was placed over this substrate and microneedle samples were similarly inserted by using a force gauge to determine the force of insertion; the percentage of microneedle insertion was determined by trypan blue staining method.

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Microneedle Penetration in Full-Thickness Pig Ear Skin and Human Skin

The microneedle arrays of each base diameter that attained highest penetration percentages in rat skin from both hard and soft substrates were then inserted into pig ear skin and human skin in a manner as described earlier. This is carried out to ascertain the closeness of mechanical properties of both substrates to full-thickness pig and human skin model. Pig skin was obtained from NUS Animal Center. Full-thickness cadaver human skin was obtained from Science Care (Phoenix, AZ, USA). The use of human skin for this study has been approved by the National University of Singapore Institutional Review Board (no. 13-167E). The human skin tissues were excised from the back of a white, female cadaver, who died of debility at the age of 75 years. Similarly, post microneedle insertion, trypan blue staining method was used to ascertain the percentage of microneedles on an array that successfully penetrated the skin.

In Vitro Drug Release from Microneedles

Lidocaine hydrochloride was encapsulated *in situ* within both the microneedle shafts and the backing layer of the most optimized geometry of microneedles. This was achieved by dissolving LH in the prepolymer solution at a concentration of 3.47% w/w and 4.31% w/w. The fabricated microneedles were then tested for *in vitro* release of LH from the microneedles. The microneedles were immersed in 15 mL of 1 × PBS in an incubator maintained at 37°C. The release solutions from LH were sampled at 1, 3, 6, 18, and 24 h. At each sampling point, all release solution was withdrawn and replaced with 15 mL of fresh 1 × PBS. Release samples were stored at 4°C until analysis. In the analysis of LH, each sample was transferred to a quartz cuvette was and analyzed by absorbance measurements at 254 nm with an UV–visible spectrophotometer (U-1900, Hitachi, Tokyo, Japan). Cumulative release and cumulative percentage release were calculated.

In Vitro Permeation Assay with Horizontal Diffusion Cell

To study the ability of optimized geometry to enhance transdermal permeation, microneedle array encapsulated with 4.3% w/w LH was inserted into excised rat abdominal skin. Each microneedle array contained 2.51 ± 0.229 mg of LH. The horizontal diffusion cell (TK-6H1, Shanghai Kai Kai Science and Technology Company Ltd., People's Republic of China) was run with circulating water maintained at 37°C for both donor and receptor solutions to mimic the body temperature. 4.5 mL of 1 × PBS with 0.005% w/v sodium azide was added to the receptor compartment. The permeated solutions for LH were periodically sampled for a period of 48 h. At each sampling point, 1 mL of the receptor solution was withdrawn and replaced with 1 mL of fresh 1×PBS with 0.005% w/v sodium azide.

The amount of LH permeated was determined by using Hitachi L2000 LaChrome Elite HPLC system with a Hypersil ODS C_{18} reverse column (ODS hypersil, Thermo Scientific; 4.6 × 250 mm, 5 μm). The C_{18} reverse column and the solvents were maintained at ambient temperature. The mobile phase consisted of water and acetonitrile (30:70% v/v) containing 5.5% triethylamine and the flow rate was set at 0.7 mL/min. The injection volume was 20 μL for each sampling and ultraviolet detection was performed at a wavelength of 254 nm.[22] The standard LH solutions were prepared by series dilution. Various standard curves were prepared to bracket all possible concentrations from the permeation studies. Under these conditions, the peak of LH appeared at 5.8 min. The permeation samples were analyzed by comparing peaks with standards peaks.

Statistical Analysis

Results were used to compute mean and standard deviation and all experiments performed were at least triplicates. Student's *t* test was used to analyze the statistical significance between specific pairs of data. For analyzing multiple groups of data or statistical differences, one-way analysis of variance was used. Results with p value of less than 0.05 were considered to be statistically significant. PASW Statistics 18 (SPSS) software was used for computing statistical results.

RESULTS

Geometric Properties of Microneedles

Microneedles of varying lengths, base diameters, and spacing could be conveniently fabricated by changing the fabrication setup and photomask patterns. It was observed that as the spacing was increased, a relative larger dose of UV light was required to fabricate microneedles with sufficicent strength for subsequent penetration studies (Supporting Information, SI 1 in the Supplementary Material). Figures 1a–1c show the microneedles of 200, 300, and 400 μm base diameter with a center-to-center spacing of 6× base diameter. When fabricated in scaffold of particluar spacer thickness,[19] mcironeedle length for different base diameters (200–400 μm) ranged between 827.9 ± 7.3 and 873.3 ± 12.3 μm ($p > 0.05$), while the tip diameter increased proportionally with the base diameter, ranging between 131.3 ± 11.8 and 276.0 ± 26.0 μm (Fig. 1d). For all microneedles fabricated via this method, a constant base-to-tip ratio of approximately 1.5 was observed across all base diameters (Fig. 1e). The fabricated mcironeedles also showed a relatively constant needle length across all base diameters and a relatively constant tip diameter amongst same base diameters (SI 2 and SI 3 in the Supplementary Material).

Microneedle Penetration in Rat Skin Using a Hard Substrate (Clay)

We observed a consistent increase in the percentage of microneedles penetrating the skin with the increase in spacing, and the penetration percentage increased with the increase in microneedle base diameter [Figs. 2ai–2ci and SI 4 (in the Supplementary Material)]. For 200 μm base diameter, penetration varied between 5% and 44%, for 300 μm base diameter, penetration varied between 9% and 64%, and for 400 μm base diameter, it varied between 12% and 79%, with the increase in spacing between microneedles spread over an array of constant area of 1.44 cm^2.

To explore this further, force per needle was calculated by dividing the force applied on the microneedle array by the number of microneedles on an array. As the number of microneedles on an array decreased with increased spacing, an incremental force per needle and higher percentage of penetration were observed (Figs. 2aii–2cii). Force per needle increased from 0.19 to 1.33 N (200 μm base diameter), 0.24–4.46 (300 μm base diameter), and 0.84–7.17 N (400 μm base diameter) with the increase in spacing. The percentage of penetration was thus linearly correlated to force per needle as observed in Figures 2aiii–2ciii.

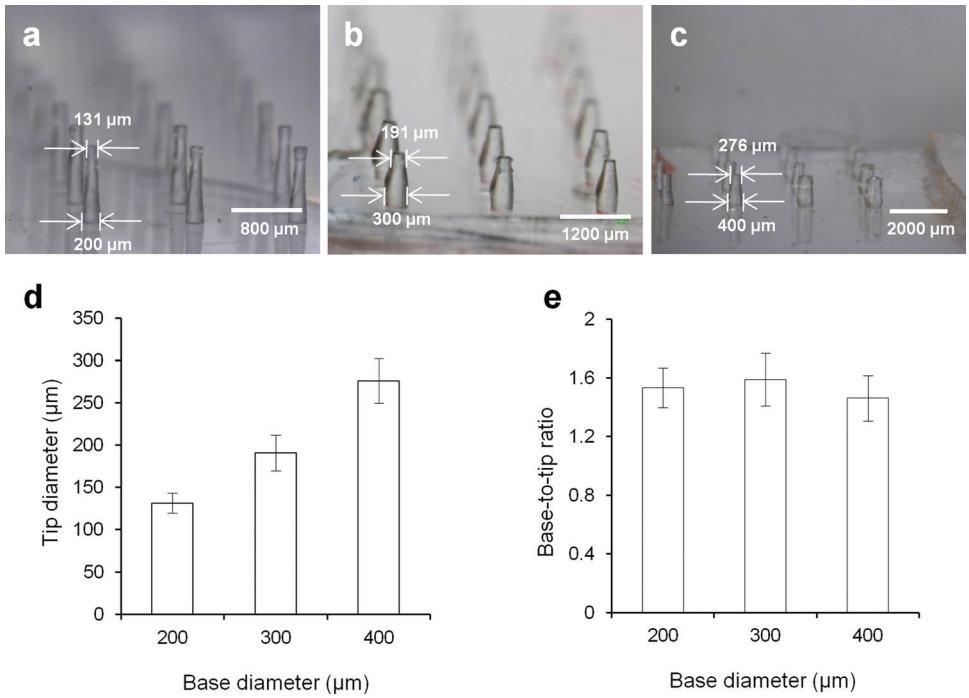

Figure 1. (a–c) images of 200, 300, and 400 µm base diameter needles, respectively, with a center-to-center spacing of 6× base diameter. Relationship between base diameter and (d) tip diameter, and (e) base-to-tip ratio of needle which is the ratio of base diameter to tip diameter remained consistent with no significant differences between different base diameters ($p > 0.05$).

Histological sectioning of the skin confirmed microneedle penetration in skin (SI 6 in the Supplementary Material).

Microneedle Penetration in Rat Skin Using a Soft Substrate (PDMS)

In an attempt to characterize the effect of substrate stiffness, a softer substrate was used for rat skin for *in vitro* microneedle penetration experiments. Although a similar trend of increased penetration with increased spacing and force per needle was observed across all geometries, the penetration percentage was lower than what was observed when clay was used as a substrate for rat skin. Penetration percentage ranged from 2% to 5% for 200 µm base diameter, 1%–52% for 300 µm base diameter, and 9%–37% for 400 µm base diameter [Figs. 3ai–3ci and SI 5 (in the Supplementary Material)]. The penetration force per needle increased from 0.07 to 0.37 N for 200 µm base diameter, 0.13–1.10 N for 300 µm base diameter, and 0.29–1.59 N for 400 µm base diameter microneedles (Figs. 3aii–3cii). A similar correlation was observed between penetration percentage and force per needle data (Figs. 3aiii –3ciii).

Microneedle Penetration in Full-Thickness Pig Ear Skin and Human Skin

With different substrates under rat skin resulting in different extents of penetration, we further explored to find the optimal substrate with close approximation of mechanical properties to full-thickness pig ear and human skin. This would enable the use of a substrate that best estimates microneedle penetration in *in vitro* penetration experiments with microneedles. We chose the microneedle geometries with highest penetration capabilities from both substrates. Thus microneedles with base diameters of 200, 300, and 400 µm with center-to-center spacing of 1200, 1800, and 2400 µm, respectively, were inserted on stretched samples of full-thickness pig ear and human skin using a similar method used for rat skin with two different substrates. Although it has been widely reported in literature that pig skin has similar mechanical properties to human skin, we observed that percentage of penetration of microneedles on human skin was slightly higher than on pig skin (Figs. 4 and 5). On the other hand, because rat skin is most often used as a substitute, we found that PDMS as a substrate under rat skin more closely approximates the penetration in human skin as compared to the use of clay as a substrate (Fig. 6). This indicates that PDMS has suitable biomechanical properties similar to subcutaneous tissues.

In Vitro Drug Release from Microneedles

Microneedles measuring 300 µm base diameter and 1800 µm center-to-center spacing were used for studying drug encapsulation purposes. LH was used as a model drug compound. The release of LH in PBS over 24 h resulted in a 93.9% release (3.04 mg) for 3.47% w/w LH-containing microneedles and 91.3% release (3.77 mg) for 4.31% w/w LH-containing microneedles at the end of 24 h (SI 7 in the Supplementary Material). An initial burst release was observed for the first 3 h, with a gradual increase over time and relatively constant release thereafter until 24 h.

In Vitro Permeation Assay with Horizontal Diffusion Cell

Microneedles containing 4.31% w/w of LH were tested for their ability to enhance permeation across rat skin as compared to a propylene glycol solution containing 4.31% w/w LH. 320.8 ± 147.5 µg of LH permeated through rat skin after a period of 48 h, with a 78.2-fold increase in permeation amount over control (SI 8 in the Supplementary Material). Microneedles delivered a consistently higher amount of LH, while

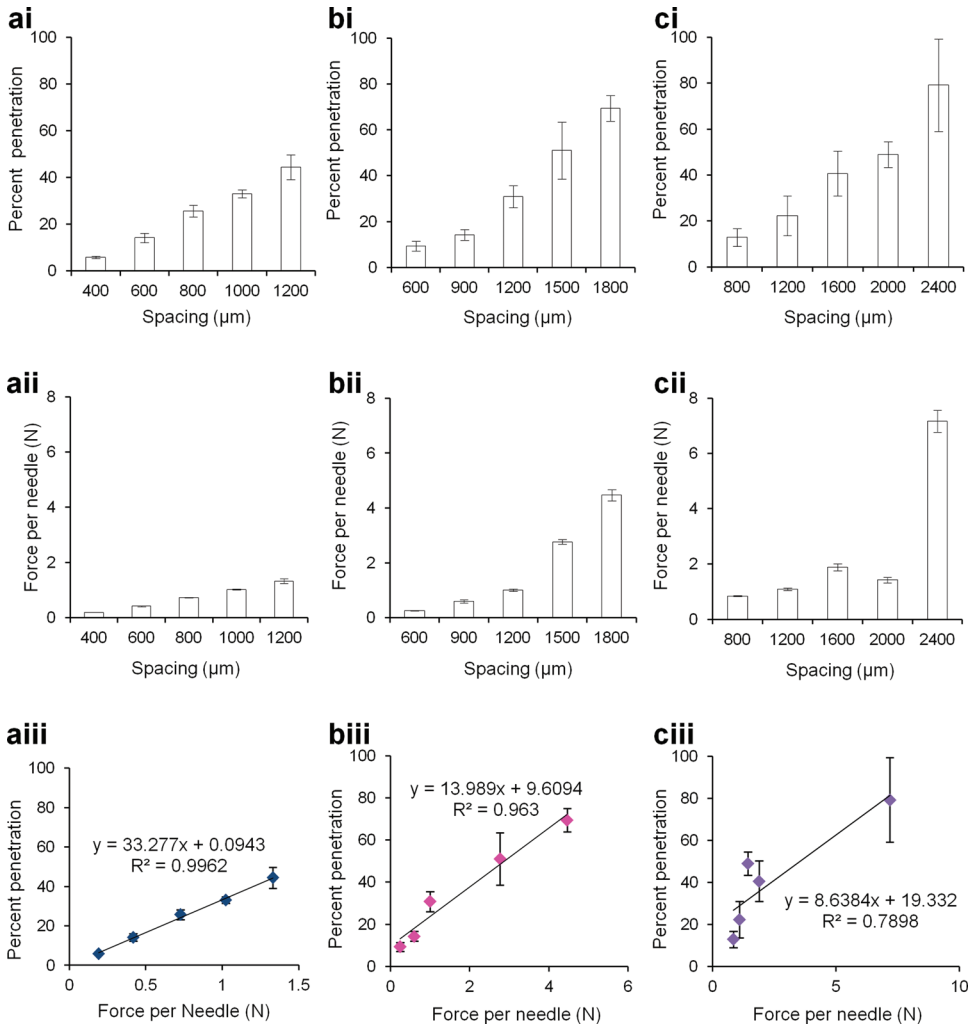

Figure 2. The first row shows percentage penetration for needles against centre-to-centre spacing of 2×/3×/4×/5×/6× of base diameters for base diameters of (ai) 200, (bi) 300, and (ci) 400 μm. The second row shows graphs of needle penetration force against centre-to-centre spacing of 2×/3×/4×/5×/6× for base diameters of (aii) 200, (bii) 300, and (cii) 400 μm. The third row shows linear regressions of percentage penetration against force per needle for base diameters of (aiii) 200, (biii) 300, and (ciii) 400 μm.

passive diffusion resulted in a much lower permeation of the drug. While the amounts released from microneedles in PBS were much higher, due to inherent barrier properties of skin and interaction of drugs with skin components, the permeated amounts were lower.

DISCUSSION

Mechanically, skin behaves like a viscoelastic tissue,[23] and behaves more as an elastic tissue with light applied loads.[24] Microneedle geometry is critical to ensure efficent penetration through skin. It has been observed previously that microneedle insertion in skin led to skin retraction and a 1080 μm insertion in skin using single microneedles caused indentations on the skin with only 100–300 μm penetrating the skin.[10] While this effect was observed for single microneedles, the situation is even more complex when using a microneedle array. At the same time, geometrical dimensions of microneedle shafts play a critical role in efficient penetration through the skin. These factors motivated us to study microneedle geometric properties in detail.

Using our photolithographical approach,[19] we fabricated microneedles possessing a tapered vertical profile, which could be explained through the loss of energy as the UV light travels a distance through the setup. The photopolymerization is governed by the inverse square law of light, where the intensity of photons emitted from the light source is inversely related to the square of the distance from the light source.[25] However, we observed that irrespective of the base diameter of microneedles, the decrease in intensity and hence the tapering of microneedles shafts remained consistent, giving a uniform base-to-tip ratio (Fig. 1).

In this study, microneedle geometry in terms of varying center-to-center spacing has been shown to be a crucial factor in the penetration profile into skin. An increase in force per needle was seen with increased center-to-center spacing of needles for all base diameters; suggesting that needles with higher center-to-center spacing are able to penetrate the skin better. This correlates well with the findings by Olatunji et al.[15] where

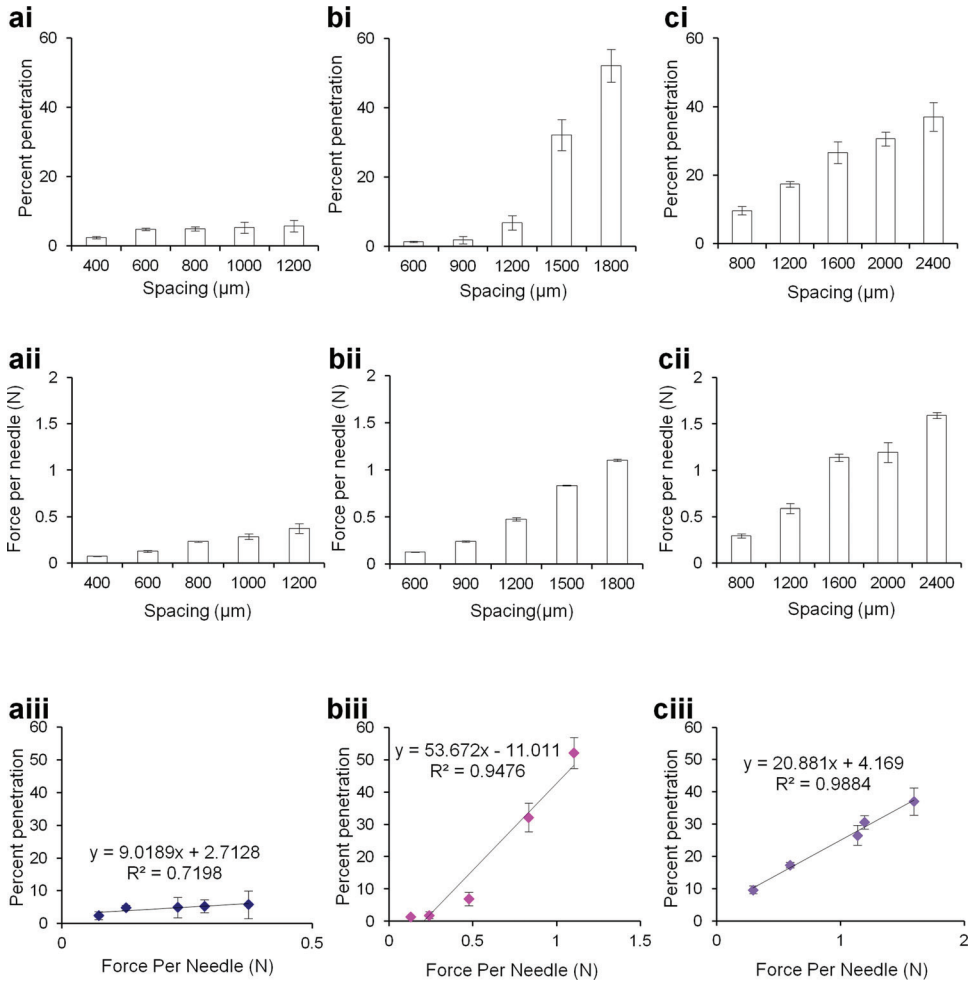

Figure 3. The first row shows percentage penetration for needles against centre-to-centre spacing of 2×/3×/4×/5×/6× of base diameters for base diameters of (ai) 200, (bi) 300, (ci) 400 μm. The second row shows graphs of needle penetration force against centre-to-centre spacing of 2×/3×/4×/5×/6× for base diameters of (aii) 200, (bii) 300, and (cii) 400 μm. The third row shows linear regressions of percentage penetration against force per needle for base diameters of (aiii) 200, (biii) 300, and (ciii) 400 μm.

an increase in needle interspacing results in lower resistance for penetration in skin, which could result in higher needle penetration rates, and thus, a greater quantity of drug delivery. Apart from center-to-center spacing, 300 and 400 μm base diameter needles showed the most promising penetration profiles with high penetration rates, while 200 μm base diameter needles had a low penetration rate, which could be attributed to its thin needle profile that results in a weaker structure which buckles more easily upon insertion onto the surface of the skin than for needles of larger base diameters. Microneedles with 300 μm base diameter, 1800 μm spacing, and 400 μm base diameter, 2400 μm spacing gave consistent and relatively high percentage of microneedles penetrating the skin. In addition, because sharper needles have a larger length-to-base aspect ratio in our study, needles with a smaller length-to-base aspect ratio penetrate skin better. (In this study, all needles have the same length and a sharper needle has a smaller base diameter).

It has been previously reported that decrease in the tip diameter enhanced microneedle penetration irrespcetive of center-to-center spacing.[26,27] This is true for microneedles fabricated from robust materials like silicon and metals. However, for polymeric microneedles, it has been reported that decreased tip diamater led to microneedle shaft weakness and easy fracture, which may potentially impact microneedle insertion.[28] In our study, we also observed that for lower tip diameters, microneedle shafts were weaker and hence lower extent of penetration was observed for the microneedles with 200 μm base diameter (Figs. 2 and 3).

At the same time, our study highlights the effect of microneedles made from Poly(ethylene glycol) -based material, and hence the mechanical properties of harder materials may influence microneedle penetration differently. While harder materials like silicon and metal are not influenced much by microneedle geometry, polymeric microneedles require a careful calibration of geometrical properties to enhance their penetration efficacy.

The availability of human skin samples for research purposes has been limited, prompting the use of rat skin as a substitute for human skin. As these human skin suurogates do not mimic the mechanical properties, it is essential to develop suurogate models to be used with rat skin. To our knowledge, this is the first such study to investigate the extent to which certain substrates placed under excised rat skin mimic the mechanical tissue support of human skin.

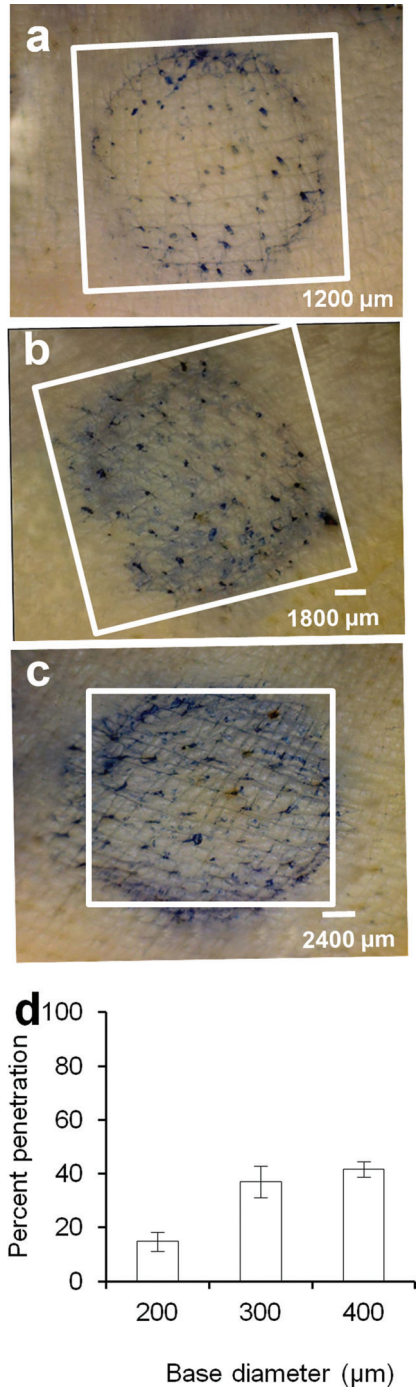

Figure 4. Microneedle penetration performed on pig ear skin for (a) 200 µm base diameter, 1200 µm center-to-center spacing, (b) 300 µm base diameter, 1800 µm center-to-center spacing, and (c) 400 µm base diameter, 2400 µm center-to-center spacing microneedles. (d) the percentage of needles penetrated into pig ear skin for each needle base diameter. Triplicates were used for each base diameter.

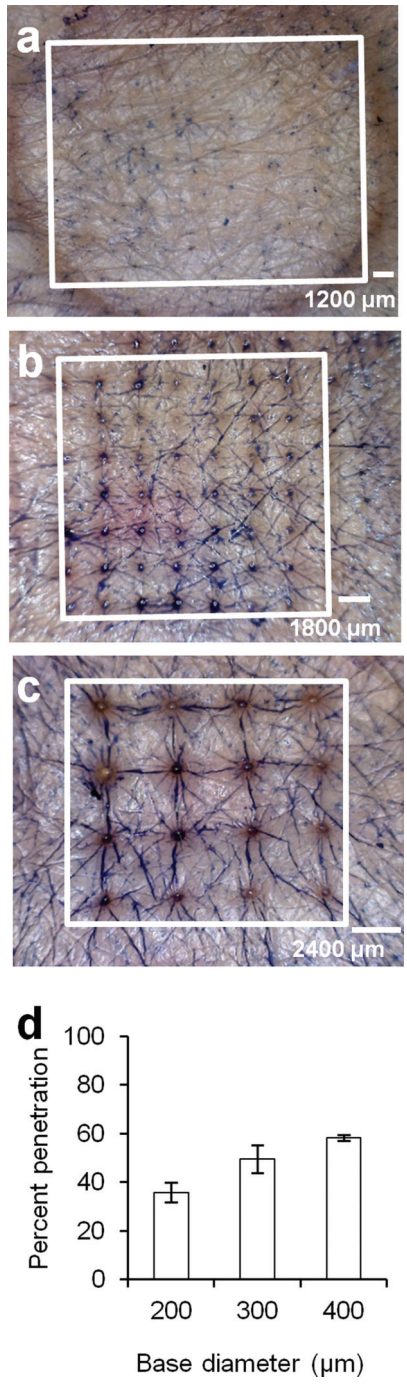

Figure 5. Microneedle penetration performed on full-thickness human cadaver skin for (a) 200 µm base diameter, 1200 µm center-to-center spacing, (b) 300 µm base diameter, 1800 µm center-to-center spacing, and (c) 400 µm base diameter, 2400 µm center-to-center spacing microneedles. (d) the percentage of needles penetrated into human skin for each needle base diameter. Triplicates were used for each base diameter.

Due to factors such as different mechanical properties and elasticity of various layers of skin as well as the force of application, microneedle arrays may penetrate to different extent during *in vitro* studies. We observed the hard substrate (clay) used resulted in an overestimation of the penetration ability of the microneedles (Fig. 2), while the soft substrate (PDMS) (Fig. 3) and full-thickness pig skin (Fig. 4) gave a slight underestimate of the penetration ability of the microneedles on full-thickness human skin (Fig. 5).

Thus, we propose that apart from pig skin, PDMS will be a reliable substrate for tissue-like mechanical support under skin to mimic *in vivo* human skin conditions for the purpose of future microneedle studies.

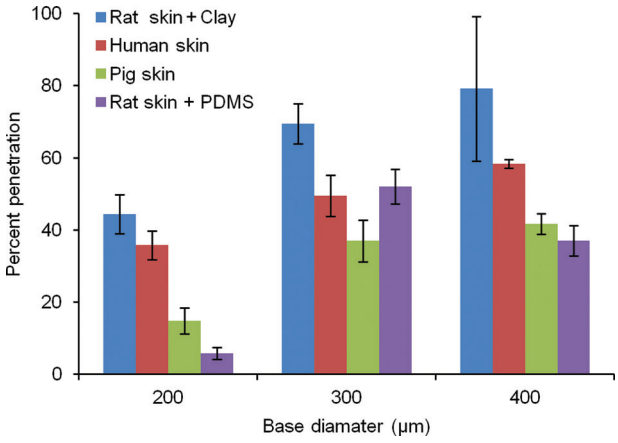

Figure 6. Comparison of percentage of microneedle penetration for various base diameter of microneedles on different animal skin models and human skin.

In the drug release and skin permeation study, the microneedles were effective in releasing LH into PBS, with nearly all drug (93.9% and 91.3%) released after 24 h. While previous studies at our lab observed a slower release of rhodamine B encapsulated in polymeric microneedles into PBS which reported nearly 60% release after a week,[19] possible reasons for high LH could be due to the greater hydrophilicity of LH (log $P = -1.41$)[29] as compared to rhodamine B (log $P = 2.43$). This demonstrates that bolus delivery or sustained delivery could be a possibility with such transdermal microneedle patches. The optimized geometries of microneedles were also effective in delivering LH through rat skin, showing a 78.2-fold higher permeation amount than control. This demonstrated the potential of the polymeric microneedles to deliver drugs for therapeutic use through skin.

CONCLUSIONS

We demonstrated that microneedle geometry can be controlled by using PEGDA macromers through a photolithographical process. Geometry was shown to be critical for efficient penetration through the skin, and an increase in spacing between microneedles results in an increased force of application per needle, resulting in higher penetration through the skin. In addition, we showed the importance of substrate stiffness for *in vitro* skin permeation studies using substitutes like rat skin. PDMS, possibly possessing mechnical properties in close resemblance to inherent subcutaneous tissues, offered a better model than previously used harder alternatives, which overestimated microneedle penetrtaion. The optimized geometries were shown to enhance the permeation of a model drug LH, potentially making them applicable for clinical applications.

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