

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: <https://orca.cardiff.ac.uk/id/eprint/104510/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Davies, Lleucu, Gateley, Christopher, Holland, Phillip, Coulman, Sion and Birchall, James 2018. Accelerating topical anaesthesia using microneedles. *Skin Pharmacology and Physiology* 30 (6) , pp. 277-283. 10.1159/000479530

Publishers page: <http://dx.doi.org/10.1159/000479530>

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See <http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.



Accelerating Topical Anaesthesia using Microneedles

Lleucu B Davies

*Siôn A Coulman

*James C Birchall

*School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, CF10 3NB,
UK*

*Joint senior authors

Corresponding Author

James C Birchall

School of Pharmacy and Pharmaceutical Sciences

Cardiff University

Redwood Building

King Edward VII Avenue

Cardiff

CF10 3NB

Tel: +44 2920875815

Email: birchalljc@cf.ac.uk

Abstract

Background/Aims: Topical anaesthetics reduce pain during venous access procedures in children. However, clinical use is hindered by a significant anaesthetic onset time. Restricted diffusion of the topical anaesthetic through the stratum corneum barrier is the principal reason for the delayed onset. Microneedles can painlessly pierce the skin. This study evaluated microneedle pre-treatment of *ex vivo* human skin as a means to increase the rate of tetracaine permeation, in order to accelerate the onset of anaesthesia.

Methods: Franz-type diffusion cells were used to determine permeation of a commercial tetracaine formulation, Ametop gel, through human skin epidermis. Microneedle-assisted permeation was compared to untreated epidermis. Upon completion of permeation studies the epidermal membranes were visually characterised.

Results: At 30 minutes $5.43 \mu\text{g}/\text{cm}^2$ of tetracaine had permeated through untreated membrane compared to $12.13 \mu\text{g}/\text{cm}^2$ in microneedle treated membrane. Insertion of a hypodermic needle created a large single channel in the epidermis (approximately $4250\mu\text{m}^2$) whilst the punctured surface area following microneedle treatments was estimated to be $75,000\mu\text{m}^2$.

Conclusion: Pre-treatment of skin with microneedles significantly enhances the permeation of tetracaine. Microneedles have the potential to more than halve the onset time for anaesthesia when applying Ametop gel.

Keywords

Microneedles, Topical Anaesthetic, Tetracaine, Skin Permeation

1. Introduction

Venous access procedures are some of the most regularly performed invasive procedures in children [1]. However, they are described by children as some of the most painful procedures [1]. Two topical anaesthetics are commonly applied to children before undergoing venous access procedures, EMLA cream and Ametop gel [2, 3]. EMLA cream, containing a mixture of 2.5% lidocaine and 2.5% prilocaine [4, 5], requires an application time of 1 hour to be effective [5, 6] whilst Ametop gel contains 4% tetracaine base [7] and requires an application time of 30 - 45 minutes [7, 8]. These application times are required to provide a suitable depth of anaesthesia and are determined by the rate at which the anaesthetic can diffuse, passively, across the skin's stratum corneum [9]. The onset time of at least 30 minutes necessitates a two-stage clinical interaction, whereby the patient is treated topically with the anaesthetic and then after a period of 30-60 minutes is recalled for the procedure. The delayed onset is a deterrent to use of topical anaesthetics for children undergoing venous access

procedures in urgent and emergency situations [10] and increases the burden on clinical and nursing staff [11]. A number of different techniques have been employed in an attempt to enhance anaesthetic permeation through the skin, such as the use of menthol [12], menthol and ethanol [13], ultrasound [14], iontophoresis [15], electroporation [15], an occlusive transdermal patch [16] and a heated patch [11, 17].

Microneedle devices typically consist of arrays of microscopic needles that are able to pierce the stratum corneum in a minimally-invasive and painless manner, opening drug permeation pathways through the defining layer of the skin barrier [18-24]. Previous studies have shown that microneedles can improve permeation of lidocaine into the skin. These studies used microneedles to either (i) pre-treat skin before topical application of a lidocaine hydrochloride solution [25] or a hydrogel formulation [26, 27], (ii) deliver lidocaine from a dry coated microneedle surface [28] or (iii) encapsulate lidocaine within a microneedle patch [29]. The formulations used in these studies are currently not clinically approved for human use.

The focus of this study was to investigate, for the first time, whether microneedle pre-treatment of skin can increase the permeation, and thus accelerate the onset, of a commercially available topical anaesthetic, Ametop gel. The study was designed with rapid clinical translation in mind i.e. the topical anaesthetic has not been re-formulated or integrated into the microneedle device and so evaluation of the device alone should be sufficient to facilitate clinical translation.

2. Materials & Methods

2.1 Preparation of human skin epidermal membrane

Frozen human breast skin from donors aged between 60 and 73, obtained from mastectomy or breast reduction surgery under informed patient consent and ethical approval, was allowed to thaw at room temperature for 1 hour. Subcutaneous fat was removed by blunt dissection before the skin was placed in 60°C deionised water for 55 seconds. The epidermis was then manually separated from the dermis using forceps. The isolated epidermal membrane was stored at -20°C before use.

2.2 Evaluating tetracaine permeation from a commercial topical formulation through intact or punctured epidermal membrane

Frozen human epidermal membrane was allowed to thaw at room temperature for 20 minutes. Areas of membrane of approximately 2.5 cm², were prepared and either pre-treated with i) an array of 30 stainless steel microneedles, each measuring 500µm in length and 200µm in width (Figure 1), inserted for 10 seconds; ii) a single puncture with a 26G hypodermic needle (BD, Oxford, England); or (iii) left untreated. The membranes were mounted in Franz-type diffusion cells and the receptor phase of each was filled with phosphate buffered saline (PBS). The diffusion cells were left to equilibrate for 30 minutes before Ametop gel (Smith & Nephew, London, England), 0.5g, was weighed into the donor compartment of the diffusion cells. The donor compartment was then occluded with parafilm (Fisher Scientific, Loughborough, England) and the cells were maintained at 37°C in a water bath, with the receptor phase being continuously stirred. Samples, 200µl, were withdrawn from the sampling arm at regular time points between 5 and 360 minutes and an equal volume of PBS was replaced. In a second experiment intact epidermal membrane (N=8) was compared against microneedle-treated skin (N=9). No hypodermic control was used in this study to permit larger sample numbers.

Absorption spectra of different concentrations of Ametop gel, diluted in PBS, were measured on an Agilent Cary 60 UV-Vis spectrophotometer (Agilent Technologies, Cheshire, England), to determine

the peak absorption wavelength for tetracaine (310nm). A range of Ametop gel concentrations (diluted in PBS) were then evaluated at 310nm to create a calibration curve that was subsequently used to determine tetracaine concentration in experimental samples. Paired t-tests and repeated measures ANOVA were performed; a p-value < 0.05 was considered significant.

Figure 1: The microneedle device used to puncture the skin in this study.

The microneedles are arranged in three rows of ten needles and are held in a bespoke applicator prepared by additive manufacturing.

2.3 Imaging epidermal membranes

Following Franz diffusion studies, epidermal membranes were retrieved and imaged using a scanning electron microscope (SEM; Philips XL30) to characterise the channels created by microneedle devices and hypodermic needles. A non-contact optical profiler (Zeta) was also used to confirm the sizes of the channels and to obtain accurate surface area measurements. The Zeta optical profiler uses five separate measurement techniques to provide accurate dimensions and thicknesses of different elements of the sample being imaged, and also the roughness of the sample surface, resulting in 3-dimensional profiles of the samples being imaged [30].

3 Results and Discussion

3.1 Tetracaine permeation from a commercial topical formulation through intact or punctured epidermal membrane

The transdermal permeation of tetracaine through human epidermal membranes, having undergone different pre-treatments, was compared. Figure 2 shows a significant difference between the transdermal permeation of tetracaine at 360 minutes through skin pre-treated with microneedles (N=3) compared to both non-treated skin (N= 3, p=0.026, paired 1-tailed t-test) and skin pre-treated with a

hypodermic needle (N=3, $p=0.01$, paired 2-tailed t-test). There was no significant difference in tetracaine permeation between the non-treated and hypodermic needle treated skin ($p=0.067$, paired 1-tailed t-test). However, at the more clinically relevant 60 minute time point there were no significant differences between the control and microneedle pre-treatment ($p=0.052$, paired 1-tailed t-test), control and hypodermic needle pre-treatment ($p=0.101$, paired 1-tailed t-test) or microneedle and hypodermic needle pre-treatment results ($p=0.097$, paired 2-tailed t-test). Nevertheless data (Figure 2) from this preliminary experiment, with low replicates (N=3), suggested that microneedle pre-treatment had the potential to enhance tetracaine permeation.

Figure 2: Transdermal permeation of tetracaine following topical delivery to intact, hypodermic needle treated or microneedle treated human epidermal membranes.

The transdermal permeation of tetracaine following topical application of Ametop gel to intact (negative control), hypodermic needle treated or microneedle treated epidermal membranes. Permeation over 360 minutes and 60 minutes (insert) are shown. (n=3 from 3 donors. Values are mean \pm standard deviation.)

Increased experimental replicates in a further study enabled more robust statistical comparison of tetracaine permeation through microneedle treated and untreated skin. Figure 3 shows the transdermal permeation of tetracaine through intact (control) and microneedle pre-treated (microneedle) epidermal membranes at time points up to 6 hours. Tetracaine permeation was significantly enhanced by microneedle treatment of the tissue over the duration of the study i.e. 360 minutes (Figure 3a); $p=0.004$, repeated measures ANOVA). More notably, permeation through microneedle treated membrane was also significantly greater over 60 minutes (Figure 3b); $p=0.006$, repeated measures ANOVA). This is clinically significant as Ametop gel is currently applied to the skin for 30 to 60 minutes before venous puncture [7]. However, enhancements to clinical practice would necessitate acceleration of tetracaine permeation at earlier time points and therefore the most clinically significant result is the statistically significant difference ($p=0.027$, repeated measures ANOVA) in tetracaine permeation between intact skin and microneedle skin as early as 10 minutes after addition of the Ametop gel (Figure 3b), where $3.89\mu\text{g}/\text{cm}^2$ permeates through microneedles treated skin compared to $0.82\mu\text{g}/\text{cm}^2$ in untreated skin.

Figure 3: Transdermal permeation of tetracaine following topical delivery to intact or microneedle treated human epidermal membranes.

The transdermal permeation of tetracaine following topical application of Ametop gel to intact (negative control) or microneedle treated epidermal membranes. Permeation over 360 minutes (a) and 60 minutes (b) are shown. (n=8 for untreated; n=9 for microneedle treated; from 3 donors. Values are mean \pm standard deviation.)

3.1.2 Predicting tetracaine onset time

It has been suggested that topical anaesthetics are under-utilised in children in accident and emergency situations because the delay in anaesthetic onset time disrupts patient flow through the department [10]. A reduced topical anaesthetic onset time could lead to the more wide spread use of Ametop gel in children, especially in emergency situations where short onset times are required. A line of best fit was therefore plotted through the data pertaining to microneedle treatment of skin and this was used to predict the permeation values for tetracaine at 30 and 60 minutes. These values were compared to the permeation values achieved using untreated skin. The results (Table 1) show that equivalent tetracaine permeation can be achieved in approximately half the time when human skin is treated with microneedles before application of the Ametop gel, thus suggesting that in the clinic microneedles have the potential to halve the onset time of the locally applied anaesthetic.

The results of this study are consistent with extant literature investigating microneedle-assisted permeation of local anaesthetics. Coated [28], encapsulated [29], and dissolving [31] microneedles all provide novel drug delivery systems that can potentially facilitate increased levels of anaesthetic permeation. Nayak et al [26] have also used a novel topical lidocaine formulation in combination with microneedles to accelerate anaesthetic permeation. However, all of these studies employ medicinal products that require formulation or re-formulation of the topical anaesthetic and therefore regulatory bodies are likely to consider them to be new medicinal products. This would necessitate extensive development and validation, in both pre-clinical and clinical testing, prior to commercial clinical readiness. The alternative, investigated in this study, is the use of a microneedle device i.e. a medical device, to pre-treat the skin surface prior to use of a commercially available topical anaesthetic cream in a clinically approved manner. Ametop gel was selected for this study, due to a more rapid onset than its clinical counterparts such as EMLA cream, and the aim was to enhance its permeation further

by pre-treatment of the skin with a microneedle. The simplicity of this approach is likely to expedite clinical translation and the minor deviation from current clinical protocol, i.e. pre-treatment with a microneedle, is also likely to encourage early clinical adoption. Doubling the rate of permeation, as witnessed in Figure 3: is likely to significantly reduce the time to anaesthesia, and this is supported by studies conducted by Li et al [32].

Table 1: Transdermal Permeation Comparison

The transdermal permeation of tetracaine in non-pre-treated epidermal membrane after 30 minutes and 60 minutes, and the equivalent time it would take to reach these levels in microneedle pre-treated epidermal membrane

Transdermal Permeation of Tetracaine ($\mu\text{g}/\text{cm}^2$)	Time (min)	
	No Pre-Treatment	Microneedle Pre-Treatment
5.43	30	12.13
15.03	60	31.99

3.2 Membrane Imaging

The mechanism of enhanced permeation is physical disruption of the skin barrier and therefore, further work was conducted to characterise skin puncture. SEM images were able to characterise the perforations created in the epidermal membrane by the 500 μm (length) x 200 μm (base width) microneedles and the standard hypodermic needle (10mm length and 450 μm width) (Figure 4). The Zeta profiler was used to confirm, and provide further texture to, the perforation morphologies shown by SEM (Figure 5). Measurements obtained using these imaging modalities were used to estimate the total surface area of epidermal membrane disruption during microneedle treatment (Table 2).

Although a single hypodermic needle channel has a greater surface area than a single microneedle channel, multiple microneedle punctures results in a greater total puncture area (Table 2), and thus explains the enhancement in tetracaine permeation (Figure 2). It is important to note that changes in the density of the array and the shape of microneedles may therefore facilitate further enhancements to permeation. It is also worth noting that the flexibility of microneedle manufacture enables microneedle arrays of different shapes and sizes to be produced relatively easily. This would potentially enable the clinician to select a microneedle device appropriate to the area being treated.

Whilst this study has only investigated one length of microneedle, it is also worth noting that microneedle length may be an important factor in the onset time for local anaesthesia. Further studies, in human volunteers, should therefore be performed to optimise microneedle length for venous access procedures in children. It is also worth noting that local anaesthetics are used at various sites of the body for a number of clinical applications. If microneedles were to be employed in these circumstances the length of the microneedle protrusion may have to be modified accordingly, although this requirement is likely to be easily satisfied by the flexible manufacturing approach that is often utilised predominantly for microneedle production.

Figure 4: SEM images of skin epidermal membranes and needles

A microneedle channel is shown in a), a hypodermic needle channel is shown in b), and a control membrane with no channels is shown in c). Microneedles are shown in d) and a hypodermic needle is shown in e).

Figure 5: Images of skin epidermal membrane using an optical profiler.

Skin epidermal membrane was imaged using a Zeta non-contact optical profiler. A microneedle channel is shown in a) and a hypodermic needle channel is shown in b).

Table 2: Estimated area of disruption to skin surface by microneedles and a hypodermic needle

Needles	Imaged width of channel μm	Imaged length of channel μm	Estimated area of a single channel μm^2	Estimated area for all channels μm^2
Microneedles	50	50	2,500	75,000
Hypodermic Needle	25	170	4,250	4,250

4 Conclusions

Microneedles do not penetrate as deeply into the skin as hypodermic needles, and cause significantly less pain and trauma than hypodermic needles [9, 33]. Nevertheless, microneedle treated skin has a greater total surface area of barrier disruption and thus facilitates increased permeation of a topically applied local anaesthetic. Pre-treatment of skin with microneedles has the potential to more than halve the onset time for anaesthesia with Ametop gel. [The next stage is to demonstrate this effect in a proof-](#)

of-concept clinical study whereby the depth and kinetics of anaesthesia is assessed after application of the topical anaesthetic, both with and without microneedle pre-treatment. The intervention described in the current study is a simple method using a simple medical device, with no requirement to reformulate the medicinal product. This may expedite clinical translation of this approach and could lead to the more routine use of Ametop gel in children, when fast anaesthetic onset times are necessary.

Acknowledgements

The authors gratefully acknowledge the assistance of Ian Holton (Acutance Scientific) and Vamsi Velidandla (Zeta Instruments) in conducting the optical profiling studies.

Conflicts of Interest

This work was funded by a grant from the South East Wales Academic Health Science Partnership's Health Technology Challenge Scheme. There are no conflicts of interest.

References

1. Wong DL and Baker CM, *Pain in children: comparison of assessment scales*. *Pediatr Nurs*, 1988. **14**(1): p. 9-17.
2. Ormandy S and Doyle E, *A comparison of EMLA cream and amethocaine gel for topical cutaneous analgesia in children*. *Acute Pain*, 1998. **1**(3): p. 28-30.
3. Wilks Z and Brekle B. *Peripheral venous cannulation of children*. 2005 25 March 2014 [cited 2016 11/01/2016]; 4:[Available from: <http://www.gosh.nhs.uk/health-professionals/clinical-guidelines/peripheral-venous-cannulation-children>].
4. Friedman PM, Mafong EA, Friedman ES, and Geronemus RG, *Topical anesthetics update: EMLA and beyond*. *Dermatol Surg*, 2001. **27**(12): p. 1019-26.
5. *Summary of Product Characteristics - EMLA*. 2013 25/03/2013 [cited 2016 19/01/2016]; Available from: <http://www.mhra.gov.uk/home/groups/spcpil/documents/spcpil/con1452230688112.pdf>.
6. Woolfson AD, McCafferty DF, McClelland KH, and Boston V, *Concentration-response analysis of percutaneous local anaesthetic formulations*. *Br J Anaesth*, 1988. **61**(5): p. 589-92.

7. *Summary of Product Characteristics - Ametop*. 2011 01/2011 [cited 2016 19/01/2016]; Available from: <http://www.mhra.gov.uk/home/groups/spcpil/documents/spcpil/con1450416853878.pdf>.
8. O'Brien L, Taddio A, Lyszkiewicz DA, and Koren G, *A critical review of the topical local anesthetic amethocaine (Ametop) for pediatric pain*. *Paediatr Drugs*, 2005. **7**(1): p. 41-54.
9. Gupta J, Denson DD, Felner EI, and Prausnitz MR, *Rapid local anesthesia in humans using minimally invasive microneedles*. *Clin J Pain*, 2012. **28**(2): p. 129-35.
10. MacLean S, Obispo J, and Young KD, *The gap between pediatric emergency department procedural pain management treatments available and actual practice*. *Pediatr Emerg Care*, 2007. **23**(2): p. 87-93.
11. Sawyer J, Febbraro S, Masud S, Ashburn MA, and Campbell JC, *Heated lidocaine/tetracaine patch (Synera, Rapydan) compared with lidocaine/prilocaine cream (EMLA) for topical anaesthesia before vascular access*. *Br J Anaesth*, 2009. **102**(2): p. 210-5.
12. Liu Y, Ye X, Feng X, Zhou G, Rong Z, Fang C, and Chen H, *Menthol facilitates the skin analgesic effect of tetracaine gel*. *Int J Pharm*, 2005. **305**(1-2): p. 31-6.
13. Fang C, Liu Y, Ye X, Rong ZX, Feng XM, Jiang CB, and Chen HZ, *Synergistically enhanced transdermal permeation and topical analgesia of tetracaine gel containing menthol and ethanol in experimental and clinical studies*. *Eur J Pharm Biopharm*, 2008. **68**(3): p. 735-40.
14. Zempsky WT, Robbins B, and McKay K, *Reduction of topical anesthetic onset time using ultrasound: a randomized controlled trial prior to venipuncture in young children*. *Pain Med*, 2008. **9**(7): p. 795-802.
15. Hu Q, Liang W, Bao J, and Ping Q, *Enhanced transdermal delivery of tetracaine by electroporation*. *Int J Pharm*, 2000. **202**(1-2): p. 121-124.
16. McCafferty DF and Woolfson AD, *New patch delivery system for percutaneous local anaesthesia*. *Br J Anaesth*, 1993. **71**(3): p. 370-4.
17. Ravishankar N, Elliot SC, Beardow Z, and Mallick A, *A comparison of Rapydan(R) patch and Ametop(R) gel for venous cannulation*. *Anaesthesia*, 2012. **67**(4): p. 367-70.
18. Birchall JC, Clemo R, Anstey A, and John DN, *Microneedles in clinical practice--an exploratory study into the opinions of healthcare professionals and the public*. *Pharm Res*, 2011. **28**(1): p. 95-106.
19. Coulman SA, Barrow D, Anstey A, Gateley C, Morrissey A, Wilke N, Allender C, Brain K, and Birchall JC, *Minimally invasive cutaneous delivery of macromolecules and plasmid DNA via microneedles*. *Curr Drug Deliv*, 2006. **3**(1): p. 65-75.
20. Donnelly RF, Morrow DIJ, McCarron PA, Woolfson AD, Morrissey A, Juzenas P, Juzeniene A, Iani V, McCarthy HO, and Moan J, *Microneedle-mediated intradermal delivery of 5-aminolevulinic acid: Potential for enhanced topical photodynamic therapy*. *J Control Release*, 2008. **129**(3): p. 154-162.
21. Donnelly RF, Singh TR, Tunney MM, Morrow DI, McCarron PA, O'Mahony C, and Woolfson AD, *Microneedle arrays allow lower microbial penetration than hypodermic needles in vitro*. *Pharm Res*, 2009. **26**(11): p. 2513-22.
22. van der Maaden K, Jiskoot W, and Bouwstra J, *Microneedle technologies for (trans)dermal drug and vaccine delivery*. *J Control Release*, 2012. **161**(2): p. 645-655.
23. Henry S, McAllister DV, Allen MG, and Prausnitz MR, *Microfabricated microneedles: a novel approach to transdermal drug delivery*. *J Pharm Sci*, 1998. **87**(8): p. 922-5.
24. Prausnitz MR and Langer R, *Transdermal drug delivery*. *Nat Biotechnol*, 2008. **26**(11): p. 1261-8.
25. Duan D, Moeckly C, Gysbers J, Novak C, Prochnow G, Siebenaler K, Albers L, and Hansen K, *Enhanced delivery of topically-applied formulations following skin pre-treatment with a hand-applied, plastic microneedle array*. *Curr Drug Deliv*, 2011. **8**(5): p. 557-65.
26. Nayak A, Das DB, and Vladisavljevic GT, *Microneedle-assisted permeation of lidocaine carboxymethylcellulose with gelatine co-polymer hydrogel*. *Pharm Res*, 2014. **31**(5): p. 1170-84.
27. Nayak A, Short L, and Das DB, *Lidocaine permeation from a lidocaine NaCMC/gel microgel formulation in microneedle-pierced skin: vertical (depth averaged) and horizontal permeation profiles*. *Drug Deliv Transl Res*, 2015. **5**(4): p. 372-86.

28. Zhang Y, Brown K, Siebenaler K, Determan A, Dohmeier D, and Hansen K, *Development of lidocaine-coated microneedle product for rapid, safe, and prolonged local analgesic action*. Pharm Res, 2012. **29**(1): p. 170-7.
29. Kochhar JS, Lim WX, Zou S, Foo WY, Pan J, and Kang L, *Microneedle integrated transdermal patch for fast onset and sustained delivery of lidocaine*. Mol Pharm, 2013. **10**(11): p. 4272-80.
30. *3D Profiler*. 2016 [cited 2016 18/02/2016]; Available from: <http://www.zeta-inst.com/applications/3d-imaging-profiler-applications>.
31. Caffarel-Salvador E, Tuan-Mahmood TM, McElnay JC, McCarthy HO, Mooney K, Woolfson AD, and Donnelly RF, *Potential of hydrogel-forming and dissolving microneedles for use in paediatric populations*. Int J Pharm, 2015. **489**(1-2): p. 158-69.
32. Li X, Zhao R, Qin Z, Zhang J, Zhai S, Qiu Y, Gao Y, Xu B, and Thomas SH, *Microneedle pretreatment improves efficacy of cutaneous topical anesthesia*. Am J Emerg Med, 2010. **28**(2): p. 130-134.
33. Gill HS, Denson DD, Burris BA, and Prausnitz MR, *Effect of microneedle design on pain in human volunteers*. Clin J Pain, 2008. **24**(7): p. 585-94.

Figure 1: The microneedle device used to puncture the skin in this study.

The microneedles are arranged in three rows of ten needles and are held in a bespoke applicator prepared by additive manufacturing.

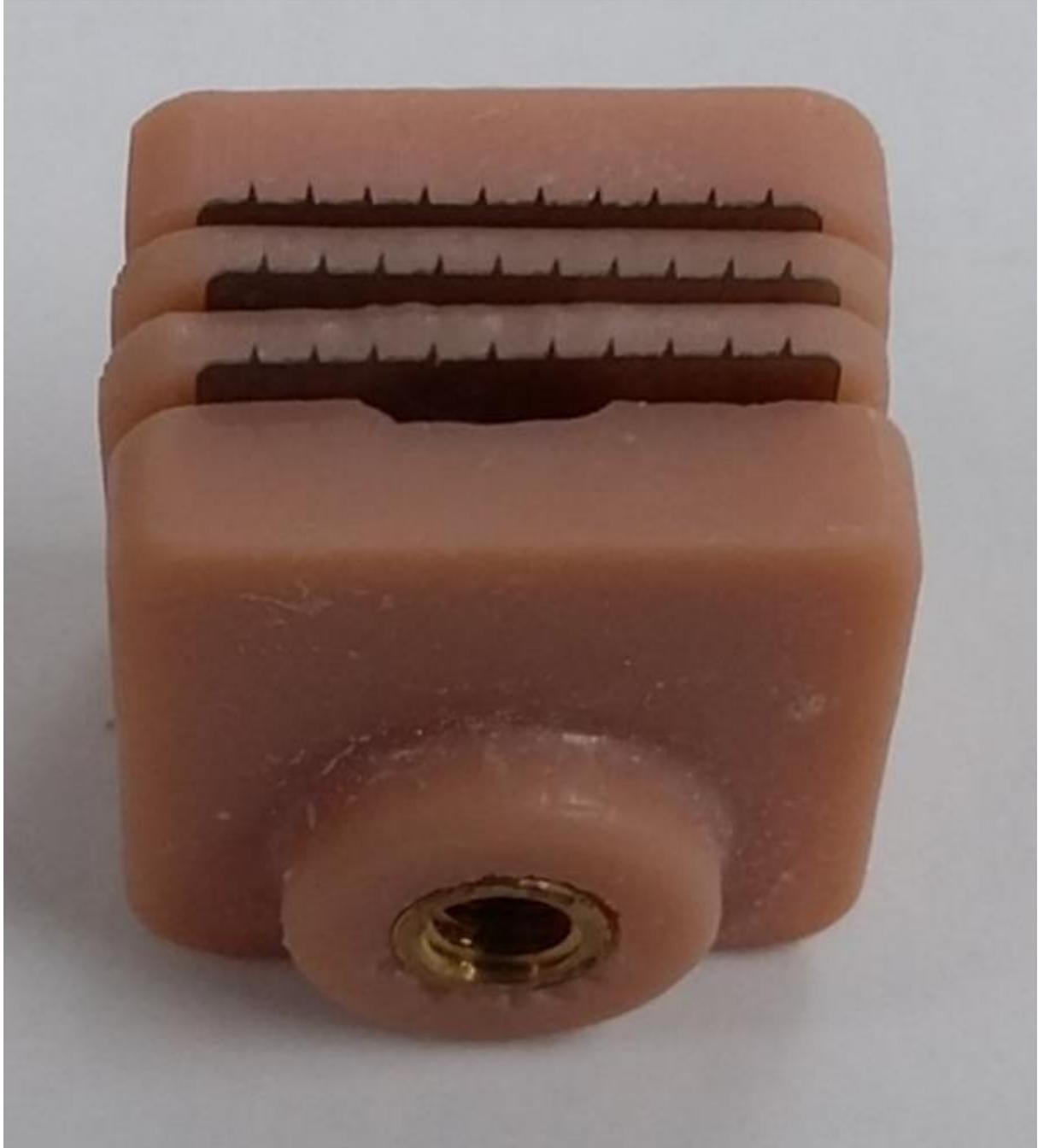


Figure 2: Transdermal permeation of tetracaine following topical delivery to intact, hypodermic needle treated or microneedle treated human epidermal membranes.

The transdermal permeation of tetracaine following topical application of Ametop gel to intact (negative control), hypodermic needle treated or microneedle treated epidermal membranes. Permeation over 360 minutes and 60 minutes (insert) are shown. (n=3 from 3 donors. Values are mean \pm standard deviation.)

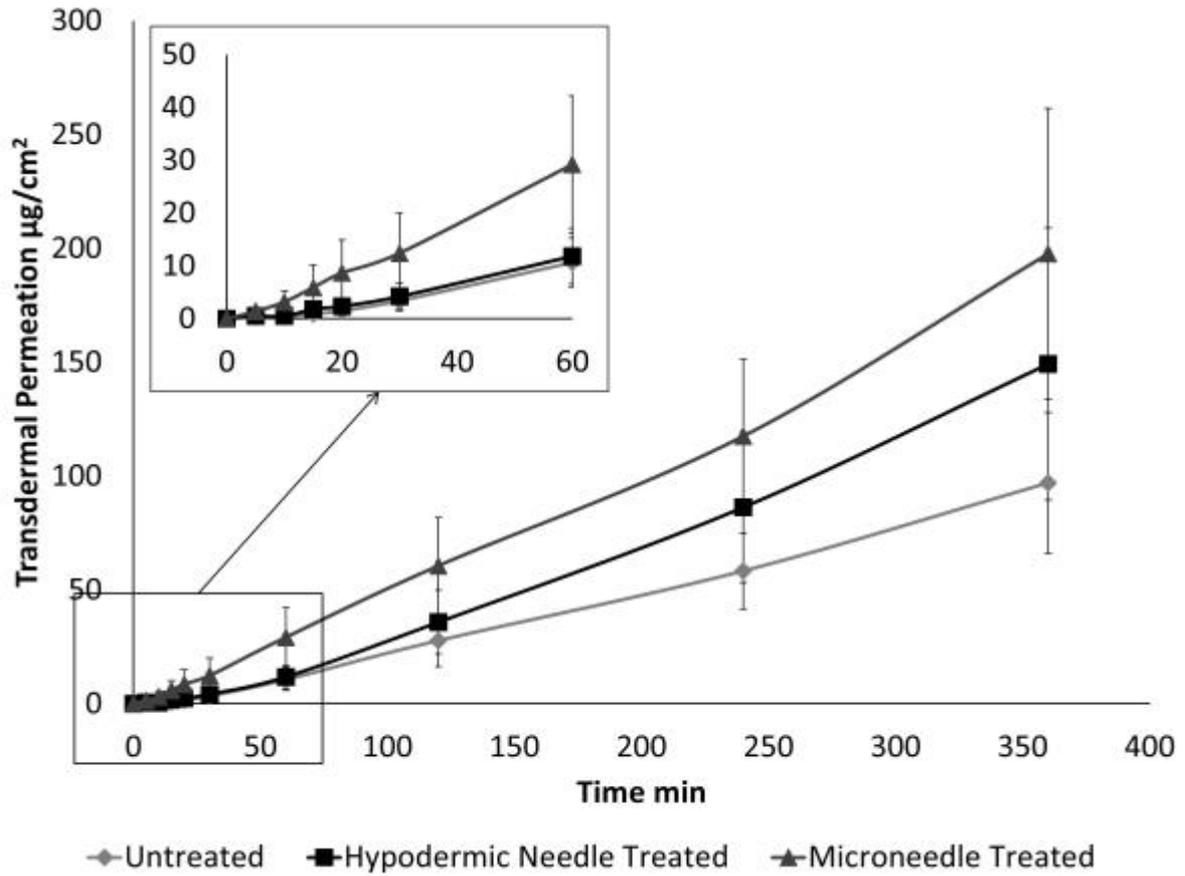


Figure 3: Transdermal permeation of tetracaine following topical delivery to intact or microneedle treated human epidermal membranes.

The transdermal permeation of tetracaine following topical application of Ametop gel to intact (negative control) or microneedle treated epidermal membranes. Permeation over 360 minutes (a) and 60 minutes (b) are shown. (n=8 for untreated; n=9 for microneedle treated; from 3 donors. Values are mean \pm standard deviation.)

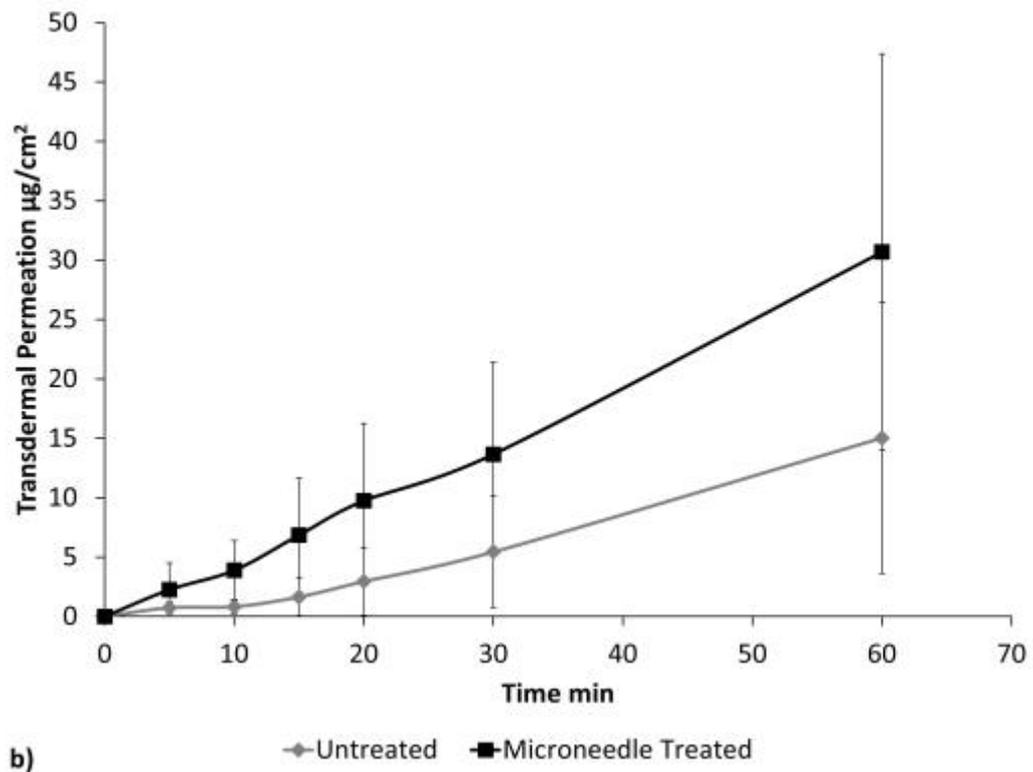
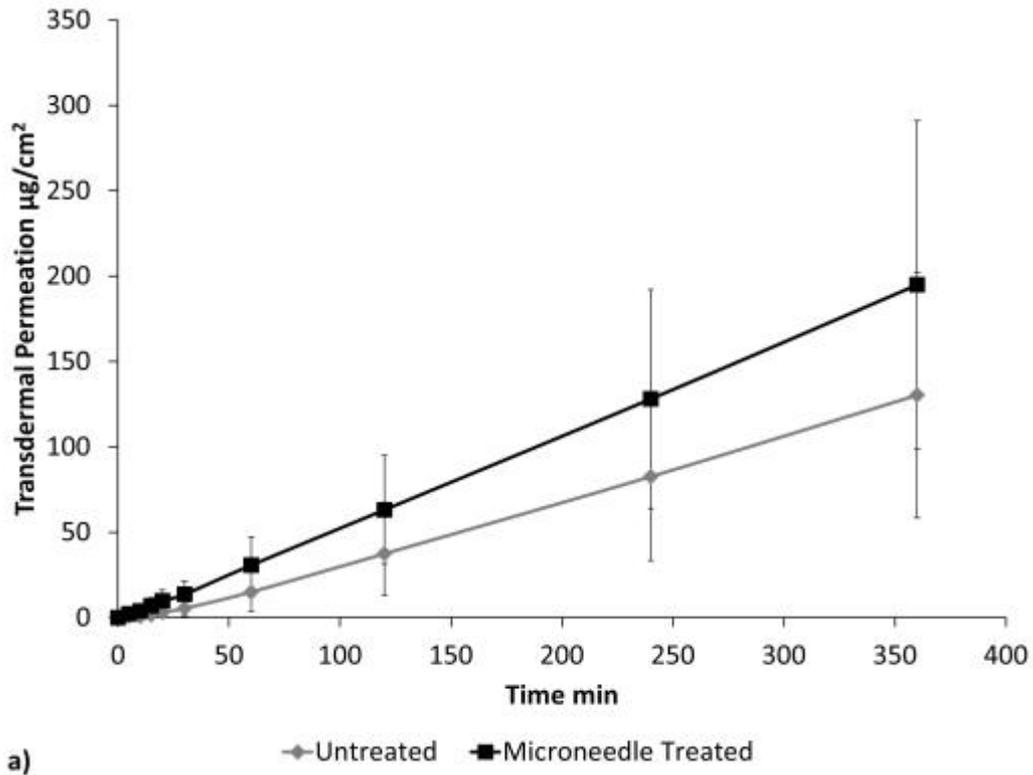
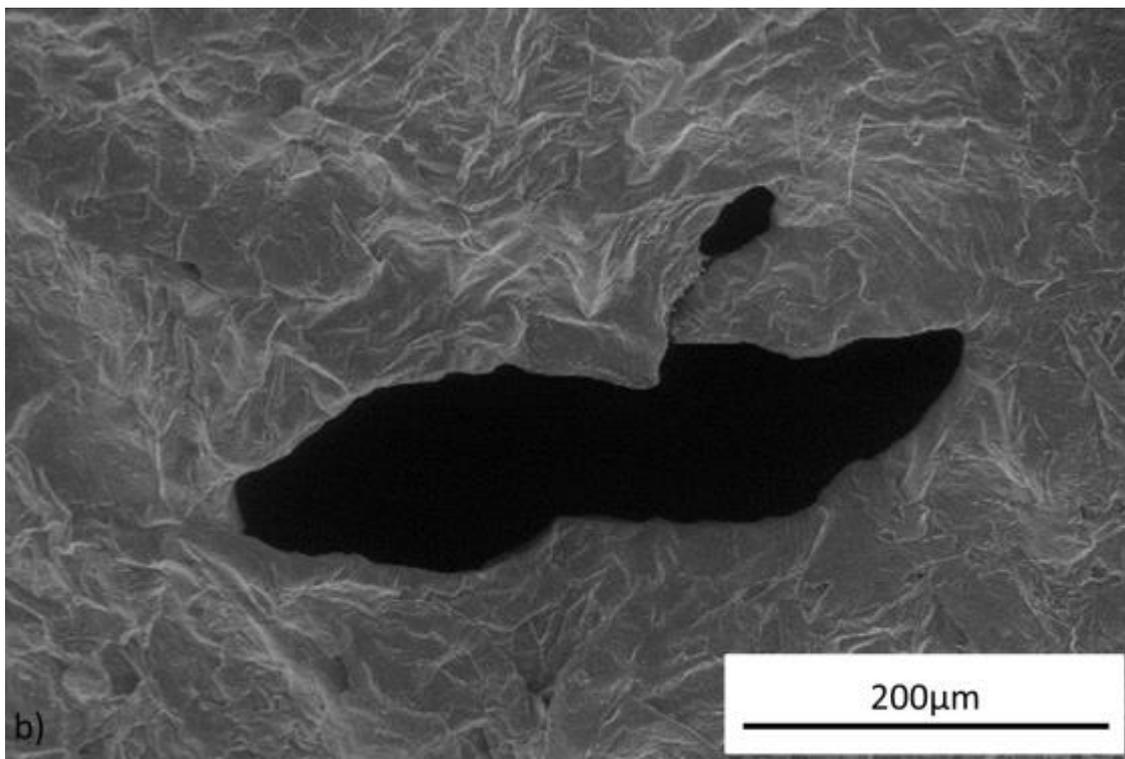
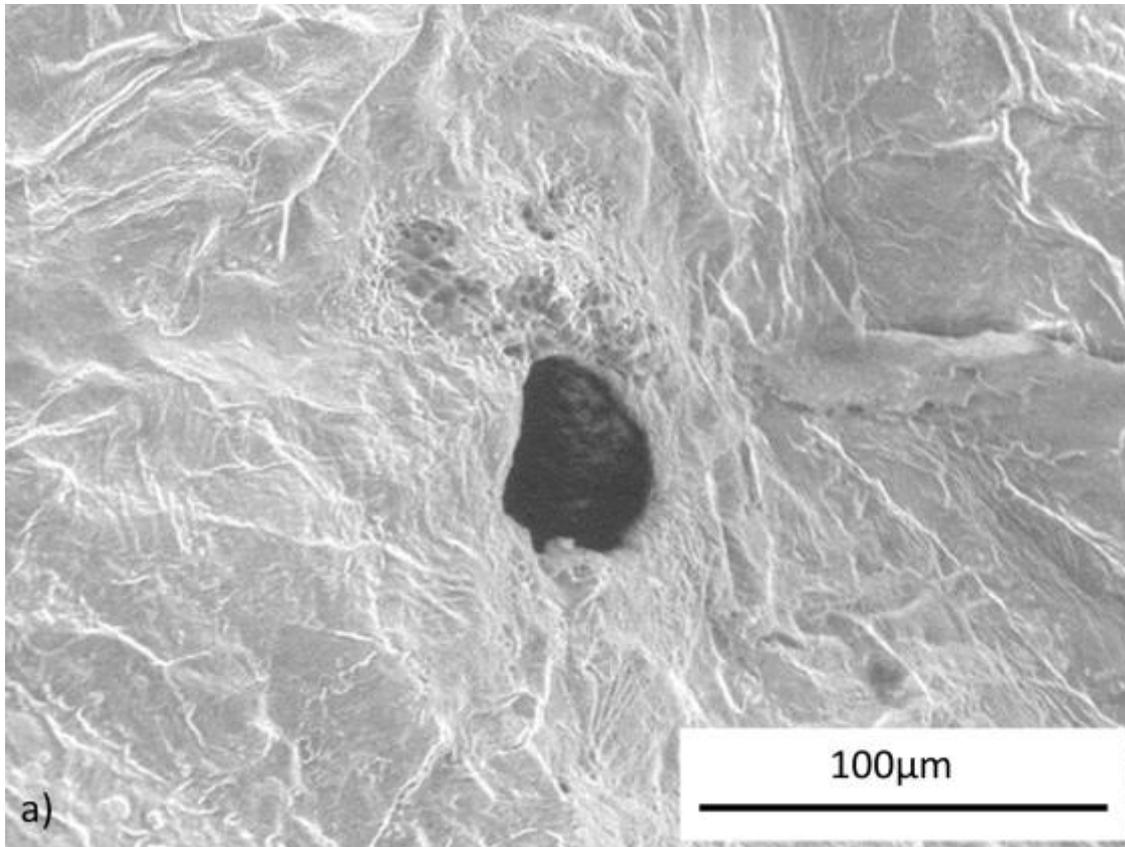
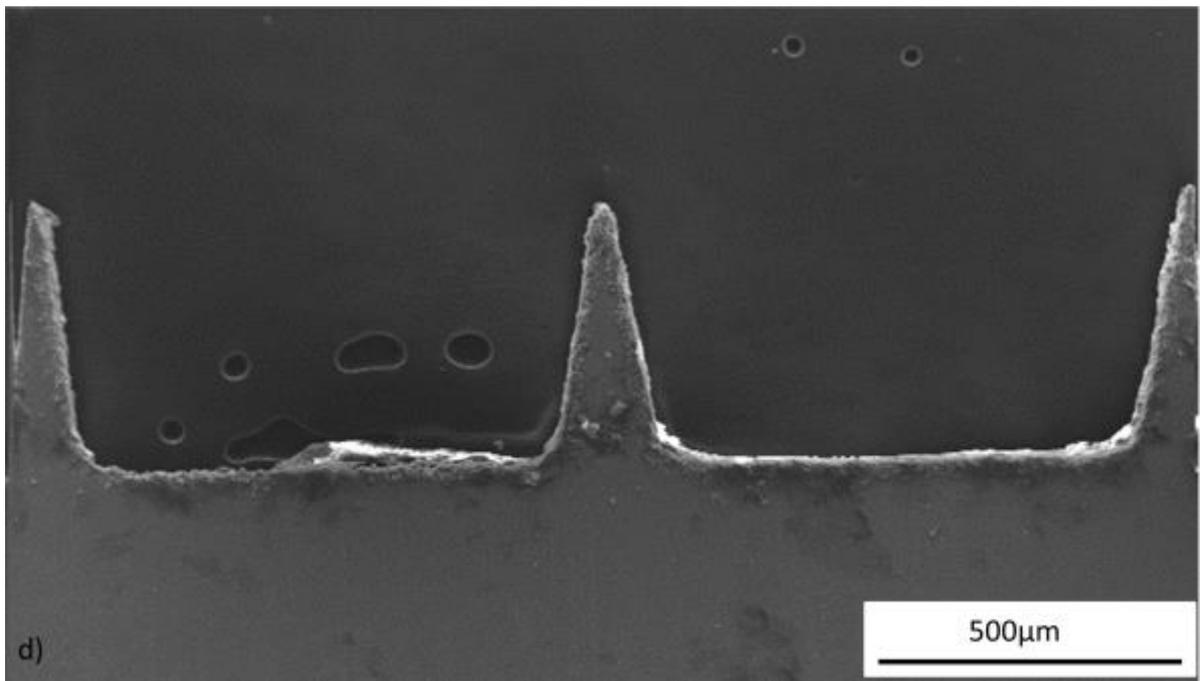
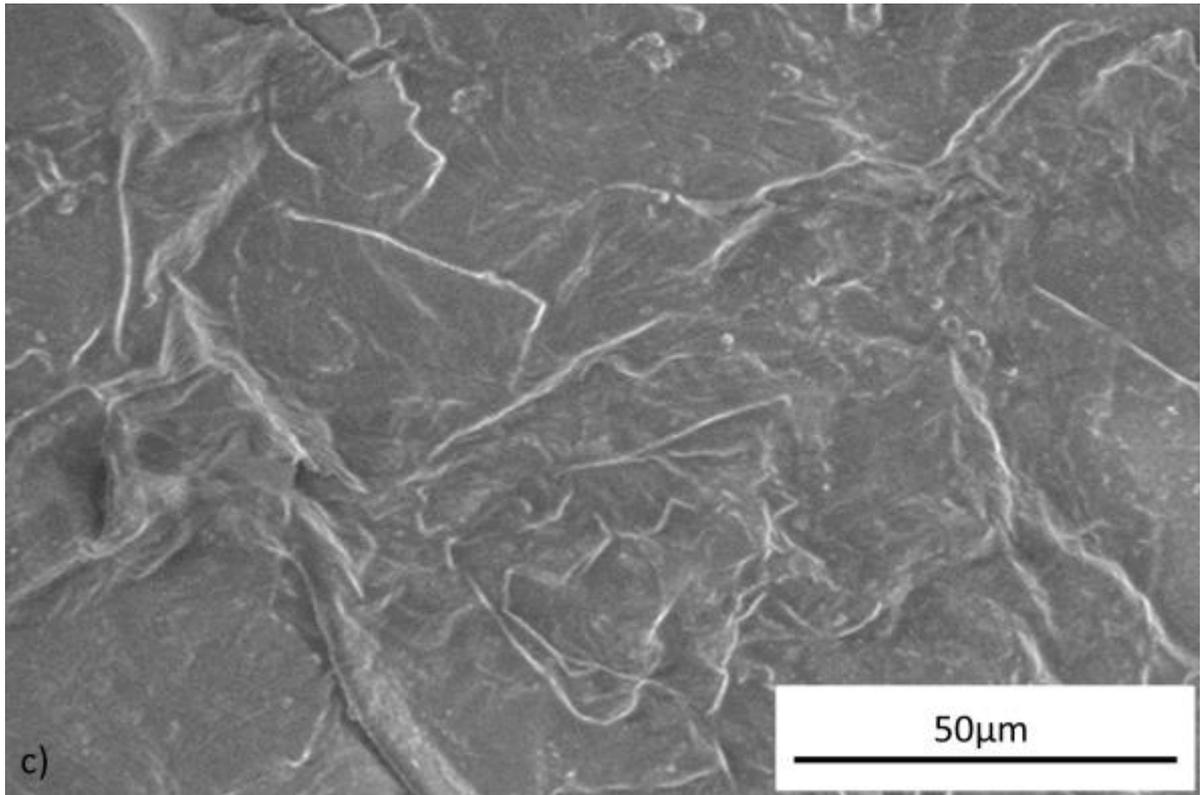
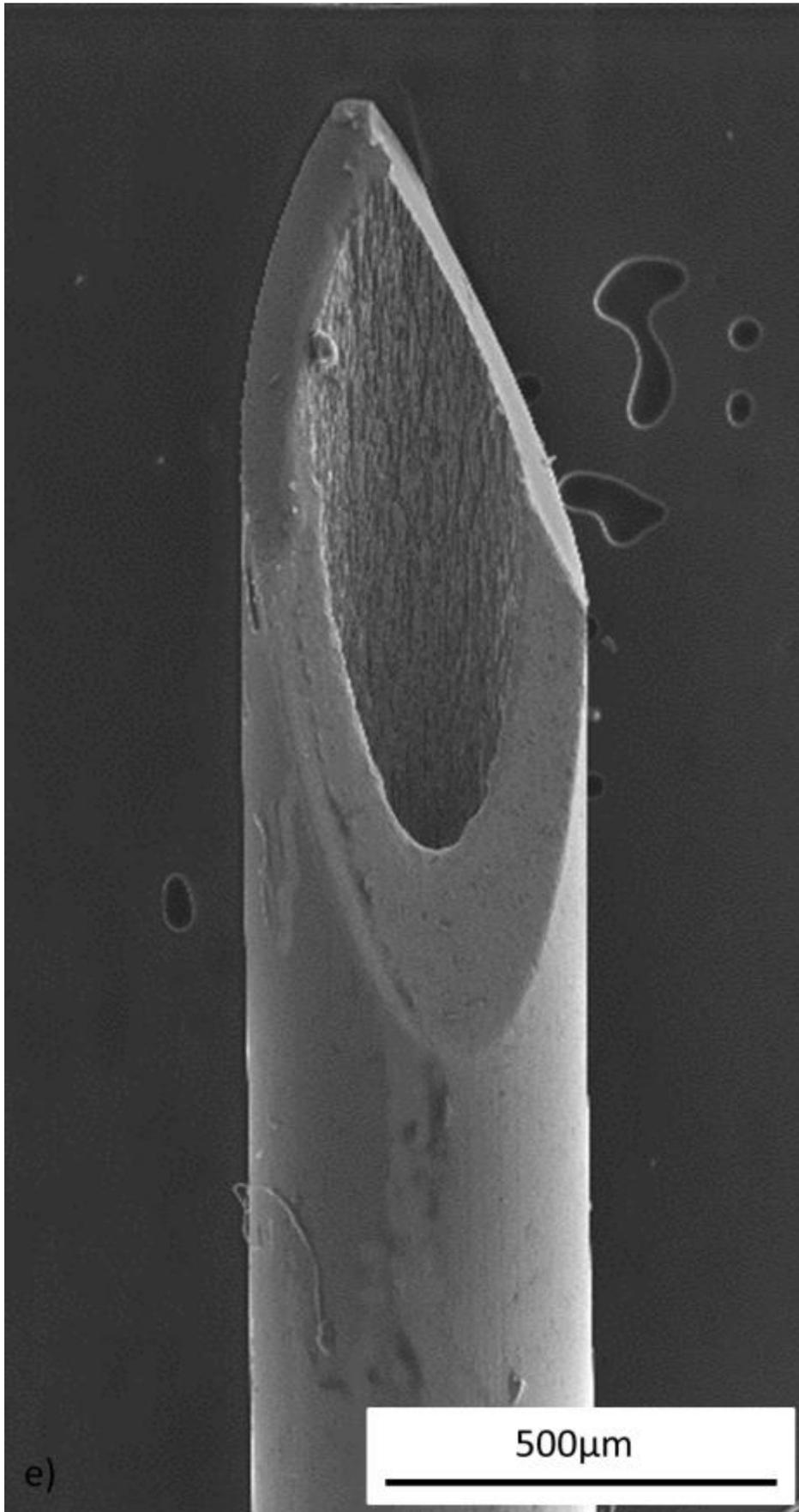


Figure 4: SEM images of skin epidermal membranes and needles.

A microneedle channel is shown in a), a hypodermic needle channel is shown in b), and a control membrane with no channels is shown in c). Microneedles are shown in d) and a hypodermic needle is shown in e).







e)

500µm

Figure 5: Images of skin epidermal membrane using an optical profiler.

Skin epidermal membrane was imaged using a Zeta non-contact optical profiler. A microneedle channel is shown in a) and a hypodermic needle channel is shown in b).

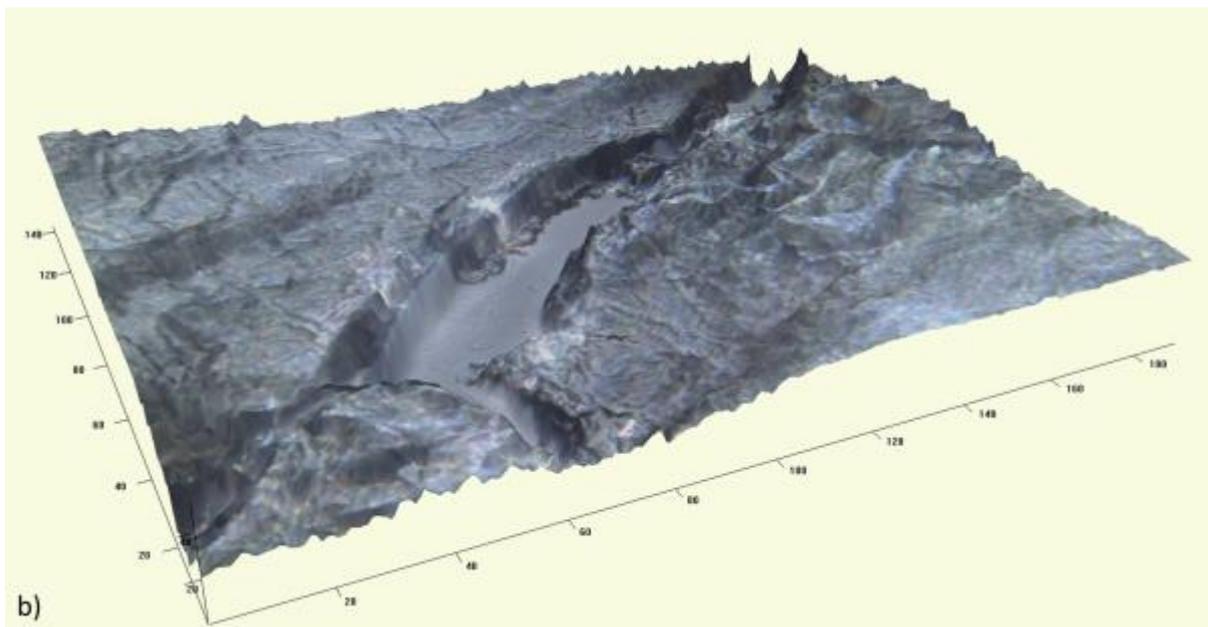
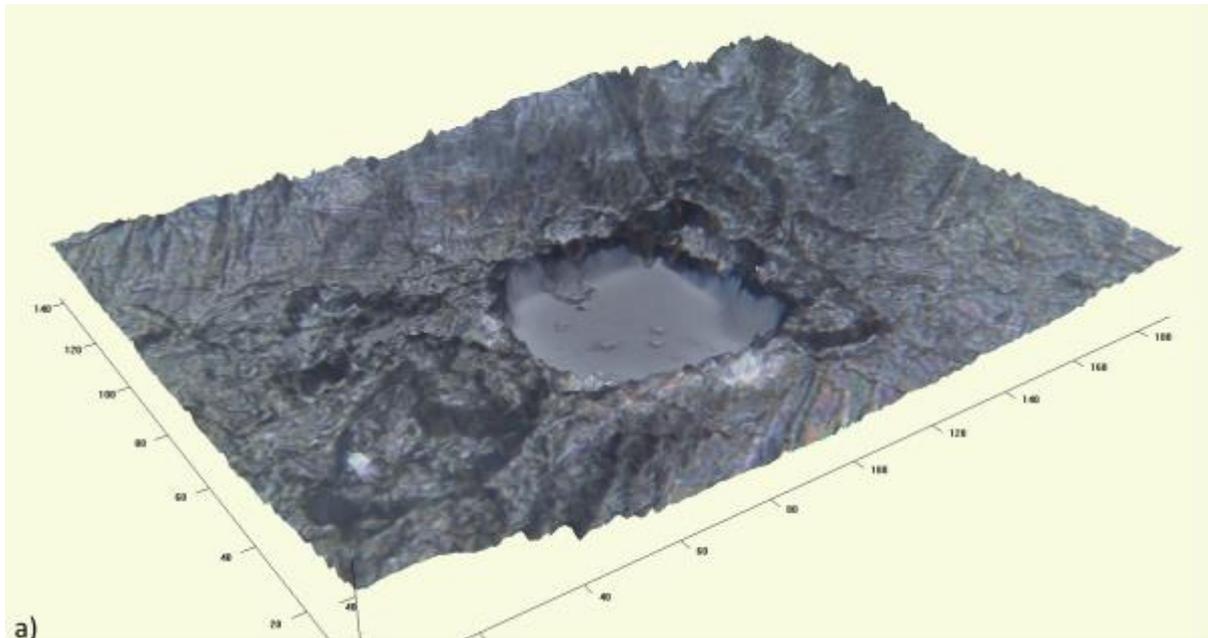


Table 1: Transdermal permeation comparison.

The transdermal permeation of tetracaine in non-pre-treated epidermal membrane after 30 minutes and 60 minutes, and the equivalent time it would take to reach these levels in microneedle pre-treated epidermal membrane.

Transdermal Permeation of Tetracaine ($\mu\text{g}/\text{cm}^2$)	Time (min)	
	No Pre-Treatment	Microneedle Pre-Treatment
5.43	30	12.13
15.03	60	31.99

Table 2: Estimated area of disruption to skin surface by microneedles and a hypodermic needle.

Needles	Imaged width of channel μm	Imaged length of channel μm	Estimated area of a single channel μm^2	Estimated area for all channels μm^2
Microneedles	50	50	2,500	75,000
Hypodermic Needle	25	170	4,250	4,250