Accelerating Topical Anaesthesia Using Microneedles

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Keywords

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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 the permeation studies, the epidermal membranes were visually characterised. **Results:** At 30 min, 5.43 µg/cm² of tetracaine had permeated through the untreated membrane compared to 12.13 µg/cm² through the microneedle-treated membrane. Insertion of a hypodermic needle created a large single channel in the epidermis (approx. 4,250 µm²) whilst

the punctured surface area following microneedle treatments was estimated to be 75,000 µm². **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.

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Introduction

Venous access procedures are some of the most regularly performed invasive procedures in children [1]. However, they are described by children as one 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 h to be effective [5, 6]. Ametop gel contains a 4% tetracaine base and requires an application time of 30–45 min [7, 8]. These application times are required to provide a suitable

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Fig. 1. The microneedle device used to puncture the skin. The microneedles are arranged in 3 rows of 10 needles and held in a bespoke applicator prepared by additive manufacturing.

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 min necessitates a two-stage clinical interaction, whereby the patient is treated topically with the anaesthetic, and then, after a period of 30–60 min, is recalled for the procedure. The delayed onset is a deterrent for the use of topical anaesthetics in children undergoing venous access procedures in urgent and emergency situations [10] and it also 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 the permeation of lidocaine into the skin. These studies used microneedles to either (i) pre-treat skin before the topical application of a lidocaine hydrochloride solution [25] or 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.

Materials and Methods

Preparation of Human Skin Epidermal Membrane

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

Evaluating Tetracaine Permeation from a Commercial Topical Formulation through an Intact or Punctured Epidermal Membrane

Frozen human epidermal membrane was allowed to thaw at room temperature for 20 min. 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 500 µm in length and 200 µm in width (Fig. 1) and inserted for 10 s; (ii) a single puncture with a 26-gauge hypodermic needle (BD, Oxford, UK); or (iii) left untreated. The membranes were mounted in Franz-type diffusion cells and the receptor phase of each was filled with PBS. The diffusion cells were left to equilibrate for 30 min before 0.5 g Ametop gel (Smith & Nephew, London, UK) was weighed into the donor compartment of the diffusion cells. The donor compartment was then occluded with Parafilm (Fisher Scientific, Loughborough, UK) and the cells were maintained at 37°C in a water bath, with the receptor phase being continuously stirred. Samples (200-µl) were withdrawn from the sampling arm at regular time points between 5 and 360 min, 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.

The absorption spectra of different concentrations of Ametop gel, diluted in PBS, were measured on an Agilent Cary 60 UV-Vis spectrophotometer (Agilent Technologies, Cheshire, UK), to determine the peak absorption wavelength for tetracaine (310 nm). A range of Ametop gel concentrations (diluted in PBS) were then evaluated at 310 nm, to create a calibration curve that was subsequently used to determine the tetracaine concentration in experimental samples. Paired t tests and repeated-measures ANOVA were performed; $p < 0.05$ was considered significant.

Fig. 2. The transdermal permeation of tetracaine after topical application of Ametop gel to intact (negative control), hypodermic needle-treated, or microneedle-treated human epidermal membranes. Permeations over 360 and 60 min (**inset**) are shown ($n = 3$ from 3 donors). Values are mean $\pm$ standard deviation.

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

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 obtain accurate surface area measurements. The Zeta optical profiler uses 5 separate measurement techniques to provide accurate dimensions and thicknesses of different elements of the sample, and also the roughness of the sample surface, resulting in 3-dimensional profiles of the samples being imaged [30].

Results and Discussion

Tetracaine Permeation from a Commercial Topical Formulation through an 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 min through skin pre-treated with microneedles ($n = 3$) and through non-treated skin ($n = 3$; $p = 0.026$; paired 1-tailed t test) or 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). At the more clinically relevant 60-min 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 ($p = 0.097$; paired 2-tailed t test) results. Nevertheless, the data (Fig. 2) from this preliminary experiment, with low replicates ($n = 3$), suggested that microneedle pre-treatment had the potential to enhance tetracaine permeation.

Increased experimental replicates in a further study enabled a 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 epidermal membranes at time points up to 6 h. Tetracaine permeation was significantly enhanced by microneedle treatment of the tissue for the duration of the study, i.e., 360 min (Fig. 3a; $p = 0.004$; repeated-measures ANOVA). More notably, permeation through the microneedle-treated membrane was significantly greater over 60 min as well (Fig. 3b; $p = 0.006$; repeated-measures ANOVA). This is clinically significant as Ametop gel is currently applied to the skin for 30–60 min before venous puncture [7]. However, enhancements to clinical practice would necessitate the 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-treated skin as early as 10 min after the addition of the Ametop gel (Fig. 3b), where $3.89 \mu\text{g}/\text{cm}^2$ permeated through microneedle-treated skin compared to $0.82 \mu\text{g}/\text{cm}^2$ in untreated skin.

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 the flow of patients through the department [10]. A reduced topical anaesthetic onset time could lead to the more widespread use of Ametop gel in children, especially in emergency situations where short onset

Table 1. Transdermal permeation comparison

Transdermal permeation of tetracaine	Time taken with no pre-treatment	Time taken with microneedle pre-treatment
$5.43 \mu\text{g}/\text{cm}^2$	30 min	12.13 min
$15.03 \mu\text{g}/\text{cm}^2$	60 min	31.99 min

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

Type of needle used	Imaged width of channel	Imaged length of channel	Estimated area of a single channel	Estimated area of all channels
Microneedles	$50 \mu\text{m}$	$50 \mu\text{m}$	$2,500 \mu\text{m}^2$	$75,000 \mu\text{m}^2$
Hypodermic needle	$25 \mu\text{m}$	$170 \mu\text{m}$	$4,250 \mu\text{m}^2$	$4,250 \mu\text{m}^2$

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 min. 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 so 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 the use of a commercially available topical anaesthetic cream in a

Fig. 4. Scanning electron microscope images of skin epidermal membranes and needles. **a** A microneedle channel. **b** A hypodermic needle channel. **c** A control membrane with no channels. **d** Microneedles. **e** A hypodermic needle.

clinically approved manner. Ametop gel was selected for this study, due to a more rapid onset than its clinical counterparts, e.g., EMLA cream, and the aim was to enhance its permeation further by pre-treatment of the skin with microneedles. 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 demonstrated in Figure 3, is likely to significantly reduce the time to anaesthesia, and this is supported by studies conducted by Li et al. [32].

Membrane Imaging

The mechanism of enhanced permeation is physical disruption of the skin barrier, and so 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) × 200 µm (base width) microneedles and the standard hypodermic needle (10 mm [length] × 450 µm [width]) (Fig. 4). The Zeta profiler was used to confirm and provide further texture to the perforation morphologies shown on SEM (Fig. 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), which explains the enhancement in tetracaine permeation (Fig. 2). It is important to note that changes in the density of the array and the shape of the 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 for the onset time of 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 require-

Fig. 5. Skin epidermal membrane was imaged using a Zeta non-contact optical profiler. **a** A microneedle channel. **b** A hypodermic needle channel.

ment is likely to be easily satisfied by the flexible manufacturing approaches that are predominantly utilised for microneedle production.

Conclusions

Microneedles do not penetrate as deeply into the skin as hypodermic needles, and they cause significantly less pain and trauma than hypodermic needles [9, 33]. However, microneedle-treated skin has a greater total surface area of barrier disruption and thus facilitates increased permeation of a topically applied local anaesthetic. The 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 micro-

needle pre-treatment. The intervention described in this study is a simple method using a simple medical device, with no requirement to re-formulate the medicinal product. This may expedite the clinical translation of this approach and could lead to the more routine use of Ametop gel in children, when fast anaesthetic onset times are desirable.

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Statement of Ethics

Human skin was obtained from surgical procedures under informed consent (South East Wales Research Ethics Committee, Ref. 08/WSE03/55, November 2008).

Disclosure Statement

There are no conflicts of interest.

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