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Research Protocol

Transdermal Microneedle Patch to Enhance Topical Anaesthesia Before Intravenous Line Insertion for Blood Transfusion in Paediatric Thalassaemia Patients

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CHAPTER I

INTRODUCTION

1.1 Transdermal drug delivery

Venepuncture causes pain and is regarded as the one of the more frequently encountered traumatic experiences among hospitalised children. The distress and fear toward needle puncture may further be aggravated in children with chronic diseases which require them to undergo regular venous cannulation or venepuncture. The pain perception in the long term may affect the psychological well-being and potentially adversely affect personal self-esteem and relationships.

Healthcare is constantly evolving as providers aim to deliver holistic care to patients. Local anaesthesia before venepuncture aligns with Good Clinical Practice and is considered a quality standard of care in children. More recently, transdermal drug delivery is rapidly evolving as a less invasive way of administering such medications in a cost-effective manner.

When it comes to transdermal drug delivery (TDD), the traditional low-cost hypodermic needles and topical creams are the most common approach to be considered. However, both have their drawbacks. For the hypodermic needle, the downsides associated include significant pain, anxiety, distress, and, worst of all, needle phobia.^{1,2} Hence, it could be challenging in children as the needle was the least favourable option among the paediatric population and the parents.^{3,4} There are also other state-of-the-art modalities to enhance transdermal delivery of local anaesthetic agents such as phonophoresis (utilising ultrasound to enhance local anaesthetic agents penetration through the skin), magnetophoresis (using magnetic field), iontophoresis (utilising electrical current to boost electrically-charged or ionic local anaesthetic agents penetration into the deeper layer of dermis) and jet injection (J-tip) (using pressurized gas to enhance subcutaneous delivery of local anaesthetic agents).⁵⁻⁸ However, the large size and the exorbitant prices of these devices warrant the utilisation of extra power sources and satisfactory training of operators before such complex devices could be handled effectively.⁹

Topical route hence appeared to be an attractive choice to be focused on. As such, topical anaesthetic creams, for example EMLA Cream (lidocaine 2.5% and prilocaine 2.5%), provide a non-invasive and convenient means of administering anaesthesia. As the drug passively diffuses across the skin, it results in a slower time of onset of effective analgesia which is around 60 minutes.^{10,11} This delayed onset of full analgesia is impractical and infeasible in a busy clinical setting. Therefore, such limitations of all available TDD methods are attributed to the human skin anatomy.

1.2 Skin structure and transdermal drug delivery

The human skin, particularly the outermost epidermal layer, is responsible for the mechanical barrier function. Unfortunately, this barrier function impedes the percutaneous entry of beneficial chemical compounds for a successful transdermal delivery of drugs.

The skin is histologically made up of 3 main layers, i.e., the outer epidermis, middle dermis, and inner hypodermis.^{12,13} For the epidermis, this avascular layer is composed of 5 keratinocytes layers and is generally 50–150 μm thick.¹² The full epidermis is constituted of the outermost *stratum corneum* (SC)

and its beneath viable epidermis (VE), with a thickness spanning from 10 to 15 μm and from 50 to 100 μm , respectively.^{13,14} In between the epidermis and dermis lies the undulating epidermal-dermal junction. The dermis is the thickest component of the skin, measuring 2 – 4 mm thick in depth.¹² The papillary and reticular dermal layers consist of active cells, connective tissues, blood vessels, lymphatics, and nerves.^{12,13} Lying underneath is the innermost hypodermis, primarily composed of subcutaneous adipose tissue as well as other histological architecture such as blood vessels, lymphatics, and nerves.¹³ Thereby, the sophisticated capillary network and nerve endings embedded in the dermis and hypodermis are substantial for systemic delivery of drugs through the transdermal route.¹² **Figure 1** illustrated the human skin structure.

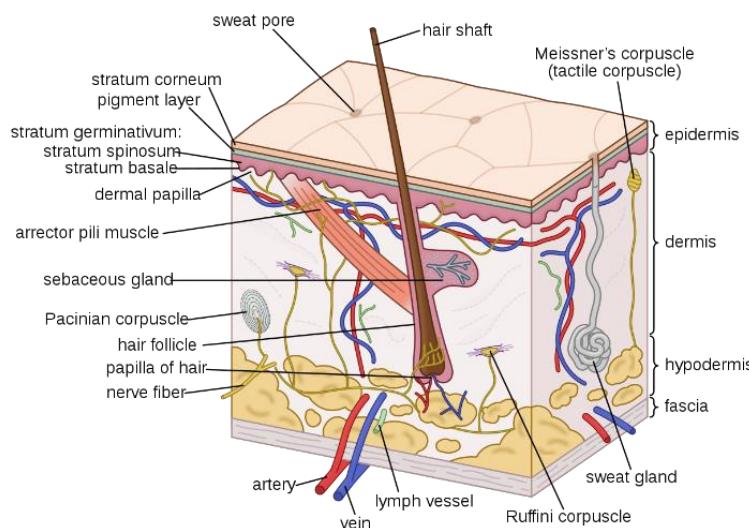


Figure 1. Human skin structure. The 3 skin layers composed of i.e., epidermis, dermis and hypodermis. The structural components in each layer were illustrated in relative to its anatomical position. *Figure courtesy: Tomáš Kebert & umimeto.org - Own work, CC BY-SA 4.0*

The *stratum corneum* (SC), made up of 15 to 20 layers of dead corneocytes, is primarily responsible for the skin barrier function.¹⁴ Hence, SC is implicated for the failure in effective systemic delivery of those lipophobic and high molecular weight active pharmaceutical ingredient (API).² Nevertheless, the role of full epidermis should also be taken into account. Basement membrane and tight junctions located at the epidermal-dermal junction may confer certain resistance on molecular transportation of API across the epidermis.¹³ In the previous literature, it is also observed that drug compounds that managed to pass through the papillary dermis could subsequently reach systemic circulation and exhibit its systemic effect.¹³

The evidence was further supported by the poor bioavailability from topical preparation, whereby only 10–20% of total drug reach the circulation. In contrast, a hypodermic needle is able to penetrate the SC layer and delivers 90–100% of the loaded drug.¹ However, it punctures deep into the dermis and may hit the pain receptors.

1.3 Emergence of microneedle and its applications

With the maturation of the microfabrication manufacturing technology, research on microneedle (MN) had been carried out extensively. MN is the mimic of hypodermic needle, composed of hundreds of

micron-sized, out-of-plane protrusions, typically arranged in arrays on a patch that can be applied to the skin.^{14,15} The mechanism behind is MN creates multiple transient microchannels across the skin and disrupt the SC in a minimally invasive manner. These micron-sized conduits increase the permeability for better API transport into the systemic circulation, enhancing the therapeutic effect.

Here lie the benefits of MN whereby the needle specifications can be tailored to avoid stimulating the nociceptors and penetrating the blood vessels. The MN can be fabricated from a variety of material. Remarkably, the recent focus towards biocompatible polymer holds excellent promise over silicon or metal. Microneedle biocompatibility is the most crucial aspect to consider in the paediatric population as the skin barrier function is immature and rapidly evolving.¹² A biocompatible MN dramatically eliminates the risk of biohazard wastage and possible complications if needle breakage occurs.

Maltose, a natural carbohydrate, is preferred for its biodegradability and dissolubility within minutes.¹⁶ Its safety profile is well-recognized, which makes maltose being extensively used in pharmaceutical formulations.^{1,16} Among the MN types, coated MN refers to the solid MN coated with drugs formulation on its surface. It has a coat-and-poke feature which means the drug coating will be continuously dissolved and then released into the skin along with the polymer.²

So far, there is a paucity of prior studies investigating the effects of combined usage of microneedle and local anaesthetic application in paediatric patients requiring regular blood transfusion for their clinical conditions. There are three prior studies investigating the use of microneedle for insulin delivery and glucose monitoring in paediatric patients (children and adolescents) with type I diabetes. Gupta, Felner and Prausnitz (2011) compared the use of a single hollow microneedle as a means for intradermal delivery of insulin with the subcutaneous administration of insulin using a catheter.¹⁷ They found no significant differences in terms of insulin's area under the curve (AUC) between those two modalities but faster absorption of insulin using the former ($t_{max} = 27 \pm 13$ minutes (MN + insulin group) vs 57 ± 20 minutes (catheter + insulin group)). They also found out that pain associated with procedures was substantially lower in the MN + insulin group. However, a study by Norman et al. (2013) demonstrated higher pain scores in children and adolescents aged between 10 and 18 years old who received insulin via MN than their counterparts who received insulin subcutaneously via insulin pump.¹⁸ Nevertheless, the difference is not statistically significant. On the contrary, in a recently-published study by Samant et al. (2020) investigating the safety and suitability of different glucose monitoring modalities in 15 paediatric patients (mean age (SD): 16.8 (3.2) years) with type I diabetes, the authors demonstrated similar VAS scores between those whose glucose levels were monitored via interstitial fluid (ISF) sampling using MN patch (MN+ISF) and fingerstick capillary blood glucose monitoring.¹⁹ Interestingly, the MN+ISF group reported a statistically-significant lower VAS score mean than those who went blood glucose monitoring using intravenous catheters.¹⁹

Hence, based on these prior findings, the utilities of microneedle in alleviating pain perception associated with routine clinical procedures in paediatric patients are still inconclusive. Therefore, further investigations are obviously warranted to bridge the gap in our current understanding of microneedle utilities in paediatric patients.

1.4 The Pharmacological properties of EMLA constituents

Both constituents of EMLA cream, lidocaine and prilocaine, are amide-type local anaesthetics. They have similar pKa (7.9 (lidocaine) and 7.7-7.9 (prilocaine)), percentage of ionization at physiological pH (75-76% (lidocaine) and 67-76% (prilocaine)) and elimination half-lives ($t_{1/2} = 1.6$ hours).²⁰⁻²² However, prilocaine has a much smaller partition coefficient (129 vs 366) and percentage of protein bound (55% vs 65%) than lidocaine, resulting in a much higher volume of distribution (2.73 L/kg vs 1.30 L/kg) and a higher clearance (2.03 L/kg per hour vs 0.85 L/kg per hour) than lidocaine.^{20,22} Most

amide-type local anaesthetic agents undergo hepatic metabolism and around 5% of their mass is removed renally without metabolic transformation, except for prilocaine which could also be metabolised in the kidney.²² Hence, liver or renal impairment may affect the elimination of both agents, resulting in an elevated risk of systemic toxicity. Prilocaine results in less vasodilation than lidocaine and therefore the use of epinephrine is not warranted to lengthen its duration of action, an advantageous feature for those whom epinephrine use is contraindicated.²²

Since both lidocaine and prilocaine are less lipophilic than other local anaesthetic agents such as bupivacaine and tetracaine, they cause less cardiotoxicity. However, prilocaine causes less direct neurotoxicity (e.g. transient neurologic symptoms (TNS) and cauda equina syndrome) than lidocaine due to its larger volume of distribution, expeditious lung uptake and swift metabolism.^{20,21} Hence, prilocaine seldom reaches toxic plasma concentration level.²³ Nevertheless, prilocaine induces methaemoglobinemia formation due to its toxic metabolite, ortho-toluidine (o-toluidine).²⁴ Consequently, neonates have the highest risk of developing methaemoglobinemia since their red blood cells have insufficient methaemoglobin reductase.²² To treat this, methylene blue or ascorbic acid, could be administered intravenously to revert the ferric ion (Fe^{3+}) in the ring-like non-protein heme group of haemoglobin to its original ferrous state (Fe^{2+}).²⁵

EMLA (Eutectic Mixture of Local Anaesthetics) is a eutectic mixture of 2.5% lidocaine and 2.5% prilocaine. Due to its eutectic property, EMLA exists in an emulsified form at room temperature. Skin analgesia is induced by the diffusion of lidocaine and prilocaine transdermally, resulting in the stabilisation of neuronal membranes via the blockade of ion fluxes that are needed for action potential initiation and transmission.²⁶ EMLA should not be used on mucous membrane since it is rapidly absorbed into systemic circulation. The speed of onset, duration and depth of skin analgesia are largely reliant upon the length of application time.²⁶ Besides, the duration and onset of action are also influenced by the thickness of the skin at the application area and local blood flow.²⁷ The effects of increasing the dosage of EMLA on the onset, duration and depth of analgesia are still unknown. A maximal analgesic effect (under occlusive dressing) is achieved 2 to 3 hours post application and this may last up to 1 to 2 hours post removal.²⁶

1.5 Pain assessments

Pain is a subjective perception, rendering its assessment difficult. Several pain assessment tools had been proposed, and each presented with specific strength properties. Ideal measurement tools should always possess good sensitivity, consistency, validity, and particularly easy to understand so that they can be straightforwardly applicable to the paediatric population.^{28,29} Self-assessment scales require certain levels of comprehension skills and cognitive function. Hence, self-assessment scales are only reliable in children aged 6 years and above.²⁸ Visual Analog Scale (VAS) is considered to be the most validated tool and correlates positively with other self-measuring scales, such as Faces-pain scale revised (FPS-R) and Numerical Rating Scale (NRS). It has been further proven as an optimal tool to evaluate pain as its values can be utilised to monitor pain progression. Besides, VAS scores are also sensitive to treatment effect, and represent real difference in pain intensity at 2 different timepoints.²⁹ **Figure 2** shows a child-friendly VAS pain score device, which further gives the children a more convenient and visual way to measure pain.

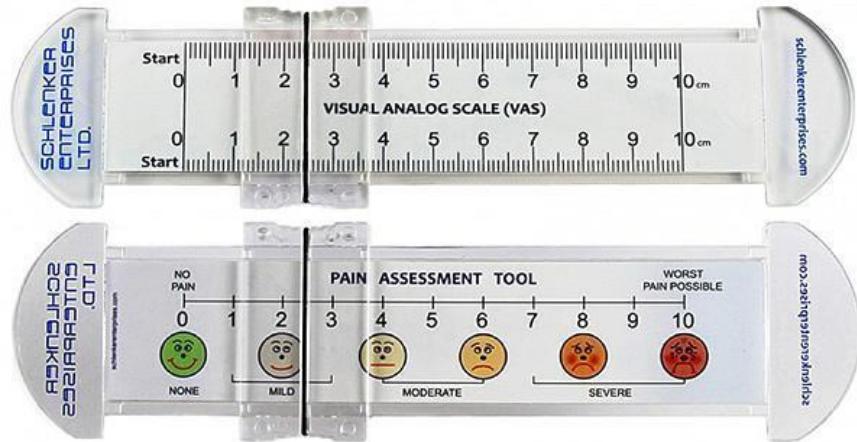


Figure 2. The child-friendly VAS pain score.

Nevertheless, scientists had set eyes on the objective approach that best measures the pain intensity. With the recent help in technology advancement, a pain monitoring device had been developed based on stress induced sweating. The device relies on the firing rate of the nociceptive nerve, mirrored by the skin conductance peaks, which is then translated to the pain stimuli perceived.³⁰ The stress induced sweating is activated through skin sympathetic nerve and is independent of external environment.

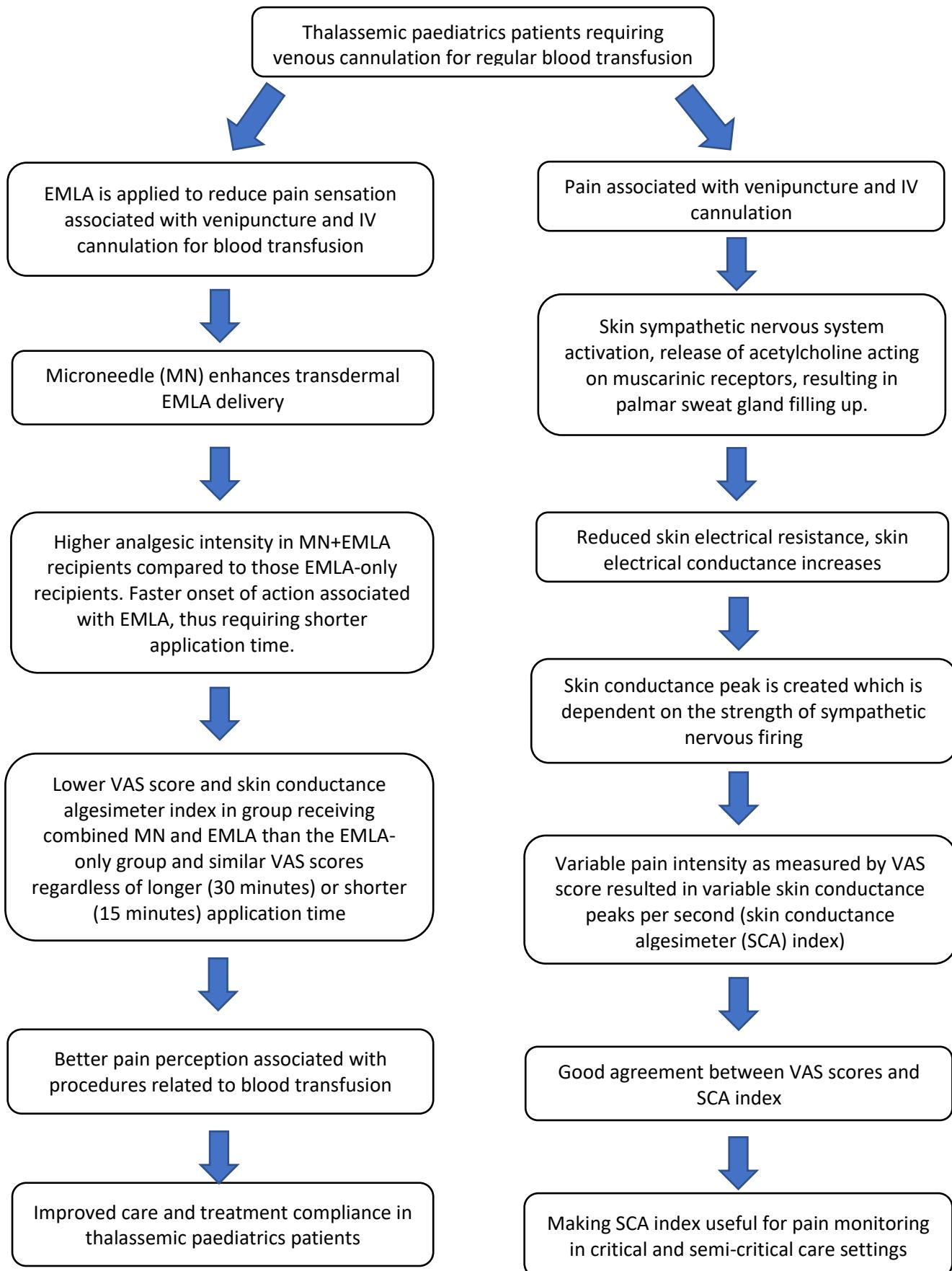
1.6 Problem statements and justifications of the research

Based on our brief review of literature, there are two problem statements summarising the identified research gaps that justify the conceptual significance of this research:

- i) The combined effects of microneedle usage reduce the dose and hasten the action of EMLA Cream on skin anaesthesia in paediatric patients requiring venous cannulation for regular blood transfusion have not been properly elucidated in previous studies. This study aimed to demonstrate a better care for paediatric patients that frequently receive blood transfusion as part of the management of their clinical conditions.
- ii) The agreement between VAS score and the physiological skin conductance algesimeter index obtained using medical devices has not been previously investigated in paediatric patients. This study aimed to highlight the potential of physiological pain monitoring in critical and semi-critical care where the patient is often either semi-conscious or unconscious.

Hence, it is anticipated that our research findings may satisfactorily provide the answers to the problem statements above, resulting in significant application of microneedle in TDS of drugs such as EMLA Cream, and the use of physiological monitoring of pain in paediatric patients. The conceptual framework (**Figure 3**) diagrammatically summarizes our research underpinnings, postulates and justifications.

1.7 Conceptual framework



CHAPTER II

STUDY OBJECTIVES

2.1 General Objective

To evaluate if the pain scores are different during venous cannulation through a pre-anaesthetized skin patch, with and without the use of microneedle.

2.2 Specific Objectives

- 2.2.1 To compare Visual Analogue Scale pain scores among Microneedle with 1 Finger Tip Unit EMLA Cream (experimental group) applied for 30 minutes, Microneedle with 0.5 Finger Tip Unit EMLA (experimental group) applied for 30 minutes, Microneedle with 1 Finger Tip Units EMLA applied for 15 minutes, and 1 Finger Tip Units EMLA Cream only applied for 30 minutes (controls) groups.
- 2.2.2 To compare skin conductance algesimeter index among Microneedle with 1 Finger Tip Units EMLA Cream (experimental group) applied for 30 minutes, Microneedle with 0.5 Finger Tip Unit EMLA (experimental group) applied for 30 minutes, Microneedle with 1 Finger Tip Units EMLA applied for 15 minutes, and 1 Finger Tip Units EMLA Cream only applied for 30 minutes (controls) groups.
- 2.2.3 To evaluate the agreement between the Visual Analogue Scale pain scores obtained and the skin conductance algesimeter index obtained via PainMonitor™ machine

2.3 Research Questions

- 2.3.1 Is there any difference in terms of VAS pain score means among the MN with 1 Finger Tip Unit (FTU) EMLA Cream (30-minute application), MN with 0.5 FTU EMLA (30-minute application), MN with 1FTU EMLA cream (15-minute application), and 1 FTU EMLA Cream only (30-minute application, controls) groups?
- 2.3.2 Is there any difference in terms of skin conductance algesimeter index means among the MN with 1 FTU EMLA Cream (30-minute application), MN with 0.5 FTU EMLA (30-minute application), MN with 1FTU EMLA cream (15-minute application), and 1 FTU EMLA Cream only (30-minute application, controls) groups?
- 2.3.3 Is there any agreement between the VAS pain score and the skin conductance algesimeter index obtained via PainMonitor™ machine?

2.4 Research Hypotheses

- 2.4.1 There is at least a difference in terms of the VAS pain score means among the MN-with-1-FTU-EMLA (30-minute application time), MN-with-1FTU-EMLA (15-minute application time), MN-with-0.5 FTU-EMLA (30-minute application time), and 1-FTU-EMLA-only (30-minute application time) groups.
- 2.4.2 There is at least a difference in terms of the skin conductance algesimeter index means among the MN-with-1-FTU-EMLA (30-minute application time), MN-with-1FTU-EMLA (15-minute application time), MN-with-0.5-FTU-EMLA (30-minute application time), and 1-FTU-EMLA-only (30-minute application time) groups.
- 2.4.3 There is agreement between the VAS pain score and the skin conductance algesimeter index obtained via PainMonitorTM machine.

CHAPTER III

RESEARCH METHODOLOGY

3.1 Research Design

This is a prospective, phase II, single-centre, single-blind, cross-over, randomized negative-controlled (without microneedle (MN)) trial at Hospital Canselor Tuanku Muhriz (HCTM), Universiti Kebangsaan Malaysia. Eligible participants will be randomized in a cross-over fashion to one of 24 treatment sequences as shown in **Figure 4**.

This crossover design is chosen over the more conventional parallel-group randomized trial since the lesser intra-patient variability than the inter-patient variability, thus minimising the required sample size. The study investigators also do not anticipate the inherent disadvantages associated with this trial design (greater attrition rate, prolonged carry-over effects and the inconsistency of participant's condition) will be present in this study.

3.2 Time period/ Duration

From August 2021 until August 2022 (52 weeks).

3.3 Study site

Paediatric day-care centre at level 4 of UKM Medical Centre.

3.4 Study population and sampling method

Thalassaemic patients who came for regular blood transfusion at HCTM who fulfil the inclusion and exclusion criteria and voluntarily provide the voluntary written informed consent. Convenient sampling will be used to obtain the study subjects to prevent insufficient recruitment caused by the paucity of eligible study participants.

3.5 Inclusion Criteria

- i) Patient aged at least six years to below eighteen years old
- ii) Patients requiring venous cannulation for blood transfusion

3.6 Exclusion Criteria

- i) Patient with a previous history of sensitization or allergy to EMLA Cream
- ii) Patient with a previous history of allergy to materials used in the study i.e., plaster, electrodes, maltose, Polyvinyl Alcohol (PVA), and Polyethylene Terephthalate (PET)
- ii) Patient exposed to analgesic usage within 24 hours prior to the procedure
- iii) Generalized skin disorder/ rash
- iii) Agitated/ fretful patient

3.7 Ethical issues

Voluntary written informed consent will be obtained from each study participant. This study will be conducted in accordance to the principles of ethics in human research laid as stipulated by the Declaration of Helsinki (18th World Medical Association General Assembly, 1964) and the Good Clinical Practice (GCP) guidelines.³¹ Ethical approval will be obtained from the UKM Research Ethics Committee (Human) (JEPUKM).

To aid the transparency of reporting, the trial will be registered at the Clinical Trials.gov registry (<https://clinicaltrials.gov/>). The full trial protocol will be made available in the same trial registry and prepared according to the Consolidated Standards of Reporting Trials (CONSORT) guideline for crossover design and the 2013 Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) statement.^{32,33} The trial data with study participant identification numbers removed (Hospital RN, Identity Card (IC) numbers, subject's identification number (SIDNO) will be made available to the public via Harvard Dataverse repository for research data (<https://dataverse.harvard.edu>) to ensure the transparent dissemination of study findings and fulfil the requirements for the future publication of the findings of this research.

3.8 Randomization procedure

For random allocation, simple randomization with random allocation rule (RAR) will be used by which a list of random numbers will be generated in a balanced 1:1 ratio (based on 24 different treatment sequences) using the randomizeR package version 20.0 executed on R platform.³⁴ The list of generated random numbers will be used to allocate the study participants to either intervention or control branch. The allocation sequence generated will be kept in a password-protected document that is only accessible to the statistician to maintain allocation concealment. To further guarantee allocation concealment is satisfactory, randomisation code will not be revealed until the participants have been definitively recruited into the trial, which is after all baseline measurements are made and all eligibility criteria are deemed fulfilled. Besides, allocation concealment is further assured by ensuring the information on the intervention allocation is only given to the person who will be administering the allocation via secured phone calls (central randomization). Consecutive recruitments will be made until the final intended sample size is reached.

For this study, the study participants, outcome assessors, care providers and data handlers will be blinded to the study interventions. Only the statistician and the person who will be administering the interventions will be unblinded to the study interventions. Besides, unique code to indicate each treatment sequence assignment will be utilised to ensure that the unintentional / intentional unblinding of one trial participant will not compromise the integrity of blinding for the rest of study participants. The main unblinded trial persons (the statistician and the key person who will be administering the intervention) are instructed not to divulge the identity of the allotted treatments to other blinded trial persons. The success of blinding will be determined by asking the blinded trial persons (e.g. study participants, care providers, outcome assessors) to guess the interventions received and then compare the results obtained with what would be anticipated by chance. Blinding indices such as James' Blinding Index or Bang's blinding Index could also be computed to objectively evaluate the success of blinding.^{35,36}

3.9 Clinical protocols

3.9.1 Fabrication of solid maltose microneedle

The MN array patch size is measured 1 cm x 1 cm, containing 36 microneedles with 1 mm needle gap in between. The maltose (Hayashibara, Okayama, Japan) MN dimension is designed to be around 400 μm in height, base width 100 μm , with a 3- μm tip radius. The standard deviation of needle heights within the patch is controlled to be less than 3%. The total patch thickness is therefore 0.8 mm. The MNs are grown on a soft cushion on top of Polyvinyl Alcohol (PVA) material (Kanto Chemical, Tokyo, Japan), with a Polyethylene Terephthalate (PET) patch (Acrysunday, Tokyo, Japan) to support the soft PVA patch as below. The base patch spans 125% larger than the array MN patch, with an estimated patch size of 1.5 cm x 1.5 cm. The overall size of the microneedle device is thus 1.7 cm (width) x 4 cm (length) x 0.5 cm (height). Figure 3 illustrates the schematic representation of microneedle prototypes that will be used in this study.

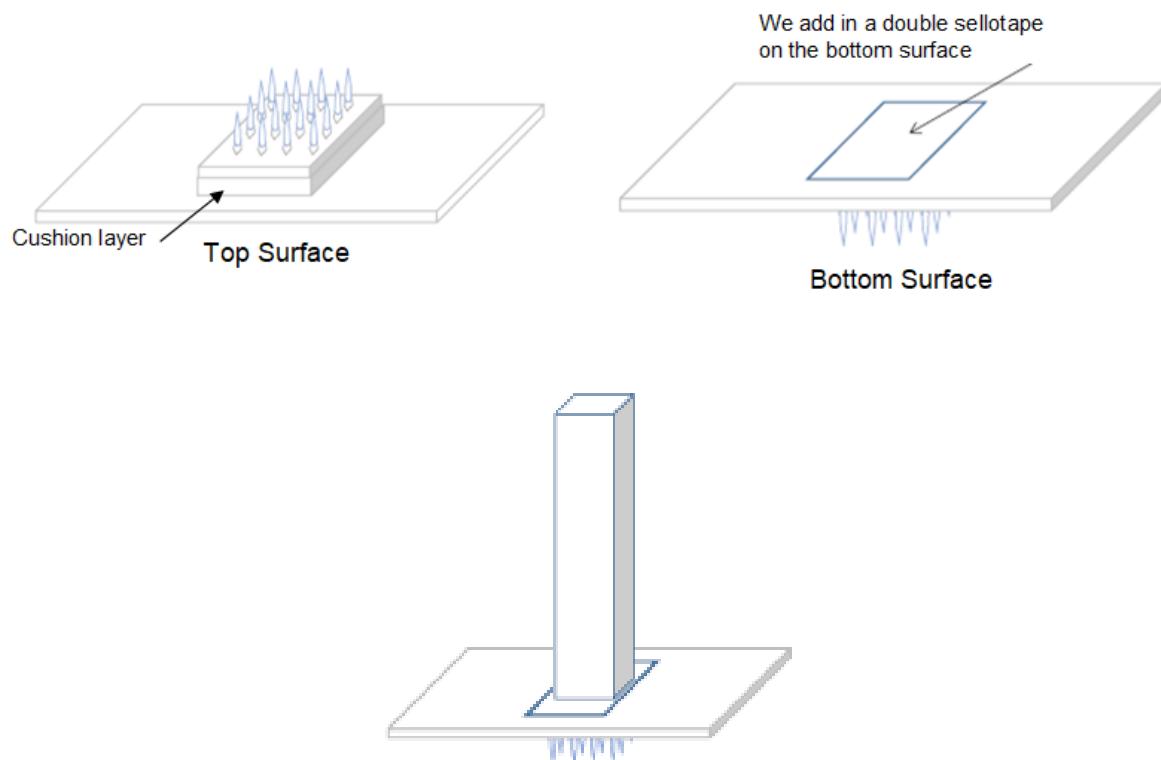


Figure 3. The schematic representation of microneedle prototypes.

Since the MN patch will be manually applied to the skin with a normal thumb force, various studies reported the hole diameter should be around $1/3$ of MN length.^{37,38} Therefore, this research's specification is speculated to achieve the desired penetration depth around 160 μm where the epidermal-dermal junction lies. To ensure a uniform force is applied all over the MN patch, the interventionist will stick a pillar handler of a size of 1 cm x 1cm x 4.9 cm on the bottom surface of MN patch with a double sellotape stuck onto it. The application of MN to the skin thus mimics a stamping action.

The maltose dissolving time is about 15s at a temperature between 25 and 30 °C and a humidity of more than 60%. Hence, in normal condition, the maltose MN is able to dissolve into the skin within 1 minute. For storage, the maltose MN is recommended to be stored at room temperature (upper limit not exceeding 40 °C) and at room humidity of less than 60% to avoid melting. The shelf time can last for more than 8 years, provided that the storage temperature is maintained at 25 °C and a room humidity of less than 10%.

3.9.2 Administration of interventions / controls

Prior to the administration of intervention / control, relevant clinic-demographic profiles (age, gender, ethnicity, anthropometric measurements, presence of comorbidities, thalassemia types etc) will be recorded and entered in the case report forms (CRFs) that are specifically designed for this study. This research study uses EMLA Cream (lidocaine 2.5% and prilocaine 2.5%) as the topical anaesthetic agent. EMLA Cream is a eutectic emulsion mixture of lidocaine and prilocaine at 1:1 ratio (i.e. each gram of EMLA cream contains lidocaine and prilocaine, 25 mg each). A eutectic mixture has a lower melting temperature than each constituent's melting temperature. The anaesthetic efficacy of EMLA cream will be assessed via pain induced by intravenous cannulation and the primary endpoint is the participant's VAS score measured after applying EMLA Cream (with and without MN application) for 15 and 30 minutes.

The window period given to EMLA Cream for its effect to work will be based on the usual clinical practice observation where it is usually applied for 30 minutes prior to intravenous catheterization. The rationale behind it is due to logistical issues and for the day care's operational convenience. Nevertheless, in a busy clinical setting, the application time is sometimes shortened to 15 minutes for slight anaesthetic effect. Thus, the study investigators postulate that, with the aid of microneedle, the time to onset of action for EMLA Cream could be greatly reduced, thus requiring less time to achieve its maximal effects.

According to the routine hospital protocol, all study participants received their blood transfusion based on the Good Clinical Practice (GCP) guidelines. For each participant, the individual will be randomized to one of the 24 treatment sequences (**Figure 4** and **Table 1**) and there will be a minimum of 3-weeks washout period before administering the next intervention. **Figure 5** illustrated the complete clinical flow for the first and subsequent visits, which includes the preliminary recruitment and randomization phases.

The investigator identified and drew a grid of 1cm × 1cm at the dorsum hand, which served as an ideal site for cannulation. The administrator of intervention (procedurist) will apply either 1 FTU of EMLA Cream (approximately 0.68 g/cm²) or 0.5 FTU (approximately 0.369 g/cm²) over the preparation area. If the patient is subjected to MN patching at his/her visit, the MN patch will be applied by thumb force and pressed firmly against the hand surface for 5 seconds to patch MN to the skin entirely before applying EMLA cream. Otherwise, an empty (i.e. without MN) PVA-containing PET sham patch will be applied instead. Besides, the height-to-base ratio (4:1) used for MN will also optimally minimise its adverse effects (pain, redness), thus preserving the masking (blinding) of study participants from knowing the types of interventions received. The preparation area will be covered with an adhesive dressing (Tegaderm™; 3M, Maplewood, Minnesota, USA) after EMLA cream application. After the allocated application time (15 or 30 minutes), the attending medical officer will set up the transfusion line with a 22-gauge hypodermic needle inserted into the dorsum hand. Throughout the process, the parents/guardians will be allowed to stay by the patient's side at all times.

3.9.3 Pain assessment

After a random treatment sequence was assigned to the study participants, the study participants will be guided on the operating manual for a 10-points, 100mm VAS pain score. The children will be presented with a ruler that contains 100-mm slots with “No Pain” written on the left side and “Worst Pain” on the opposite right side.

Before applying MN patch and EMLA Cream, the patients will be attached with the PainMonitor™ (Med-Storm Innovation AS, Oslo, Norway) device whereby the electrodes will be attached to the hypothenar eminence of the opposite hand not receiving the blood transfusion. The skin conductance peaks (in microSiemens (μ S) and the average rise time (in microSiemens per second (μ S/s)) will be recorded. Those parameters indicate the skin’s sympathetic nerve block induced by the applied EMLA cream.

After each procedure, the children then will be asked to move and place the slider in the slot that accurately describes his/her pain at the following time points: 1) 1 minute after application of MN/ sham patch and before EMLA Cream application (baseline VAS score); 2) 1 minute after IV cannulation. The investigator will record the location of the slot where the slider is placed in (millimetres (mm), clearly printed on the ruler’s backside) and this will be the participant’s VAS score. Throughout the process, there will be a trained nurse standing by at the day-care to assist the verification of the pain scale and to aid the participants who require additional assistance.

3.10 Interventional safety assessment

3.10.1 Adverse Events (AEs) / Serious Adverse Events (SAEs): Definitions

For this study, the study investigators define adverse events as “an abnormal sign, symptom, laboratory test, syndromic combination of such abnormalities, untoward or unplanned occurrence (e.g. an accident), or any unexpected deterioration of concurrent illness”.³⁹ For serious adverse events (SAEs), they are defined according to the USA Code of Federal Regulations Title 21, Section 312.32 which is “adverse events result in the following outcomes: 1) death; 2) life-threatening AEs; 3) inpatient hospitalization or prolongation of existing hospitalization; 4) a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly / birth defect”.⁴⁰ Other significant medical events are also considered as SAEs if the events compromise the study participants and result in the requirement of medical or surgical intervention to prevent one of the outcomes above. As an example, allergic bronchospasm requiring intensive treatment in an emergency setting to prevent the outcomes above.

3.10.2: The likelihood of AEs / SAEs: Classification of causality

The likelihood of AEs and SAEs are classified based on the modified Naranjo et al. 1981 recommendations as follows:⁴¹

- a) **Unrelated:** The AEs / SAEs are unlikely related to microneedle or EMLA application.
- b) **Possible:** The connection between AEs / SAEs with microneedle or EMLA cannot be excluded with absolute certainty:

- i) There is a temporal relationship between the microneedle / EMLA application with the occurrence of AEs / SAEs.
- ii) However, there is an alternative factor (e.g. characteristic of patient's disease / clinical state) that can possibly or likely explain such a relationship or a significant uncertainty exists over the cause of AEs / SAEs
- c) **Probable:** The connection between AEs / SAEs with microneedle or EMLA has a high degree of certainty
 - i) There is a temporal relationship between the microneedle / EMLA application with the occurrence of AEs / SAEs
 - ii) AEs / SAEs disappear or decreases upon the withdrawal of microneedle or EMLA application but do not reappear upon subsequent microneedle / EMLA application
 - iii) Alternative causes cannot reasonably explain the relationship between microneedle / EMLA application with the occurrence of AEs / SAEs.
- e) **Definite:** The AEs / SAEs are obviously linked to microneedle / EMLA application with the occurrence of AEs / SAEs
 - i) There is a temporal relationship between the microneedle / EMLA application with the occurrence of AEs / SAEs
 - ii) AEs / SAEs disappear or decreases upon the withdrawal of microneedle or EMLA application and reappears on subsequent microneedle or EMLA application.
 - iii) No other alternative causes.

3.10.3 Methods for assessing and recording of AEs / SAEs

The study protocol will be halted at any moment of the study period if the study participants develop any sudden (expected or unexpected) severe complications / adverse events. The withdrawal of a study participant from the trial will be documented on the adverse event page of the CRF and the participant will be further followed up for the study outcomes and included in the analysis as per the original randomization group (intention-to-treat analysis). All AEs / SAEs will be recorded and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5 and US FDA's Toxicity Grading Scale Healthy Adults and Adolescents Volunteers Enrolled in Preventive Vaccine Clinical Trials criteria as shown in Appendix III and IV.^{42,43}

3.10.4 Local skin reaction

Prior to the IV cannulation and after blood transfusion, the adhesive dressing will be removed and skin antisepsis will be performed. The investigator will visually examine the tested site for any evidence of skin irritation. Photos will be taken for further documentation. Among local AEs / SAEs that will be solicited and documented are as follows:

- Pain
- Erythema (redness)

- Ecchymosis (bruising)
- Swelling
- Pruritus (itchiness)
- Tenderness (pain upon touching the cannulation site)

The patients will be followed up via phone call within 24 hrs after the procedure to obtain any subsequent evidence or occurrence of skin irritation. Any local skin reaction will be described and documented in terms of its maximum intensity, duration of reaction, subsequent action taken to mitigate any adverse event, potential causal link with the study intervention / control and the evolution of the skin reaction: (1) continues; 2) recovered with sequel; 3) recovered without sequel; 4) unknown.

3.10.5. Systemic Reaction

Among systemic AEs / SAEs associated with study interventions that will be solicited and documented are as follows:

- Fever
- Irritability
- Tiredness
- Anorexia
- Vomiting
- Tachycardia
- Seizure
- Hypotension

Similar safety protocol will be adhered to as in 3.10.4 with adjustments made for systemic reactions.

3.10.6 Reporting of AEs / SAEs to the Institution Review Board (IRB)

All AEs will be recorded in the CRFs. AEs of grade 3 and above will be reported to the UKM Human Ethics Committee within 5 business days. All SAEs (including Serious Unexpected Suspected Adverse Events (SUSARs)) will be reported within 24 hours of occurrence (expedited reporting), to the UKM Human Ethics Committee. The reporting procedures for SAEs should adhere to the following steps:

- i) The SAEs initial report form should be filled in by the assessors (medical officers) and reviewed and signed by the PI (Prof Cheah Fook Choe) or the co-investigator (Dr Doris Lau).
- ii) The SAEs initial report should then be submitted to the UKM Ethics Committee in an expedited fashion.
- iii) SAEs should be reviewed and followed to resolution by the medical officers / PI / co-investigator in charge of study participant
- iv) A subsequent follow-up report will be submitted that documents any additional important information, including the resolution of SAEs.

3.10.8 AEs / SAEs follow-up at the end of study period

If AEs / SAEs occur or are still ongoing at the end of the study, the study participants will still be continuously followed up until the resolution of AEs / SAEs occur, except the PI deems that no further follow-up is necessary. The follow-up may take the form of 1) additional subject visits to the trial centre / hospital; 2) telephone calls to the subjects; 3) additional reporting in the form of letters from the treating physicians.

3.10.7 Study Halting Criteria

Participant enrolment and allocation and institution of interventions will be stopped if one of the following occur:

- a) Death related to microneedle or EMLA application
- b) Any participant experiences bronchospasm, laryngospasm or anaphylaxis within 24 hours post microneedle or EMLA application
- c) Any SAE related to microneedle or EMLA application
- d) Any AE of grade 3 and above or any SAE that cannot obviously be implicated on other causes
- e) Any study participant who develops abscess / ulceration / erosion at the site(s) of microneedle or EMLA application

3.11 Quality Control

To ensure the quality of data collection, the following procedures will be followed: 1) all parties involved in this study (including outcome assessors) will be required to attend a training session to standardize procedures and data collection; 2) the CRFs will be continuously checked for data accuracy and consistency, missing data and other data collection-related issues on fortnightly basis; 3) the biostatistician (Dr Irfan) will be notified on missing data or any data inaccuracy and tasked with formulating remedial measures; 4) the principal investigators and the biostatistician will conduct a final review of the detailed reports on screening, enrolment, follow-up and any data completeness / accuracy; 5) upon the clearance obtained from the principal investigators and biostatistician, the data from each CRF will be eventually entered into the SPSS spreadsheet.

3.12 Sample size calculation

The determination of sample size is carried out using G*Power software, version 3.1.9.6 (Universitat Kiel, Germany; February 2020) using the F-test family, Repeated Measure (RM) ANOVA within and within-between interaction sub-options. The type I error (α) and the study power ($1 - \beta$, where β = type II error) are fixed at 0.05 and 0.80, respectively.

Due to scarcity of information from previous literature on paediatric population, we could not use the parameter estimates (standard deviation of the differences, mean differences between pairs) from prior studies as guides for our sample size calculation. However, to circumvent this, we utilise Cohen's guideline on choosing the effect size for this study.⁴⁴ We assumed that the Cohen's d is large ($d = 0.8$) and used this as our effect size. It had been demonstrated by a

previous study on the efficacy of microneedle as a delivery method for 5% lidocaine dental gel (Septodont, UK) that the effect size could be that large, thus justifying our choice of effect size in general.⁴⁵ For objectives 1 and 2, we used the RM-ANOVA within-group comparison sub-option and fix the number of groups and measurements to 1 and 4, respectively. Based on prior guidelines, moderate-to-high correlations between the within-group measurements ($r = 0.7$) are assumed.^{46,47} For non-sphericity correction (ϵ), a value of 0.75 is assumed since the sphericity assumption is rarely fulfilled in practical and actual trial setting due to unequal correlations between measurement pairs and the chosen ϵ value is midway between its lower bound of 0.5 and maximal value of 1 to fairly preserve the RM-ANOVA test against inflated type I error rate (α) when non-sphericity occurs.⁴⁸⁻⁵¹ Hence, for objectives 1 and 2, we require a sample size of 23 subjects. Assuming a 10% attrition (drop-out) rate, the total sample size is 26 ($n_{\text{total}} = 26$).

For objective 2, we could not calculate the sample size since we don't have the estimate of the standard deviation of the differences (denoted by (s) henceforth) between the two methods for pain evaluation (the VAS score and the skin conductance algesimeter index obtained via PainMonitor™). Information on (s) is required since the sample size formula for evaluating limit of agreement require s parameters to be explicitly specified. However, we could roughly assess the precision of the limit of agreements (as per (s) magnitude) based on the sample size proposed for objective 1 and 2 using the formula recommended by the original authors.⁵² Based on Bland and Altman (1986), the 95% CI of limit of agreement is given by:

$$\pm 1.96 * \sqrt{3/n} * s^{52},$$

where s = standard deviation of the differences between VAS score and the skin conductance algesimeter index. If we assume a sample size of 24, then the precision of the limit of agreement is:

$$\pm 1.96 * \sqrt{3/26} * s = \pm 0.67 * s,$$

which we deem a satisfactory precision in our case.

However, due to absence of an estimate of (s) from prior studies, the actual precision could not be accurately estimated, thus highlighting the importance of (s) in the actual sample size calculation. Thus, it is hoped the estimate of (s) obtained from this study will be used as a guide for calculating a sample size for a future follow-up study that can answer this study objective more reliably. However,

Hence, the biggest overall sample size required for this study is 26 ($n_{\text{total}} = 26$).

3.13 Operational definitions of study variables

a) Independent variables

i) **Age:** The age of a study participant at the first study visit. The variable will be measured in years and month and modelled as a continuous numerical variable and will not be categorised into separate age groups.

ii) **Gender:** A categorical variable that will be recorded in the SPSS data frame as 0 = female (base category) and 1 = male. Missing data shall be recorded as 999

iii) **Ethnicity:** A categorical variable that will be recorded in the SPSS data frame as 0 = Malay (base category); 1 = Chinese; 2 = Indian; and 3 = Other ethnicity. Missing data shall be recorded as 999.

iv) **Body mass index (BMI):** A continuous numerical variable that is calculated using the standard body mass index formula; $BMI = \text{kg} / \text{m}^2$. This variable will be categorised according to Kuczmarski et al. (2000) classification⁵³:

<5% percentile = underweight (SPSS code: -1)

5% - <85% percentile = normal BMI (SPSS code: 0; base category)

85% - 95% percentile = Overweight (SPSS code: +1)

>95% percentile = Obese (SPSS code: +2)

The categorized variable will then be used as a predictor variable for statistical modelling purposes. Missing data will be recorded as 999 in the SPSS spreadsheet.

iv) **Intervention groups:** A categorical variable representing the types of interventions received by the study participants in each study visit. This variable will be recorded in the SPSS data frame as 0 = 1-FTU-EMLA only group (control, base category); 1 = MN + 0.5FTU EMLA (30 minutes), 2 = MN + 1FTU EMLA (15 minutes), 3 = MN + 1FTU EMLA (30 minutes).

v) **Baseline VAS score:** A continuous numerical variable that will be measured before the administration of intervention. This will be used as a predictor variable to control the confounding effect of heterogenous baseline VAS scores among study participants. Missing data will be recorded as 999 in the SPSS spreadsheet.

vi) **Baseline pain score obtained via PainMonitor™ device:** A continuous numerical variable that will be measured before the administration of intervention. This will be used as a predictor variable to control the confounding effect of heterogenous baseline VAS scores among study participants. Missing data will be recorded as 999 in the SPSS spreadsheet.

b) Dependent (outcome variables)

i) **VAS score (15- and 30-minutes post EMLA application during IV cannulation):** A continuous numerical variable that will be measured during each visit; 15 minutes after EMLA application and 30 minutes after EMLA application. Missing data will be recorded as 999 in the SPSS spreadsheet.

ii) **Pain score from PainMonitor™ device (15- and 30-minutes post EMLA application):** A continuous numerical variable that will be measured during each visit: 15 minutes after EMLA application and 30 minutes after EMLA application. Missing data will be recorded as 999 in the SPPS spreadsheet.

3.14 Planned Statistical Analysis

The data will be initially checked and cross-examined with the information in the data collection sheet for accuracy, consistency and the amount of missing data. Missing data will be treated as Missing At Random (MAR) and multiple imputation will be considered to impute the missing values in order to reduce the biasness of the parameter estimates. Any variable with serious missing data problem (more than 40%) will be dropped from further analysis.^{54,55} All patients will be analysed according to the original randomization group, based on the intention-to-treat (ITT) analysis.

All data will be descriptively summarized as mean and standard deviation (SD) for the normally-distributed numerical variables (or median and interquartile range (IQR) for the non-normally distributed numerical variables) and frequency and percentage for categorical variable. For numerical data, the normality assumptions will be evaluated subjectively using the histogram with overlying normal distribution curve, box and whisker plot and steam-and-leaf diagrams and objectively using the Kolmogorov-Smirnov and Shapiro-Wilks tests ($p>0.05$ indicates normal distribution assumption is met). Fisher's coefficient of skewness will be subsequently utilized to assess the severity of skewness in non-normally distributed numerical variables using the following formula:

Fisher's coefficient of skewness: Skewness / standard error of skewness

Any value outside ± 2 range will indicate extreme skewness.⁵⁶

For the first and second objectives, repeated measure ANOVA and generalized mixed effect model (GLMM) with an identity link function will be used to assess and estimate the differences in terms of VAS score means between the periods of interventions, considering intra-subject correlations. For repeated measure ANOVA, Mauchly's test of sphericity will be used to assess the sphericity assumption. If sphericity assumption is violated, the Greenhouse-Geisser correction will be used if $\varepsilon < 0.75$ or the Huynh-Feldt correction if $\varepsilon > 0.75$.⁵⁷ Within-group analysis will be used to assess the significance of mean VAS score differences between treatment pairs. To assess the presence of carryover effect, the within group-between treatment sequence interaction effect will be assessed for statistical significance.

For GLMM, the Restricted Maximum Likelihood (REML) estimator will be used to estimate the model parameters. The unadjusted and adjusted estimates of the parameters obtained using the GLMM will be examined, with and without the consideration of periodic effects. Random intercept model and robust variance estimators will be used for the calculation of standard errors. Likelihood ratio test will be utilized to compare the nested models and Akaike Information Criteria (AIC) and Bayesian Information Criteria will be used for comparing non-nested models. Multicollinearity between fixed effects (age, sex and treatment effects) will be evaluated using bivariate correlations. Besides, interaction effects (effect modification) between significant fixed predictors will also be assessed by evaluating the significance of interaction terms. Residual diagnostics will also be performed to ensure the validity of model assumptions and the presence of outliers and influential points will be investigated to ensure that they do not influence the model parameter estimates. The presence of any influential point will then be removed or corrected if these are due to measurement errors or wrong data entry or retained if the values are authentic. We will also carry out sensitivity analyses to investigate the effects of missing data under the Missing At Random (MAR) framework.

For the third and fourth objectives, the correlation between VAS pain score and PainMonitor™ device is tested using Pearson correlation coefficient if both VAS pain scores and PainMonitor™ device are normally distributed (Gaussian) and Spearman coefficient will be used if at least one pain scores (obtained by either measuring modality) are not normally distributed. Intraclass correlation coefficient (ICC) will be computed to assess the agreement between the VAS scores obtained using the two different modalities (VAS pain score vs PainMonitor™ device) and the results will be interpreted according to the Koo-Li criteria.⁵⁸ The Bland and Altman (B&A) plot will be obtained to further assess the agreement between the two scores. The limits of agreement obtained will be assessed to ensure there is an acceptable agreement between the pain measurement modalities.

For each analysis, the significant threshold for p value is fixed at 0.05 and 95% confidence interval (CI) will be obtained for each parameter estimates. Data analysis will be performed using Statistical Package for Social Science (SPSS™) (IBM Corp. Released 2020. IBM Statistics for Windows, Version 27.0, Armonk, NY: IB Corp) and STATA™ version 15 (StatCorp. 2017. Stata Statistical Software: Release 15. College Station, Texas: StataCorp LP).

3.15 Study flow

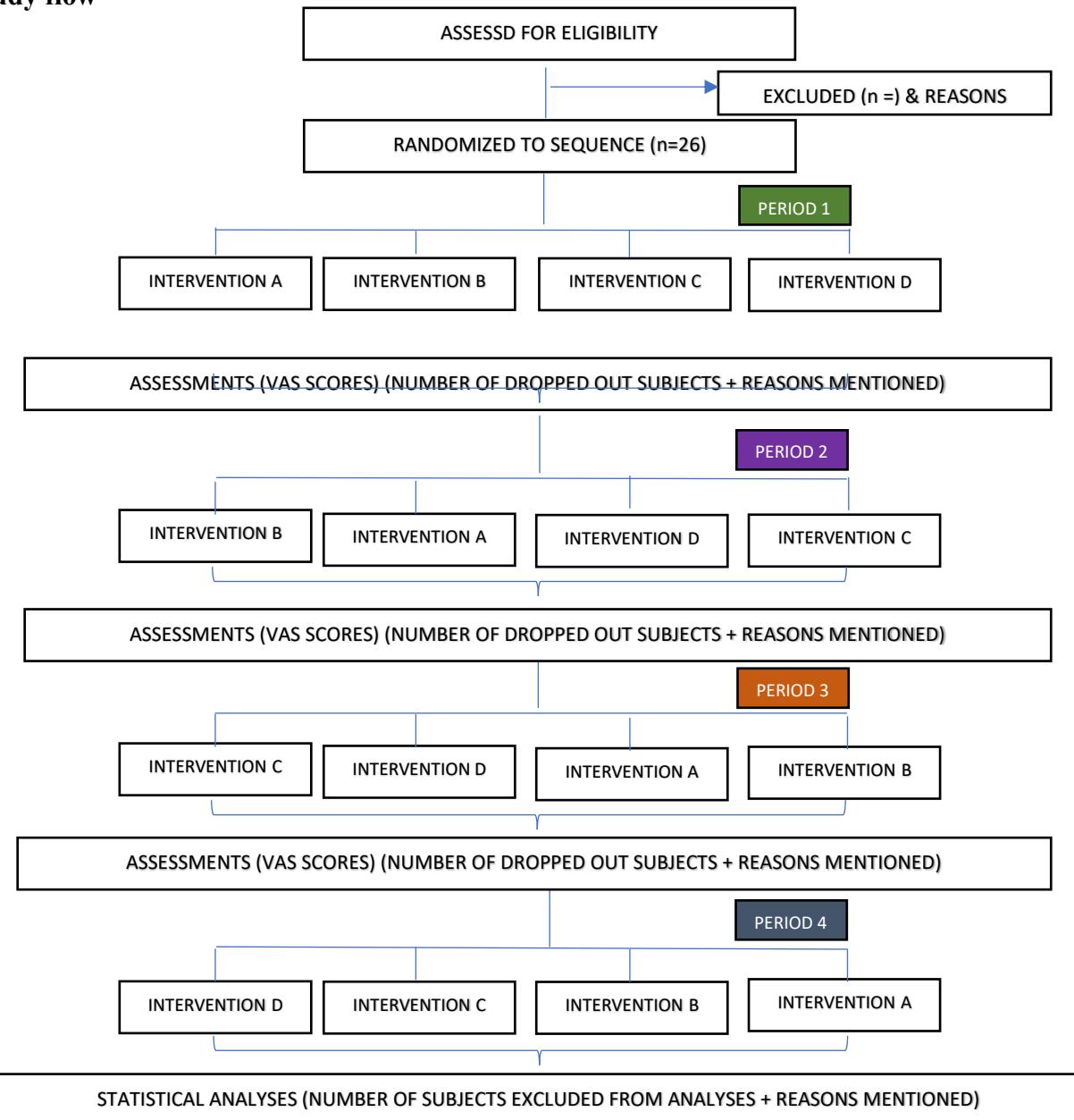


Figure 4. The flowchart of this randomized crossover trial showing 4 random intervention sequences out of 24 permutations generated. Intervention A: MN + 1 FTU EMLA 30 minutes application; Intervention B: MN + 0.5 FTU EMLA 30 minutes application; Intervention C: MN + 1 FTU EMLA 15 minutes application; Intervention D: 1 FTU EMLA Only 30 minutes (controls)

| | | | |
|------|------|------|------|
| ABCD | BACD | CADB | DABC |
| ACBD | BADC | CABD | DACB |
| ABDC | BDAC | CBAD | DBAC |
| ACDB | BDCA | CBDA | DBCA |
| ADBC | BCAD | CDAB | DCAB |
| ADCB | BCDA | CDBA | DCBA |

Table 1. The exhaustive list of permutations of intervention sequences that will be randomized to the participants of this trial. Intervention A: MN + 1 FTU EMLA 30 minutes application; Intervention B: MN + 0.5 FTU EMLA 30 minutes application; Intervention C: MN + 1 FTU EMLA 15 minutes application; Intervention D: 1 FTU EMLA Only 30 minutes (controls)

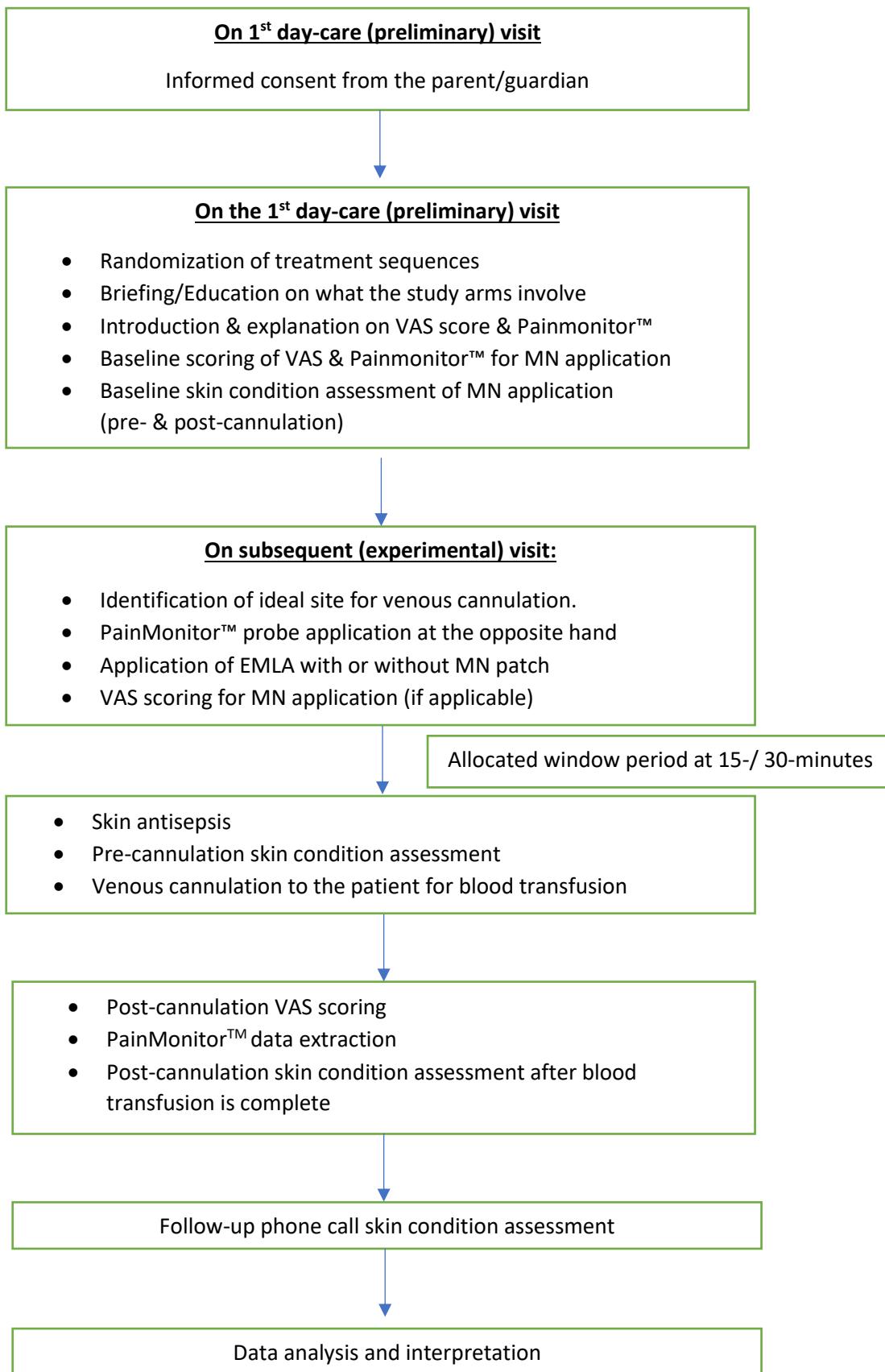


Figure 5. Schematic flow of the clinical trial.

GANTT CHART

| Project Activities | Timeline of event | | | | | | | | | | | | | | |
|---------------------------------------|-------------------|---|---|---|---|---|---|---|----|----|----|---|------|---|---|
| | 2021 | | | | | | | | | | | | 2022 | | |
| | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 1 | 2 | 3 | |
| Proposal preparation | ● | ● | ● | ● | ● | | | | | | | | | | |
| Ethics approval | | | | | ● | ● | | | | | | | | | |
| Microneedle fabrication | | | | | ● | ● | ● | | | | | | | | |
| Purchasing of PainMonitor | | | | | ● | ● | ● | | | | | | | | |
| Patients' recruitment | | | | | | ● | ● | ● | | | | | | | |
| Launching of clinical trial | | | | | | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● |
| Data analysis and interpretation | | | | | | | | ● | ● | ● | ● | ● | ● | ● | ● |
| Manuscript preparation and submission | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● | ● |
| End of research project | | | | | | | | | | | | ● | ● | ● | |

BUDGET

| No. | Description | Unit price (RM) | Quantity | Price (RM) |
|-----|---|-----------------|----------|-----------------|
| 1 | RESEARCH MATERIALS & SUPPLIES | | | |
| 1.1 | Schlenker VAS Pain Scale Rulers 0-100mm with Slider | 40 | pcs | 4 160 00 |
| 1.2 | EMLA Cream 5%, 5G, 1s x 5 | 160 | Box | 12 1920 00 |
| 1.3 | Hansaplast Sensitive Kids Plaster 20's | 6 | box | 7 42 00 |
| | | | | |
| 2 | PROFESSIONAL SERVICE | | | |
| 2.1 | Educational souvenir to patient | 10 | pcs | 26 260 00 |
| | | | | |
| 3 | RENTAL | | | |
| 3.1 | PainMonitor™ | 33850 | pcs | 1 33850 00 |
| 3.2 | Microneedle machine | | | |
| | | | | |
| | Total | | | 36232 00 |

REFERENCE

- 1 Waghule, T. *et al.* Microneedles: A smart approach and increasing potential for transdermal drug delivery system. *Biomedicine & Pharmacotherapy* **109**, 1249-1258, doi:<https://doi.org/10.1016/j.biopha.2018.10.078> (2019).
- 2 Tucak, A. *et al.* Microneedles: Characteristics, Materials, Production Methods and Commercial Development. *Micromachines* **11**, 961, doi:[10.3390/mi11110961](https://doi.org/10.3390/mi11110961) (2020).
- 3 Mooney, K., McElnay, J. C. & Donnelly, R. F. Children's views on microneedle use as an alternative to blood sampling for patient monitoring. *International Journal of Pharmacy Practice* **22**, 335-344, doi:[10.1111/ijpp.12081](https://doi.org/10.1111/ijpp.12081) (2013).
- 4 Pires, L. R., Vinayakumar, K., Turos, M., Miguel, V. & Gaspar, J. A Perspective on Microneedle-Based Drug Delivery and Diagnostics in Paediatrics. *Journal of Personalized Medicine* **9**, 49, doi:[10.3390/jpm9040049](https://doi.org/10.3390/jpm9040049) (2019).
- 5 Kim, T.-Y., Jung, D.-I., Kim, Y.-I., Yang, J.-H. & Shin, S.-C. Anesthetic effects of lidocaine hydrochloride gel using low frequency ultrasound of 0.5 MHz. *J Pharm Pharm Sci* **10**, 1-8 (2007).
- 6 Murthy, S. N., Sammeta, S. M. & Bowers, C. Magnetophoresis for enhancing transdermal drug delivery: mechanistic studies and patch design. *Journal of Controlled Release* **148**, 197-203 (2010).
- 7 Zempsky, W. T., Sullivan, J., Paulson, D. M. & Hoath, S. B. Evaluation of a low-dose lidocaine iontophoresis system for topical anesthesia in adults and children: a randomized, controlled trial. *Clinical therapeutics* **26**, 1110-1119 (2004).
- 8 Cooper, J., Bromley, L., Baranowski, A. & Barker, S. Evaluation of a needle-free injection system for local anaesthesia prior to venous cannulation. *Anaesthesia* **55**, 247-250 (2000).
- 9 Kim, Y.-C., Park, J.-H. & Prausnitz, M. R. Microneedles for drug and vaccine delivery. *Advanced drug delivery reviews* **64**, 1547-1568 (2012).
- 10 De Waard-van der Spek, F. B., Van Den Berg, G. M. & Oranje, A. P. EMLA Cream: An Improved Local Anesthetic. Review of Current Literature. *Pediatric Dermatology* **9**, 126-131, doi:<https://doi.org/10.1111/j.1525-1470.1992.tb01228.x> (1992).
- 11 Houck, C. S. & Sethna, N. F. Transdermal analgesia with local anesthetics in children: review, update and future directions. *Expert review of neurotherapeutics* **5**, 625-634, doi:[10.1586/14737175.5.5.625](https://doi.org/10.1586/14737175.5.5.625) (2005).
- 12 Duarah, S., Sharma, M. & Wen, J. Recent advances in microneedle-based drug delivery: Special emphasis on its use in paediatric population. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V* **136**, 48-69, doi:[10.1016/j.ejpb.2019.01.005](https://doi.org/10.1016/j.ejpb.2019.01.005) (2019).
- 13 Ita, K. Transdermal Delivery of Drugs with Microneedles—Potential and Challenges. *Pharmaceutics* **7**, 90-105 (2015).
- 14 Ali, R. *et al.* Transdermal Microneedles—A Materials Perspective. *AAPS PharmSciTech* **21**, 12, doi:[10.1208/s12249-019-1560-3](https://doi.org/10.1208/s12249-019-1560-3) (2019).
- 15 Caffarel-Salvador, E. *et al.* Potential of hydrogel-forming and dissolving microneedles for use in paediatric populations. *International journal of pharmaceutics* **489**, 158-169, doi:[10.1016/j.ijpharm.2015.04.076](https://doi.org/10.1016/j.ijpharm.2015.04.076) (2015).
- 16 Kolli, C. S. & Banga, A. K. Characterization of Solid Maltose Microneedles and their Use for Transdermal Delivery. *Pharmaceutical Research* **25**, 104-113, doi:[10.1007/s11095-007-9350-0](https://doi.org/10.1007/s11095-007-9350-0) (2008).
- 17 Gupta, J., Felner, E. I. & Prausnitz, M. R. Rapid pharmacokinetics of intradermal insulin administered using microneedles in type 1 diabetes subjects. *Diabetes technology & therapeutics* **13**, 451-456 (2011).
- 18 Norman, J. J., Brown, M. R., Raviele, N. A., Prausnitz, M. R. & Felner, E. I. Faster pharmacokinetics and increased patient acceptance of intradermal insulin delivery using a single hollow microneedle in children and adolescents with type 1 diabetes. *Pediatric diabetes* **14**, 459-465 (2013).
- 19 Samant, P.P. *et al.* Sampling interstitial fluid from human skin using a microneedle patch. *Sci Transl Med* **12**, eaaw0285. doi: [10.1126/scitranslmed.aaw0285](https://doi.org/10.1126/scitranslmed.aaw0285) (2020).

20 Rosenberg, P. H., Veering, B. T. & Urmey, W. F. Maximum recommended doses of local anesthetics: a multifactorial concept. *Regional Anesthesia & Pain Medicine* **29**, 564-575 (2004).

21 Hudson, A. E. & Hemmings Jr, H. C. in *Pharmacology and Physiology for Anesthesia* 44-69 (Elsevier, 2019).

22 Peck, T. & Harris, B. *Pharmacology for anaesthesia and intensive care*. (Cambridge University Press, 2021).

23 Manassero, A. & Fanelli, A. Prilocaine hydrochloride 2% hyperbaric solution for intrathecal injection: a clinical review. *Local and regional anesthesia* **10**, 15 (2017).

24 Brunton, L. L., Hilal-Dandan, R. & Knollmann, B. C. *Goodman & Gilman's the pharmacological basis of therapeutics*. (McGraw-Hill Education New York, 2018).

25 Anderson, C. M., Woodside, K. J., Spencer, T. A. & Hunter, G. C. Methemoglobinemia: an unusual cause of postoperative cyanosis. *Journal of vascular surgery* **39**, 686-690 (2004).

26 EMLA Cream [package insert]. Parsippany, NJ: Actavis Pharma, Inc. Nov 2018.

27 Taddio, A., Ohlsson, A., Einarson, T. R., Stevens, B. & Koren, G. A systematic review of lidocaine-prilocaine cream (EMLA) in the treatment of acute pain in neonates. *Pediatrics* **101**, e1-e1 (1998).

28 Beltramini, A., Milojevic, K. & Pateron, D. Pain Assessment in Newborns, Infants, and Children. *Pediatric Annals* **46**, e387-e395, doi:doi:10.3928/19382359-20170921-03 (2017).

29 Haefeli, M. & Elfering, A. Pain assessment. *Eur Spine J* **15 Suppl 1**, S17-S24, doi:10.1007/s00586-005-1044-x (2006).

30 Storm, H. Changes in skin conductance as a tool to monitor nociceptive stimulation and pain. *Current opinion in anaesthesiology* **21**, 796-804, doi:10.1097/ACO.0b013e3283183fe4 (2008).

31 *Malaysian Guideline for Good Clinical Practice*. 4th edn, (National Committee for Clinical Research (NCCR), 2018).

32 Dwan, K., Li, T., Altman, D. G. & Elbourne, D. CONSORT 2010 statement: extension to randomised crossover trials. *BMJ* **366**, l4378, doi:10.1136/bmj.l4378 (2019).

33 Chan, A.-W. *et al.* SPIRIT 2013 statement: defining standard protocol items for clinical trials. *Annals of internal medicine* **158**, 200-207 (2013).

34 Uschner, D., Schindler, D., Hilgers, R.-D. & Heussen, N. randomizeR: An R Package for the Assessment and Implementation of Randomization in Clinical Trials. *2018* **85**, 22, doi:10.18637/jss.v085.i08 (2018).

35 James, K. E., Bloch, D. A., Lee, K. K., Kraemer, H. C. & Fuller, R. K. An index for assessing blindness in a multi-centre clinical trial: disulfiram for alcohol cessation--a VA cooperative study. *Stat Med* **15**, 1421-1434, doi:10.1002/(sici)1097-0258(19960715)15:13<1421::aid-sm266>3.0.co;2-h (1996).

36 Bang, H., Ni, L. & Davis, C. E. Assessment of blinding in clinical trials. *Controlled clinical trials* **25**, 143-156 (2004).

37 Nayak, A., Das, D. B. & Vladisavljević, G. T. Microneedle-assisted permeation of lidocaine carboxymethylcellulose with gelatine co-polymer hydrogel. *Pharm Res* **31**, 1170-1184, doi:10.1007/s11095-013-1240-z (2014).

38 Loizidou, E., Inoue, N., Ashton-Barnett, J., Barrow, D. & Allender, C. Evaluation of geometrical effects of microneedles on skin penetration by CT scan and finite element analysis. *European Journal of Pharmaceutics and Biopharmaceutics* **107**, doi:10.1016/j.ejpb.2016.06.023 (2016).

39 Aronson, J.K. Medication errors: what they are, how they happen, and how to avoid them. *QJM* **102**, 513-21. doi: 10.1093/qjmed/hcp052 (2009).

40 Code of Federal Regulation, Title 21, Section 312.32 (21CFR312.32). (2020). Accessible from: <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=312.32>. Date of access: 31st / 08 / 2021

41 Naranjo, C.A., Bustó, U., Sellers, E.M., Sandor, P., Ruiz, I., Roberts, E.A., Janecek, E., Domecq & C., Greenblatt, D.J. A method for estimating the probability of adverse drug reactions. *Clin Pharmacol Ther* **30**, 239-45. doi: 10.1038/clpt.1981.154 (1981).

42 US Department of Health and Human Services. Common Terminology Criteria for Adverse Events (CTCAE) version 5. (2017) Accessible from:

43 https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick
 _Reference_5x7.pdf. Date of access: 31 / 08 / 2021

44 Center for Biologics Evaluation and Research, US Food and Drug Administration (US FDA).
 Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers
 Enrolled in Preventive Clinical Trials. (Sept 2007). Accessible from:
 https://www.fda.gov/media/73679/download. Date of access: 31st August 2021

45 Cohen, J. *Statistical Power Analysis for the Behavioral Sciences*. (Elsevier Science, 2013).

46 Daly, S. *et al.* Randomised controlled trial of a microneedle patch with a topical anaesthetic for
 relieving the pain of dental injections. *Journal of dentistry* **107**, 103617,
 doi:https://doi.org/10.1016/j.jdent.2021.103617 (2021).

47 Mukaka, M. M. Statistics corner: A guide to appropriate use of correlation coefficient in
 medical research. *Malawi Med J* **24**, 69-71 (2012).

48 Akoglu, H. User's guide to correlation coefficients. *Turkish Journal of Emergency Medicine*
18, 91-93, doi:https://doi.org/10.1016/j.tjem.2018.08.001 (2018).

49 Vitor-Costa, M. *et al.* Improving Cycling Performance: Transcranial Direct Current Stimulation
 Increases Time to Exhaustion in Cycling. *PloS one* **10**, e0144916,
 doi:10.1371/journal.pone.0144916 (2015).

50 Chang, H. A. *et al.* Attenuated vagally-mediated heart rate variability at rest and in response to
 postural maneuvers in patients with generalized anxiety disorder. **50**, 1433-1441,
 doi:10.1017/s0033291719001302 (2020).

51 Oberfeld, D. & Franke, T. Evaluating the robustness of repeated measures analyses: The case
 of small sample sizes and nonnormal data. *Behavior Research Methods* **45**, 792-812,
 doi:10.3758/s13428-012-0281-2 (2013).

52 Bland, J. M. & Altman, D. G. Statistical methods for assessing agreement between two methods
 of clinical measurement. *Lancet* **1**, 307-310 (1986).

53 Kuczmarski, R. J. *CDC growth charts: United States*. (US Department of Health and Human
 Services, Centers for Disease Control and ..., 2000).

54 Jakobsen, J. C., Gluud, C., Wetterslev, J. & Winkel, P. When and how should multiple
 imputation be used for handling missing data in randomised clinical trials – a practical guide
 with flowcharts. *BMC Medical Research Methodology* **17**, 162, doi:10.1186/s12874-017-0442-1 (2017).

55 Clark, T. G. & Altman, D. G. Developing a prognostic model in the presence of missing data:
 an ovarian cancer case study. *Journal of clinical epidemiology* **56**, 28-37,
 doi:https://doi.org/10.1016/S0895-4356(02)00539-5 (2003).

56 George, D. & Mallory, P. *SPSS for Windows step by step : a simple guide and reference, 17.0
 update*. 10th ed. edn, (Boston : Allyn & Bacon, 2010).

57 Girden, E. R. *ANOVA: Repeated Measures Series: Quantitative Applications in the Social
 Sciences*. (SAGE Publications, 1992).

58 Koo, T. K. & Li, M. Y. A Guideline of Selecting and Reporting Intraclass Correlation
 Coefficients for Reliability Research. *Journal of chiropractic medicine* **15**, 155-163,
 doi:10.1016/j.jcm.2016.02.012 (2016).

APPENDIX I

PATIENT INFORMATION SHEET

Research Title:

Transdermal Microneedle Patch to Enhance Topical Anaesthesia Before Intravenous Line Insertion for Blood Transfusion in Paediatric Thalassaemia Patients

Introduction:

You are invited to participate in a research study. Before participating in this study, it is crucial that you thoroughly read and understand the information provided in this sheet. However, before you take part or agree to continue in this research study, the study will be verbally explained to you, and you will be allowed to ask questions. After you are adequately satisfied that you understand this study and you wish to take part or continue to participate in this study, you must sign this informed consent form. You will be given a copy of these patient information sheet and consent forms to take home with you.

Purpose of Study:

Venepuncture (cannulation of blood vessel) elicits much pain and is regarded as the most frequently traumatic experience encountered among children. The pain may be reduced by applying topical anaesthetic drugs. For example, EMLA Cream (lidocaine 2.5% and prilocaine 2.5%) is a non-invasive and convenient means of administering anaesthesia. The recommended duration of time taken for EMLA Cream to work is around 60 minutes. However, in a busy clinical setting, the time is often expedited to 30 minutes. As such, microneedles had been introduced to enhance the delivery of topical anaesthesia by puncturing the skin at a very superficial level to create multiple tiny tracts for faster action. The microneedle is a device containing multiple small needles made of sugar that will dissolve into the skin.

What will the study involve?

In this study, your child is about to undergo venepuncture for regular blood transfusion in 4 consecutive day-care visits. Depending on the sequence of interventions we have determined, your child will receive application of the topical anaesthetic drug i.e., EMLA Cream, with or without the introduction of the microneedle to the skin. This will result in numbness on the site of his/her hand intended for the blood transfusion, which then reduces pain. The incidence and severity of pain will be recorded after the cannula for blood transfusion has been inserted.

For this study, we would like to compare whether EMLA Cream alone or in combination with microneedle results in better anaesthetic effect. Therefore, there will be a maximum additional 30 minutes imposed to your child normal routine for this medicine to be applied, and for us to record the findings.

Risks:

This investigation poses minimal risk to participants and is unlikely to cause side effects. Nevertheless, the possible common side effects from the EMLA Cream application include:

- pallor,
- redness,
- alterations in temperature sensation over the application area.

Additional risks associated with microneedle application might include possible mild-to-moderate skin irritation, especially in those with sensitive skin, such as

- redness,
- swelling,
- itching.

Should your child experience any problems, we will give your child medications to reduce the side effects of interventions accordingly.

Benefits:

There will be skin anaesthesia effect from topical anaesthetic drug, i.e. EMLA Cream. Furthermore, the information from this study may add to the medical knowledge about the use of dissolvable solid maltose microneedle in transdermally delivering local anaesthetic agents which may help future patients.

Do you have to take part?

Participation in this study is absolutely voluntary. Your child's medical care is not affected if you decide not to participate in this study. He/She will have the usual standard of care according to the day-care protocol.

If you agree to participate, you will be asked to sign the "Informed Consent Form". You will be given a copy of the informed consent form and this Patient Information Sheet. Should you decide to participate, you are still free to withdraw from the study at any time without giving a reason and penalty. If you decide to cease from participating in this study, you must inform your study investigator and no new data will be collected from your child. The researcher may also remove your child from the study for various reasons. In this event, your child will not lose his/her rights as a patient and will still receive the usual standard of care.

Data & Confidentiality:

Participant's confidentiality will be maintained throughout the investigation. The personal data will be anonymized. Hence your identity will be kept confidential. Data collected and entered into the Case Report Form remain the property of UKM. In the event of any publication regarding this study, your identity will remain confidential.

By signing the Informed Consent form attached, you (or your legally acceptable representative, if relevant) are authorising such access to your study records.

Payment and compensation:

You do not have to pay, nor will you be paid to participate in this study. You do have to pay for the usual hospital charges.

Whom can I ask about the study?

If you have any questions about this study or your rights, please contact

Principal Investigators: Prof. Dr Cheah Fook Choe
Department of Paediatrics
UKM Medical Centre
Phone Number : 03-9145 5391/ 5380

Co-investigator Dr. Doris Lau Sie Chong
Department of Paediatrics
UKM Medical Centre
Phone Number : 03-9145 5387

Signatures

To be entered into this study, you or a legal representative must sign and date the signature page [APPENDIX II]

APPENDIX II

Patient/Subject Information and Consent Form (Signature Page)

Research Title: Transdermal Microneedle Patch to Enhance Topical Anaesthesia Before Intravenous Line Insertion for Blood Transfusion in Paediatric Thalassaemia Patients

Researcher's Name: Prof. Dr Cheah Fook Choe / Prof. Dr Azrul Azlan Hamzah

To become a part of this study, you or your legal representative must sign this page. By signing this page, I am confirming the following:

- I have read all of the information in this Patient Information and Consent Form, including any information regarding the risk in this study and I, have had time to think about it.
- All of my questions have been answered to my satisfaction.
- I voluntarily agree to be part of this research study, to follow the study procedures, and to provide necessary information to the doctor, nurses, or other staff members, as requested.
- I may freely choose to stop being a part of this study at any time.
- I have received a copy of this Patient Information and Consent Form to keep for myself.

Patient Name (Print or type)

Patient Initials and Number

Patient I.C No. (New)

Signature of Patient or Legal Representative
(Add time if applicable)

Date (dd/MM/yy)

Name of Individual
Conducting Consent Discussion (Print or Type)

Signature of Individual
Conducting Consent Discussion

Date (dd/MM/yy)

Name & Signature of Witness

Date (dd/MM/yy)

Note: i) All subject/patients who are involved in this study will not be covered by insurance.

APPENDIX III

| LOCAL SKIN REACTIONS | GRADES | | | | |
|----------------------|---------|--|---|--|--|
| | 0 (Nil) | 1 (Mild) | 2 (Moderate) | 3 (Severe) | 4 (Life Threatening) |
| Pain | Nil | Does not interfere with activity | Repeated use of non-narcotic pain reliever for more than 24 hours or interferes with activity | Any use of narcotic pain reliever or prevents daily activity | Emergency room (ER) visit or hospitalization |
| Erythema | Nil | 2.5 cm – 5 cm | 5.1 cm – 10 cm | >10 cm | Necrosis or exfoliative dermatitis |
| Ecchymosis | Nil | 2.5 cm – 5 cm | 5.1 cm – 10 cm | > 10 cm | ER visit or hospitalization |
| Swelling | Nil | 2.5 cm – 5 cm and does not interfere with activity | 5.1 cm – 10 cm or interferes with activity | >10 cm or prevents daily activity | Necrosis |
| Pruritus | Nil | Mild or localized, requires topical intervention | Widespread and intermittent, limiting instrumental activity of daily living (ADL), requires oral intervention | Widespread and constant, limiting self-care ADL, requires systemic corticosteroid or immunosuppressive therapy | - |
| Tenderness | Nil | Mild discomfort to touch | Discomfort with movement | Significant discomfort at rest | ER visit or hospitalization |

APPENDIX IV

| SYSTEMIC REACTIONS | GRADES | | | | |
|--------------------------------------|--------------|--|---|--|--|
| | 0 (Nil) | 1 (Mild) | 2 (Moderate) | 3 (Severe) | 4 (Life Threatening) |
| Fever | <38.0 °C | 38.0 – 38.4 °C | 38.5 – 38.9 °C | 39.0 - <40.0 °C | >40.0 °C |
| Tachycardia (bpm) | <100 | 101-115 | 116-130 | >130 | ER visit or hospitalization |
| Hypotension (systolic; mm/Hg) | 90 and above | 85-89 | 81-84 | <80 | ER visit or hospitalization for hypotensive shock |
| Seizure | Nil | Brief partial seizure and no loss of consciousness | Brief generalised seizure | New onset seizures (partial or generalised). Multiple seizures despite medical intervention | Prolonged repetitive seizures, life threatening consequences |
| Irritability | Nil | Mild; easily consolable; No interference with activity | Moderate; limiting instrumental ADL, increased attention indicated; medical intervention is not indicated | Severe abnormal or excessive response; limiting self-care ADL; inconsolable; medical / psychiatric intervention is indicated | ER visit or hospitalization |

| | | | | | |
|-----------------------------|-----|---|---|---|---|
| Tiredness / Lethargy | Nil | Mild symptoms; no interference with daily activity; reduced alertness and awareness | Moderate symptoms; limiting instrumental ADL; no medical intervention is required | Prevents daily activity; requires medical intervention | ER visit or hospitalization |
| Anorexia | Nil | Loss of appetite without alteration in eating habits | Oral intake altered without significant weight loss or malnutrition; oral nutritional supplements indicated | Associated with significant weight loss or malnutrition (e.g., inadequate oral caloric and / or fluid intake) | Life threatening consequences / urgent intervention is required |
| Vomiting | Nil | No interference with daily activity or 1-2 episodes / 24 hours | Some interference with daily activity or >2 episodes / 24 hours | Prevents daily activity, requires outpatient IV hydration | ER visit or hospitalization due to hypotensive shock |