UNIVERSITI KEBANGSAAN MALAYSIA

The National University of Malaysia

Research Protocol

Transdermal microneedle lignocaine delivery versus EMLA patch for topical analgesia before venepuncture procedure to adults in a clinic setting

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CHAPTER 1

INTRODUCTION

1.1 Transdermal Drug Delivery (TDD)

Venepuncture is one of the most commonly encountered invasive medical procedures that causes significant traumatic pain experience and stress to the hospitalised patients. The anxiety and apprehension toward needle puncture may be exacerbated in patients with chronic conditions who require frequent venepuncture. This in long term may affect their emotional well-being and eventually deter them from seeking future medical attention.

With the current advances in medical science and technology, healthcare is continually improving with innovative approach to holistically deliver treatment to the patients efficiently and effectively. Local anaesthesia prior to venepuncture is regarded as a modern approach that fits with the Good Clinical Practice (GCP) framework in providing a quality standard of care to the hospitalised patients. More recently, the emergence of transdermal drug delivery system (TDDS) has been valued for its invention as a less invasive alternative in facilitating effective drug administration.

The conventional drug-delivery route through hypodermic needle injection and topical cream are the most common strategy for systemic drug delivery through the skin surface. However, both methods are having their inherent downsides. The greatest challenge associated with hypodermic needle use is the pain, stress, and even needle phobia caused by its invasiveness which leads to lower patient compliance and acceptability.[1,2] On the other hand, the topical drug delivery with formulated cream (e.g. lignocaine cream) would be a more attractive alternative route of administration since it is painless and can be self-administered by the patients themselves. Nonetheless, as the drugs must passively diffuse across the skin, its onset of action and efficacy might be affected by its limited bioavailability at the site of action.[3] Such delayed or substandard drug efficacy would thus be impractical and unsuitable for widespread and convenient use in the modern-day fast-paced clinical setting.

Besides, previous researches have investigated various state-of-the-art TDDS alternatives such as iontophoresis (induction of electrical current to drive electrostatic diffusion of charged anaesthetic permeants through the skin layers), sonophoresis (generation of low-frequency ultrasound that facilitates the penetration of drugs), electroporation (formation of microchannels upon electric pulse to increase permeability), magnetophoresis (utilisation of magnetic field), thermophoresis (short thermal exposure to ablate the skin surface for better diffusion of drugs), and the jet injection (controlled compression of gas or spring to deliver anaesthetic drug into targeted skin layer).[4–6] However, these myriad strategies of enhancing transdermal drug delivery are hampered by several design pitfalls and undesirable adverse reactions such as second-degree burns, cross-contamination risk, limited absorption for drugs of large molecular size and others.[1] Hence, a more revolutionary approach is required to circumvent the issues above.

1.2 Skin Structure and Transdermal Drug Delivery (TDD)

Human skin is the most readily accessible and largest organ in the human body which covers and area of 1.5 - 2.0m^2 and accounts for 16% of the total body weight of an adult.[1,7] The primary role of human skin is to serve as a barrier that protects the human body against the relatively

hostile external environment. However, such organised barrier function has also led to a poor permeation of the applied topical drug across the skin layer, hence impairing effective drug delivery and onset of action.

The skin is histologically classified into 3 main compartments: (1) the outermost epidermis, (2) the middle dermal layer, and (3) the innermost hypodermis.[1,8,9] The avascular layer of epidermis predominantly consists of keratinocytes (~95% of total cell in epidermis) which are the non-viable constituents of the outermost *stratum corneum* (SC) and the viable cutaneous sublayers underneath the SC. Several sublayers of the viable epidermis, i.e. *stratum basale, stratum spinosum, stratum granulosum,* and *stratum lucidum* are collectively 50-100 µm thick and are adjoint together by tonofibrils. On the whole, the whole skin epidermal layer is 50-150 µm thick.[10]

The epidermis is partitioned from the deeper dermal layer by an undulating epidermal-dermal junction. Dermis is the thickest substructure of the skin (2-3 mm thick) and it is primarily composed with collagen and elastin fibres that confer it the strength and elasticity.[11] The papillary and reticular layers of the dermis house the blood vessels, nerves, lymphatic vessels, skin appendages, and the connective tissues.[8,9] Lying underneath it is the innermost hypodermis or the subcutaneous layer, which is an elastic layer that is mainly constituted of the adipose tissues, blood vessels and nerve endings.[9] **Figure 1** illustrated the structure and composition of the human skin.

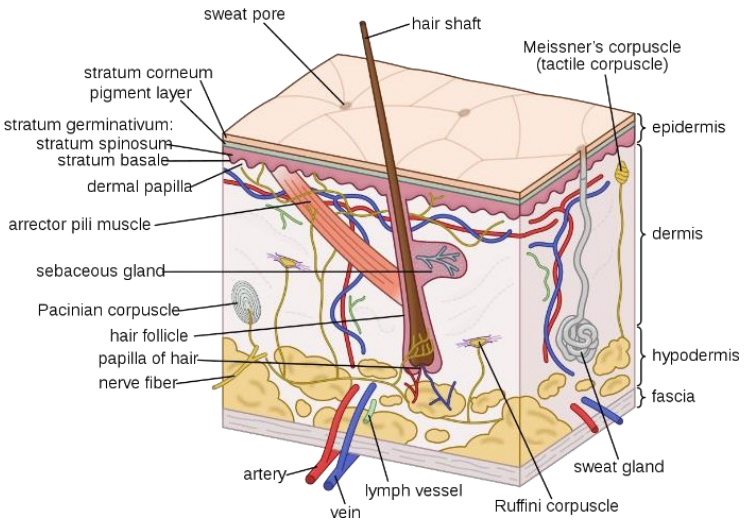

Figure 1. Human skin structure and composition. The skin is mainly comprised of 3 layers, i.e. epidermis, dermis, and hypodermis with different structural elements that reside within distinct anatomical positions. *Figure courtesy: Tomáš Kebert & umimeto.org - Own work, CC BY-SA 4.0*

In the context of TDD, the *stratum corneum* (SC) is the primary mechanical barrier that limits the uptake rate of the topically applied drugs. The SC, made up of multilayered dead corneocytes scattered throughout a lipid-rich matrix, is principally a lipophilic nature by its barrier properties and which will exhibit a selective permeability for the penetration of relatively lipophilic molecules into deeper layers of the skin.[12] Therefore, drugs that are rather

lipophobic or high in molecular weight are relatively impeded for its effective systemic delivery. Apart from that, the underlying epidermal-dermal junction would be the secondary factor conferring the resistance on the molecular transportation of drug molecules across the epidermal layer into dermis.[13] Only the drug molecules that managed to get through the SC and the papillary dermis will effectively reach the systemic circulation to exert its systemic effects.[14]

The sophisticated structure of the skin has also been implicated as the main barrier to ensuring an optimal bioavailability of the drugs delivered using TDDS. Previous study reported that the only 10-20% the total drug in a topical preparation successfully permeated across the skin layers.[15a] On the contrary, the high bioavailability of the drugs (90-100% of the total drug) has been touted as the main advantage of hypodermic needle.[15] However, its invasive and pain-inducing nature again limits its widespread usage.[15] Hence, a novel TDD system is required to overcome the aforementioned design limitations.

15a) Prausnitz MR, Langer R. Transdermal drug delivery. Nature biotechnology. 2008;26(11):1261-8.

CHAPTER 2

LITERATURE REVIEW

2.1 Emergence of Microneedle and its Applications

With the advent of the microfabrication technology, a revolutionary solution of effective transdermal drug delivery has been introduced with the use of microneedle (MN) patch. MN is a medical device that is composed of tiny micron-sized needles aligned in out-of-plane protruded arrays that can be impregnated with bioactive drug substances.[16] These micron-sized needles create multiple transdermal microchannels when they puncture through the cutaneous stratum corneum. Consequently, this innovative means of drug delivery enhances transcutaneous drug absorption, resulting in a faster onset of therapeutic action.[15]

MN offers several superior clinically-relevant benefits. The most significant advantage of MN is it minimises the pain experienced by the patients by preventing nociceptor stimulation during venepuncture. Further, the MN design could also be tailored for the intended therapeutic agent delivery.[15] Besides, MN can also be readily self-administered by the patients without the expert assistance from the healthcare professionals.[15,16] With regard to needle breakage issue associated with the conventional hypodermic needle usage, the dissolvable MN is potentially attractive in circumventing such a design imperfection. Furthermore, dissolving MN would also minimise the biohazard wastage compared to the conventional metal-based hypodermic needles.[17]

Maltose, a natural non-cytotoxic carbohydrate is one of the most common constituents utilised in MN fabrication due to its excellent biodegradability profile and quick dissolution within minutes of administration.[18,19] With a well-recognised safety record, maltose is also widely used for various pharmaceutical formulations with its proven safety and efficacy track record upon clinical application.[20] Among MN types, dissolvable MN is a type of biodegradable microneedle patch that can be encapsulated with pharmaceuticals for transdermal drug delivery. Dissolving drug-embedded microneedle patch allows a continuous dissolution of drugs into the targeted skin layer upon its insertion onto the skin surface.[21] Such drug administration strategy could also be described as a 'poke-and-release' drug delivery system.[21]

To date, the scientific endeavours investigating the effects of dissolving microneedles on delivering anaesthetics for the adult patients who requires regular blood transfusion by their clinical conditions are scarce. So far, only three prior studies evaluated the efficacy of microneedles for alleviating pain in adult patients undergoing routine vein-puncturing procedures. Rzhevskiy et al. (2022) demonstrated in a clinical trial investigating the efficacy of lidocaine delivered via a hollow microneedle (MicronJet600) prior to peripheral venous cannulation. They showed a significant 11-fold VAS score reduction in adults undergoing routine peripheral venous cannulation when 2% lidocaine was intradermally administered using MicronJet600 compared to no anaesthetic pretreatment (mean VAS score: 3.6 (MJ+L combination) vs 39.7 (placebo); Cohen's d: -.43 (95% CI -48, -3.9)) .[22] In addition, Ornelas et al. (2016) conducted a randomised, single-blinded, parallel-group clinical trial to evaluate the effect of microneedle pre-treatment in hastening the onset of cutaneously-applied 4% lidocaine cream. They demonstrated that the microneedle-assisted 4% lidocaine delivery had shortened the application time from 60 to 30 minutes and significantly minimised the pain induced by

needle lancet at 30 mins compared to sham patch (VAS score (mean ± SD): microneedle: 4 ± 1.3 mm; sham: 14.4 ± 3.8 mm).[23] In contrast, Gupta et al. (2012) compared the efficacy of lidocaine injections administered by hollow borosilicate-glass microneedle with the conventional hypodermic needle in 15 healthy adults. They established that both lidocaine delivery systems produce similar local anaesthetic effects ($p > 0.05$) across all time points, but the hollow microneedle recipients reported significantly better dermal analgesic effects compared to the recipients of conventional hypodermic needles[24]

Based on these findings, the utility of microneedles is promising for effective anaesthetic delivery and pain amelioration. However, the validity of the aforementioned evidence may be limited by several methodological shortcomings. For instance, the efficacy of transdermal microneedle in alleviating venepuncture-related pain has not been investigated in a clinical trial setting. Besides, Ornelas et al's RCT was limited to only male population, a significant methodological weakness that hampers the generalisability of the results to the whole population[23]. Apart from that, Gupta et al. (2012) stated that the hollow borosilicate-glass microneedle prototype suffers from undesirable lidocaine leakage when it was applied on their subjects[24]. Therefore, further investigations are necessary to improve the microneedle design so that the local anaesthetic delivery can be further optimised for convenient application in adults who undergo frequent routine clinical procedures venepuncture.

To our knowledge, the efficacy of lignocaine-impregnated microneedle for local anaesthetic delivery has not been studied. Hence, the safety, tolerability and pharmacodynamic property of lignocaine-embedded microneedle warrant further investigation. Therefore, our trial is designed to provide the answers to such scientific lacunae.

2.2 The general pharmacological properties of lignocaine

Lignocaine, in its solid state, possesses a crystalline and colourless structure that is dissolvable into a hydrochloric salt form.[25] It possesses anti-arrhythmic and anti-nociceptive properties mediated through voltage-gated sodium and potassium channel blockade.[26]

It is primarily bound to α-acid glycoprotein (AAG) (approximately 50%) and to lesser extent, albumin (around 25%).[27-28] Diseases such as myocardial infarction and cirrhosis and other factors, for instance smoking and age, will influence the serum AAG concentration levels which in turn will affect the fraction of free plasma lignocaine concentration.[29-32] By contrast, an increase in the AAG level will reduce the amount of unbound, active lignocaine in the plasma, resulting in a reduction of its pharmacological effects at a specific plasma lignocaine concentration.[33] The volume of distribution at steady state, V_{ss}, in normal adults is 1.32 (SD 0.27) L/kg whilst the V_{ss} in chronic heart failure (CHF), chronic liver impairment and chronic renal failure (CRF) patients are 0.88 L/kg, 2.31 L/kg and 1.2 L/kg, respectively.[34-35]

Lignocaine has a high degree of hepatic extraction ratio (62%-81%).[36] Hence, factors affecting hepatic blood flow such as metoprolol-mediated reduction in hepatic blood flow, congestive heart failure and acute myocardial infarction will influence the biotransformation rate of lignocaine.[37-38] Apart from that, lignocaine is metabolized by CYP3A4, CYP3A5 and CYP1A2 through consecutive deethylation steps.[39-40] Therefore, concurrent administration of CYP1A2 and CYP3A4 inhibitors such as amiodarone, fluvoxamine, erythromycin and others causes mild-to-major elevations of serum lignocaine concentrations.[41-43] Lignocaine dosage reduction by up to 60% is thus warranted when a CYP1A2 inhibitor (e.g. fluvoxamine) is concomitantly administered with lignocaine.[44]

Lignocaine has two major metabolites; monoethylglycinexylidide (MEGX) and glycinexylidide (GX).[45] MEGX is responsible for both lignocaine's therapeutic and toxic effects whilst GX primarily causes lignocaine-associated toxicity.[46-47] Lignocaine is mainly eliminated via hepatic metabolism and the clearance rate ranges from 0.72 (SD 0.15) L/hr/kg in healthy adults[34] to 0.25 L/hr/kg in patients with Child-Pugh class C hepatic impairment.[48]

The main adverse events (AEs) associated with toxic level of serum lignocaine affect two major organ systems; the central nervous system (CNS) and cardiovascular system (CVS). In the former, the major AEs are confusion, slurring of speech, paraesthesia around the lips and tongue, diplopia, tremor, seizures whilst sinus bradycardia, sinus arrest and disturbances in atrioventricular conduction are the major AEs in the latter.[49] The toxic effects of lignocaine will appear when serum lignocaine concentration exceeds 5 mg/L and convulsion occurs when it exceeds 10 mg/L.[50] Despite being associated with the development of nasal adenomas and tumours in murine models, lignocaine is not associated with cancer development in humans.[50-51]

In 1999, the US Food and Drug Administration (FDA) has approved the lidocaine 5% patch (Lidoderm®, Endo Pharmaceuticals Inc, Malvern, USA) for the treatment of postherpetic neuralgia.[52] Henceforth, it has been extensively investigated for the treatment of other medical conditions such as low back pain, postoperative pain control, rib fractures and for the prevention of venepuncture or injection-related pain in paediatric patients.[53-56] However, in a recent meta-analysis by Bai et al., the 5% topical lidocaine patch was ineffective as an adjunct for the management of acute and postoperative pain since no significant differences were found in terms of the mean pain intensity scores, duration of hospital stay and the postoperative opioid consumption between the 5% topical lidocaine patch and placebo recipients.[57] Hence, a new transdermal drug delivery system that can transcutaneously deliver lignocaine more effectively is required, especially in the context of alleviating pain associated with routine vein-puncturing procedures, namely venepuncture or intravenous cannulation.

2.3 Dermato-pharmacokinetics (DPK) and mathematical modelling of microneedle-assisted topical anaesthetic delivery

The dermal bioavailability of transdermally-delivered local anaesthetic agents should be accurately quantified in order to precisely evaluate their pharmacodynamic interactions with the cutaneous nociceptors. However, the quantification procedure is fraught with significant methodological challenges. Recently, Pensado et al. demonstrated that the minimally invasive stratum corneum (SC) sampling was only adequately-powered to detect a 50% difference in the mean uptake and clearance parameters and pharmacodynamic response characteristics (measured using the area above the blanching effect curve (AAEC)) between two different doses of Betnovate® (0.1% w/w BMV) cream

applied on the anterior aspects of forearms of 12 individuals, whilst a larger sample size was required to detect a smaller effect size (20% difference) for the two applied doses.[58] In contrast, the skin blanching technique produced more highly variable results and therefore was relatively indiscriminative to the two different Betnovate® cream doses.[58] Other techniques that have been proposed for dermato-pharmacokinetic (DPK) assessments of topical drugs, such as the in vitro Franz Diffusion Cell, ex vivo tape stripping, in-vivo micro-dialysis and suction blister techniques and confocal laser microscopy are also laden with serious methodological shortcomings (i.e. invasive procedure, absence of sensitive methods to analyze the analyte's concentration, technical variations in the tape removal).[59] Consequently, the DPK properties of topically-delivered local anaesthetics could not be accurately evaluated, which is the principal reason why our research will focus more on the TDD system's safety profile evaluation through the quantification of the amount of lignocaine or 5% EMLA dermal patch entering the systemic circulation (classical bioavailability).

For an infinite dose of a given local anaesthetic agent, the quantity of penetrant or permeant that has reached the receptor chamber at time t, Q(t), can be represented by the following mathematical relationship:[60]

$$Q(t) = A * p * h * c * \left[D * \frac{t}{h^2} - \frac{1}{6} - \frac{2}{\Pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} * e^{\left(\frac{-D*n^2*\Pi^2*t}{h^2}\right)} \right] \quad \text{(Equation 2.1)}$$

where A is the skin's surface area that is available for drug molecule diffusion; p is the coefficient of partition between the donor vehicle and the homogenous membrane; h is the thickness of the membrane (path length traversed by the drug molecules); C is the permeant's concentration in the donor solution; * is the multiplication symbol and D is the coefficient of diffusion for the permeant in the membrane. From equation 2.1, it can be clearly observed that the delivery of active drug molecules is proportionally related to the skin's surface area. As a result, a topically-applied local anaesthetic must be correctly applied to the intended cutaneous surface area to ensure optimal absorption and delivery of the anaesthetic drug molecules to the target site action site (i.e. skin nociceptors).

From equation 2.1, we can also deduce that decreasing h will also increase Q(t). Since microneedles puncture through the skin's SC layer, this reduces h and thus increases Q(t). We, therefore, hypothesized that the increased quantity of local anaesthetic agents (penetrant) at the skin nociceptors (receptor chamber) will also enhance the analgesic property of the local anaesthetic agents, resulting in a faster onset of action and subsequent greater pain reduction. Our postulate is further corroborated by Ronnander et al. who demonstrated that a change in the dermal concentration of sumatriptan delivered via dissolvable polyvinylpyrrolidone (PVP) microneedle with respect to time can be mathematically modelled using:[61-62]

$$\frac{dc}{dt} = -(K_L c) + 4\left(\frac{k_D tan\,\theta}{\rho * cos\,\theta}\right) h^2 \left[\frac{\beta\rho - c}{v_0 + v_{c,0} - v_c}\right] \left[c_S - \left(\frac{1-\beta}{\beta}\right) c\right], \quad \text{(Equation 2.2)}$$

where K_L is the constant of elimination; c is the drug concentration in the skin layer, k_D is the constant for the rate of dissolution of a drug matrix (in cm/h), ρ is the drug matrix's density (or the microneedle's density (in g/cm^3)) , θ is the half angle (in degree) at the dissolvable microneedle's apex (or microneedle's height-length ratio), h is the height of the dissolvable microneedle, β is the fraction of drug mass in the microneedle, $v_0 + v_{c,0} - v_c$ is the sum of the initial volume of the skin layer (cm^3) and the initial volume of the microneedle (cm^3) minus the volume of microneedle at the time when c

is measured (cm^3) and c_s is the solubility of matrix polymer in a solvent (e.g. water, measured in g/cm^3). Using the Fick's second law of diffusion, equation 2.2 could be rewritten as[62-63]:

$$\frac{\delta c}{\delta t} = D\frac{\delta^2 c}{\delta y^2} + 4\left(\frac{k_D tan\,\theta}{\rho * cos\,\theta}\right)h^2\left[\frac{\beta\rho - c}{v_0 + v_{c,0} - v_c}\right]\left[c_s - \left(\frac{1-\beta}{\beta}\right)c\right], \quad \text{(Equation 2.3)}$$

where D is the diffusion coefficient or diffusivity (cm^2/h) and $\frac{\delta^2 c}{\delta y^2}$ is the curvature of the concentration profile at a specific point (x-position) in space. The first term on the right-hand side of equation 2.2 denotes the diffusion of the drug across the cutaneous layers and the second term represents the estimate of drug released from its encapsulating polymer via dissolution.[62]

From equations 2.2 and 2.3, we could again conjecture that the concentration of a drug in the skin is dependent upon the drug loading (β), and the height of the dissolvable microneedle (h).[61-64] By increasing the height of the microneedle, the stratum corneal layer can be bypassed and the local anaesthetics can hence be rapidly delivered at the intended site of action (skin nociceptors). Therefore, we believe a lignocaine-embedded microneedle patch design may enhance the lignocaine's onset of action via a similar mechanism.

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61. Ronnander P, Simon L, Spilgies H, Koch A. Modelling the in-vitro dissolution and release of sumatriptan succinate from polyvinylpyrrolidone-based microneedles. Eur J Pharm Sci. 2018 Dec 1;125:54-63. doi: 10.1016/j.ejps.2018.09.010..

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64. Yadav PR, Han T, Olatunji O, Pattanayek SK, Das DB. Mathematical Modelling, Simulation and Optimisation of Microneedles for Transdermal Drug Delivery: Trends and Progress. Pharmaceutics. 2020 Jul 22;12(8):693. doi: 10.3390/pharmaceutics12080693.

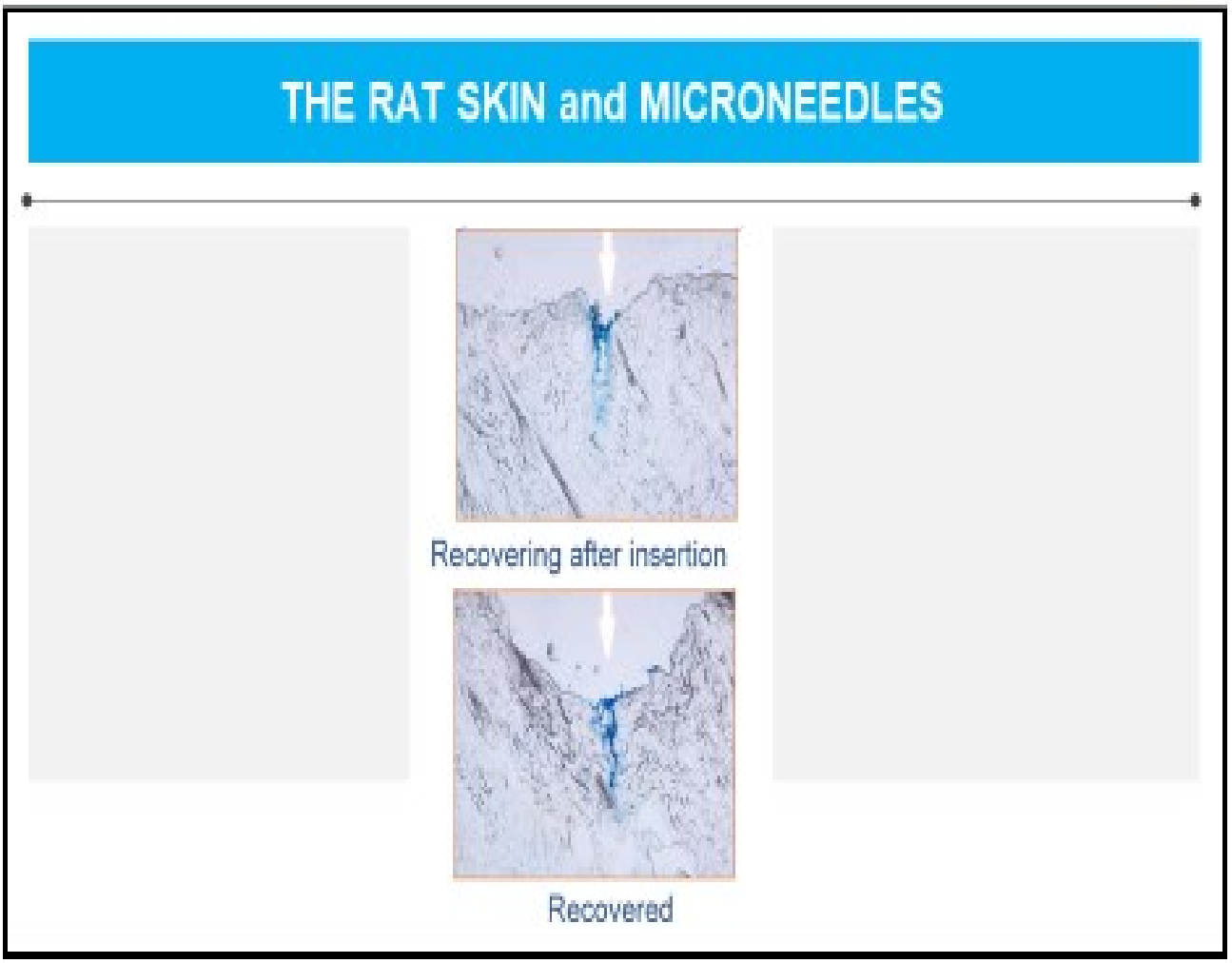
2.3 Justifications of study

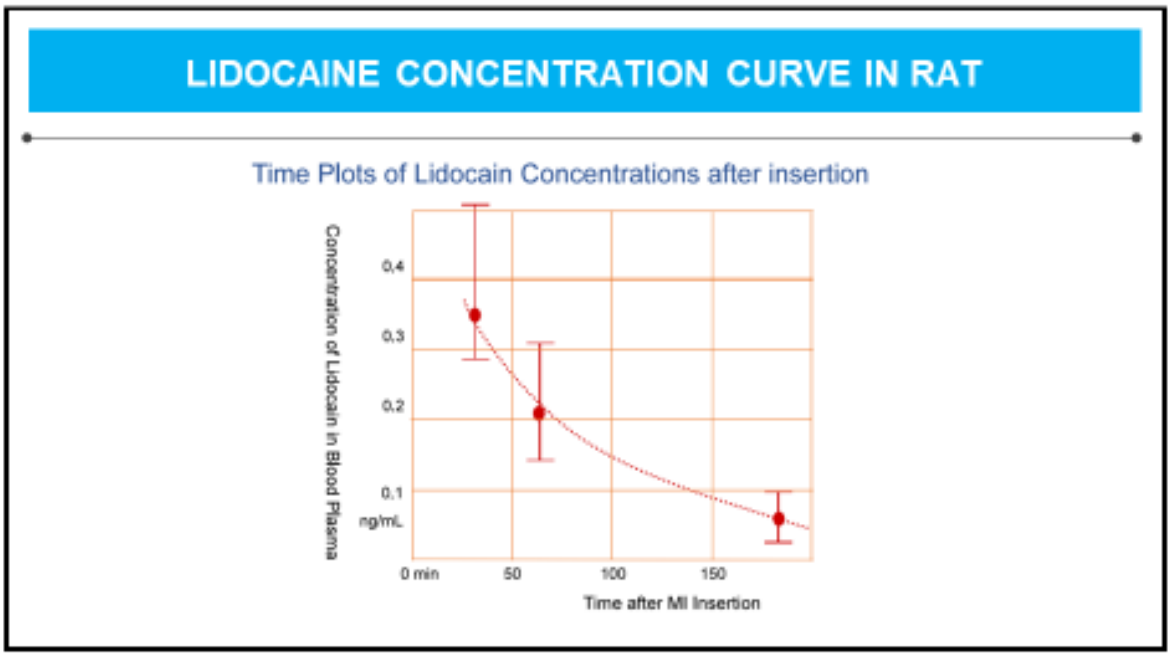
Based on our brief review of literature, we have then pointed out three problem statements in bridging the knowledge gaps from previous research with the highlighted significance of our current study.

I) The safety profile of transdermally-delivered lignocaine that is directly embedded within the matrix of dissolvable microneedle in the adult patients requiring venepuncture during routine

clinical settings have not been robustly and exhaustively tested in previous studies. This study aims to evaluate the safety and tolerability of lignocaine-embedded microneedle in this cohort of patients. The microneedle patch was designed and used (without impregnated drugs) in a previous project approved by this committee among paediatric thalassaemia patients. This trial protocol has been published in clinicaltrials.org and also in Journal of Clinical Medicine 2022, Sep 8;11(18):5291. doi: 10.3390/jcm11185291. The safety profile was good with side effects showing that out of 19 patients studied, none reported any serious adverse reactions (SARs) or sudden unexpected serious adverse reactions (SUSARs) to the microneedle, with only one case complained of mild itchiness at the patch site.

II) The pharmacokinetic properties of lignocaine that is directly delivered transdermally through embedment within dissolvable microneedle's matrix have not been investigated in the previous study. Therefore, a subset of this study patients will provide the preliminary pharmacokinetic data for lignocaine delivered via such administration route which will indicate the extent of absorption of lignocaine, if any at all, into the systemic circulation. Animal study in rats showed no scarring and complete healing after microneedle patching, and blood plasma lidocaine was subtherapeutic level, below 0.4ng/mL and diminished to almost nil after 150 minutes.

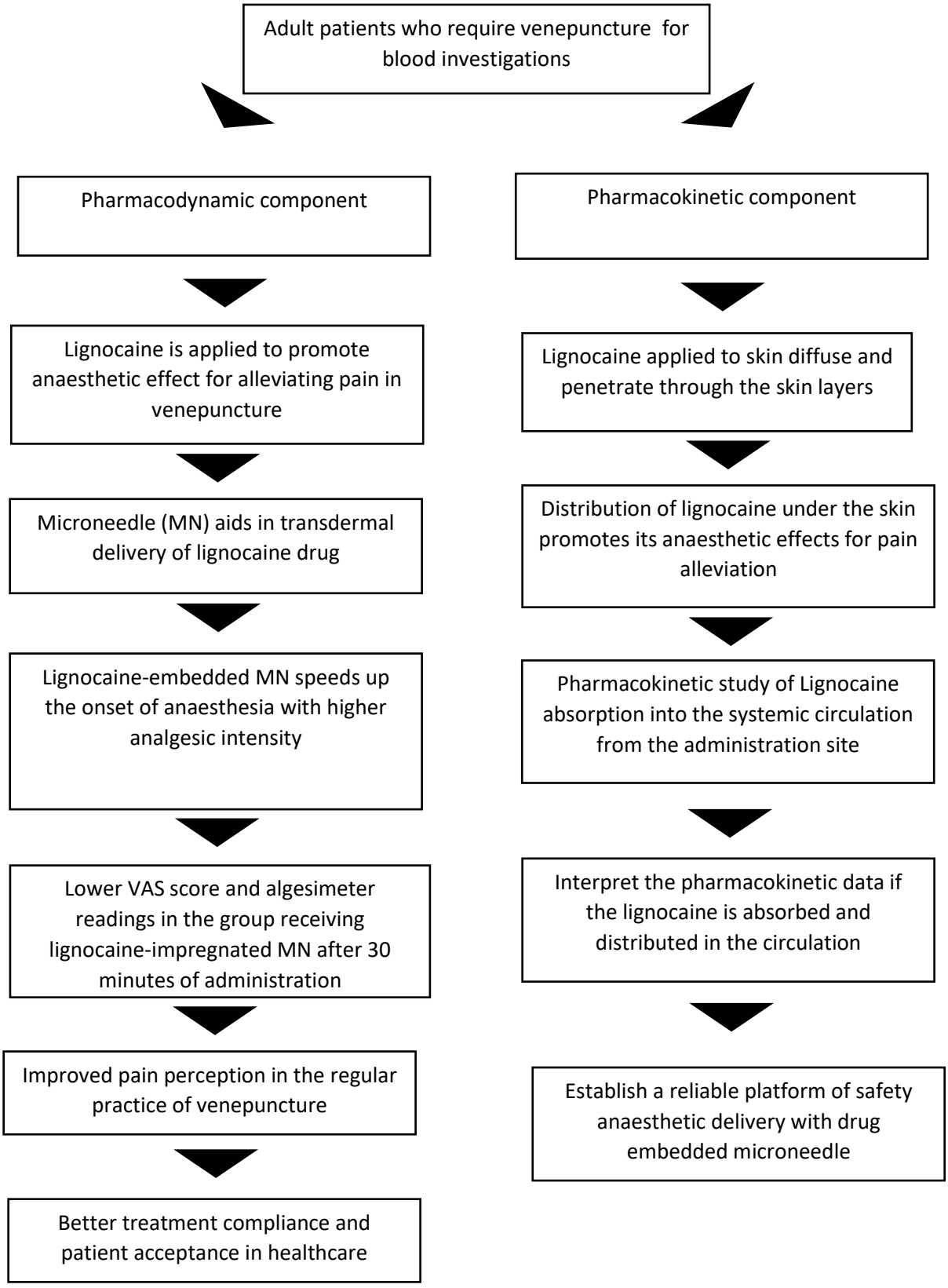




Hence, our current research is looking forward to providing the answers in response to the problem statements above as well as to highlight the potential of lignocaine-impregnated microneedle for its clinical application in topical anaesthetic administration and management. To summarize the foundations of our research, the conceptual framework of this research is shown in Section 2.4.

2.4

Conceptual Framework



CHAPTER 3

OBJECTIVES

3.1 General Objective

To assess the safety and efficacy of lignocaine-embedded microneedles as a means of pain-reduction in adult patients requiring routine venepuncture procedure.

3.2 Specific Objectives

1. To evaluate the safety profile of lignocaine-embedded microneedle patch as a means of pain reduction in adult patients requiring routine vein-puncturing procedures

2. To assess the pharmacokinetic (PK) parameters of lignocaine in the systemic circulation when the transdermal lignocaine delivery is enhanced through microneedle usage.

3. To compare the efficacy of lignocaine-embedded microneedle patch with standard 5% EMLA dermal patch for pain reduction during venepuncture procedure based on mean changes in VAS scores and skin algesimeter index (pharmacodynamic (PD) study).

3.3 Research Questions

1. What is the safety profile of lignocaine-embedded microneedle patch used for pain reduction in adult patients requiring routine vein-puncturing procedures?

2. What are the values of PK parameters (AUC_{inf}, AUC_t, $C_{max,}$ $t_{1/2}$, volume of distributions (V_d) and clearance) for the lignocaine constituent in adult patients receiving lignocaine-embedded microneedles for routine vein-puncturing procedures?

3. Are there differences between lignocaine-embedded microneedle patch and standard 5% EMLA dermal patch for pain reduction during venepuncture procedure based on mean changes in VAS scores and skin algesimeter index?

3.4 Research Hypotheses

1. H_0: The proportion of participants experiencing adverse event is not significantly different from 0.

   H_1: The proportion of participants experiencing adverse event is not significantly different from 0.

2. H_0: The values of PK parameters for the lignocaine constituent in adult patients receiving lignocaine-embedded microneedles are not bioequivalent with the values of PK parameters for the lignocaine constituent in adult participants receiving lignocaine through the standard topical route.

   H_1: The values of PK parameters for the lignocaine constituent in adult patients receiving lignocaine-embedded microneedles are bioequivalent with the values of PK parameters for the lignocaine constituent in adult participants receiving lignocaine through the standard topical route.

3. H_0: There are no differences in mean changes in VAS scores and skin algesimeter index between the trial participants receiving lignocaine-embedded microneedle patch and standard 5% EMLA dermal patch.

   H_1: There are significant differences in mean changes in VAS scores and skin algesimeter index between the trial participants receiving lignocaine-embedded microneedle patch and standard 5% EMLA dermal patch.

CHAPTER 4

METHODOLOGY

4.1 Study Design

This study can be divided into two distinct stages:

a) Stage 1: Non-randomized single-centre open-label single group clinical trial to primarily assess the safety and tolerability of lignocaine-impregnated microneedle in adult patients undergoing routine vein-puncturing related procedures (pharmacokinetic (PK) study).

b) Stage 2: A randomized single centre double blind two parallel group active controlled clinical trial to assess the efficacy of lignocaine-impregnated microneedle compared to 5% EMLA dermal patch (Pharmacodynamic (PD) study).

4.2 Study Location

The study will be carried out at the Ophthalmology Outpatient Clinic, Hospital Canselor Tuanku Muhriz (HCTM), UKM, Bandar Tun Razak.

4.3 Study Period

1st December 2022 – 30th November 2024 (24 months)

4.4 Eligibility Criteria

The inclusion and exclusion criteria of the study participants are as follows:

4.4.1 Inclusion Criteria

I) Patients aged 18 years old and above

II) Patients requiring venepuncture for blood investigations before ophthalmological procedures

4.4.2 Exclusion Criteria

I) Patient with a previous history of sensitization or allergy to lignocaine.

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)

III) Patient exposed to analgesic usage within 24 hours prior to the procedure

IV) Generalized skin disorder/ rash

V) Agitated/ fretful / uncooperative patient

VI) Uncommunicative/deaf/mute

VII) Patients on hypnotics, or chronic pain relief medications

VIII) Patients with psychiatric conditions or cognitive impairment

IX) Patients with hepatic impairment

X) Patients who are on CYP450 3A4, 3A5 or 1A2-inducing or inhibiting drugs (erythromycin, ciprofloxacin, amiodarone etc.) or pharmacotherapeutic agents that affect hepatic blood flow (metoprolol) since both may affect the metabolism of lignocaine.

XI) Failed first/single attempt at venepuncture after the application of the MN patch.

4.5 Reference Population

All adult patients undergoing vein-puncturing procedures at the Ophthalmology Outpatient Clinic in Malaysia.

4.6 Source Population

All adult patients undergoing venepuncture at the Ophthalmology Outpatient Clinic, Hospital Canselor Tuanku Muhriz (HCTM), UKM, Bandar Tun Razak.

4.7 Sampling Frame

All adult patients undergoing venepuncture at the Ophthalmology Outpatient Clinic, Hospital Canselor Tuanku Muhriz (HCTM), UKM, Bandar Tun Razak and fulfil the eligibility criteria.

4.8 Study Subjects

All adult patients undergoing venepuncture at the Ophthalmology Outpatient Clinic, Hospital Canselor Tuanku Muhriz (HCTM), UKM, Bandar Tun Razak, fulfil the eligibility criteria and consent to trial participation.

4.9 Sampling Method

Stratified random sampling, a type of probability sampling method, will be used for both stages of the trial since the sample size required (section 4.9) is smaller than the whole pool of participants. Since differential pain experience has been shown in females and male adults[58], the stratification variable is the gender of the participants to ensure that our study sample contains an equal number of female and male adults and is representative of patient population.

4.10 Sample Size Calculation

4.10.1 Pharmacokinetic (PK) study

Due to the paucity of prior information, formal sample size calculation for stage 1 (pharmacokinetic (PK)) study based on power analysis cannot be carried out. Based on recommendations by Ogungbenro and Aarons (2010) and Julious (2012), the sample size is set at 20 subjects each for the PK study[59-60]. Even though, it is recommended that the sample size can be minimally set at 12 subjects for a single-group pilot pharmacodynamic trial (Julious, 2012), the sample size is increased to 20 subjects since based on Julious 2012's results (Figure 3 of Julious 2012), the statistical asymptote is reached when the sample size is at least 20 subjects[59]. Hence, the addition of another subject will result in only non-substantial gain in the precision of the parameter estimates when the sample size of 20 participants is reached.

4.10.2 Pharmacodynamic (PD) Study

For stage II of the trial, the sample size was calculated using Power and Sample Size (PS) Program version 3.1.6 (Vanderbilt University, Nashville, Tennessee, USA; 2018). The standard deviation (SD) of the VAS score of 2.1 were obtained from a prior study and this is for patients who received 5% EMLA.[61] We consider a 1-point VAS difference between the intervention group as the minimum detectable difference (MTD) and type I error and study power (1- β) were fixed at 0.05 and 0.80, respectively. The ratio of controls to cases is fixed at 1:1 ratio. Based on these parameter values, the calculated sample size is 70 participants per group.

After accounting for a 10% drop-out rate, the final sample size is 77 participants per group (n_{total}= 154 participants) for this stage of trial.

4.11 Physical Description of the Lignocaine-Embedded Microneedle

Currently, we are able to manually produce some microneedle array patches (MAP) in a lab scale quantity. Different designed parameters MAP has been used for initial test on animal skin and also human skin. In this project, two major parts will be carried out. First, we will design a semi-auto machine to fabricate MAP. Refine parameters will be adapted from previous designs for the fabricate of the drug impregnated microneedles. Second, we will carry out a clinical trial for testing on the real implementation and application of the fabricated MAP. The details for each session are discussed as below

Part I: Preparation of Biodegradable Drug Impregnated Microneedle Array Patch

In a previous project, few parameters such as base plate size, number of microneedles per unit area, height etc. that have been determined and optimized. They will be used as a guideline for the design of new microneedle fabrication machine for better microneedle fabrication. With a new fabrication machine, we will be able to have a small-scale production to achieve a pre-commercialization technology readiness.

In this part, we will focus on the fabrication and implementation of biodegradable microneedle array patch. Sugar compounds such as sucrose, trehalose, and maltose have experimented as

biodegradable matrix materials for microneedles. In particular, maltose itself is a carbohydrate that is widely-acknowledged as a generally recognized safe excipient material for drug delivery. MAP fabricated from maltose generally demonstrate strong mechanical properties, and as such can facilitate perforation of skin and formation of micro-channels for transdermal drug delivery. Besides, for enabling the function as dissolvable MAP, maltose can rapidly dissolve in the dermal regions within minutes under body temperature, and is thus able to deliver therapeutic compounds such as proteins and peptides rapidly, safely and in an environmentally-friendly manner. The MAP that we propose as a prototype will consist of two basic but essential structures, i.e. the microneedles and the substrate (baseplate). This special design allows the microneedles to be separated from the substrate once the MAP is applied onto the skin. While the substrates can be readily disposed of after being peeled off from the skin, the microneedles and the therapeutic loads that they carry would stay on the skin and continue to dissolve and release drugs in a predetermined and sustained manner. Furthermore, as the microneedle is only 150 µm in length, thus, maximum penetration would only reach epidermis-dermis intersection. There is no possibility of the microneedles reaching the blood vessels, due to the limited length of the microneedles. Therefore, the drug distribution will follow a topical mode of distribution. No systemic distribution of drug is expected.

A. Preparation of microneedle matrix mix for the dissolving microneedle

I. To prepare the calcium ion cross-linked alginate/sugar (Ca2+/Alg-sugar) composites, sodium alginate powder is firstly dissolved in DI water at a weight ratio of 1:4 with the stir in a water bath at 60 °C until to gain the homogeneous solution.

II. And then, the 15% (w/w) $CaCl_2$ solution is added slowly with rapid mixing to cross-link alginate (CaCl2/Cross-linked alginate weight ratio = 1:10).

III. To enhance the mechanical properties of composite microneedles, 15% (w/w) maltose monohydrate or any carbohydrate equivalents is added simultaneously into the sodium alginate solution to form precursor for preparation of paste for the fabrication of drug-loaded microneedles and baseplate (substrate):

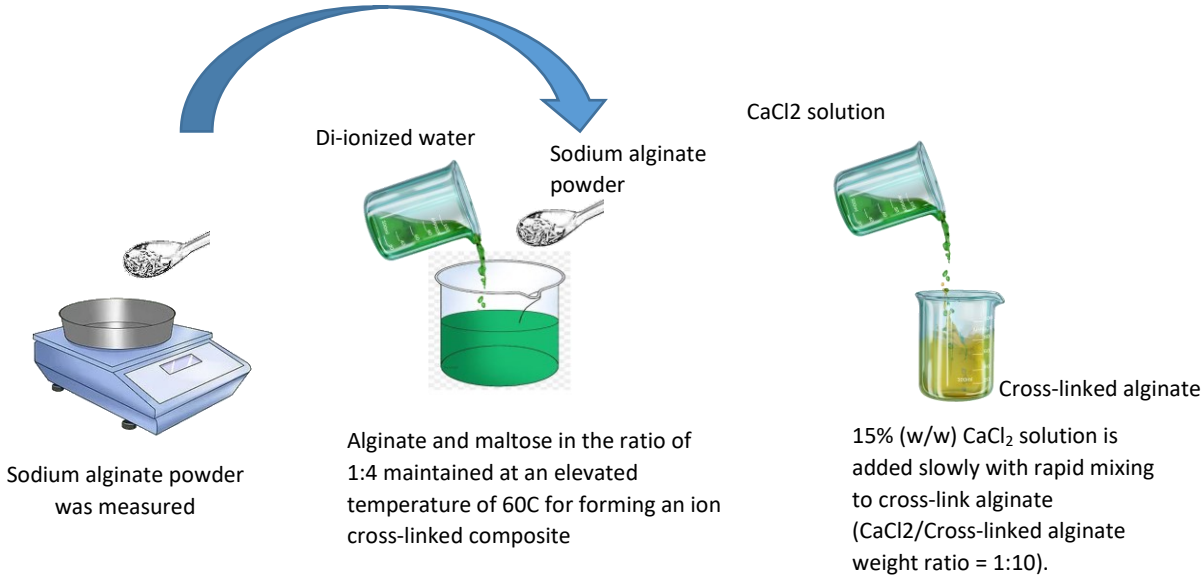
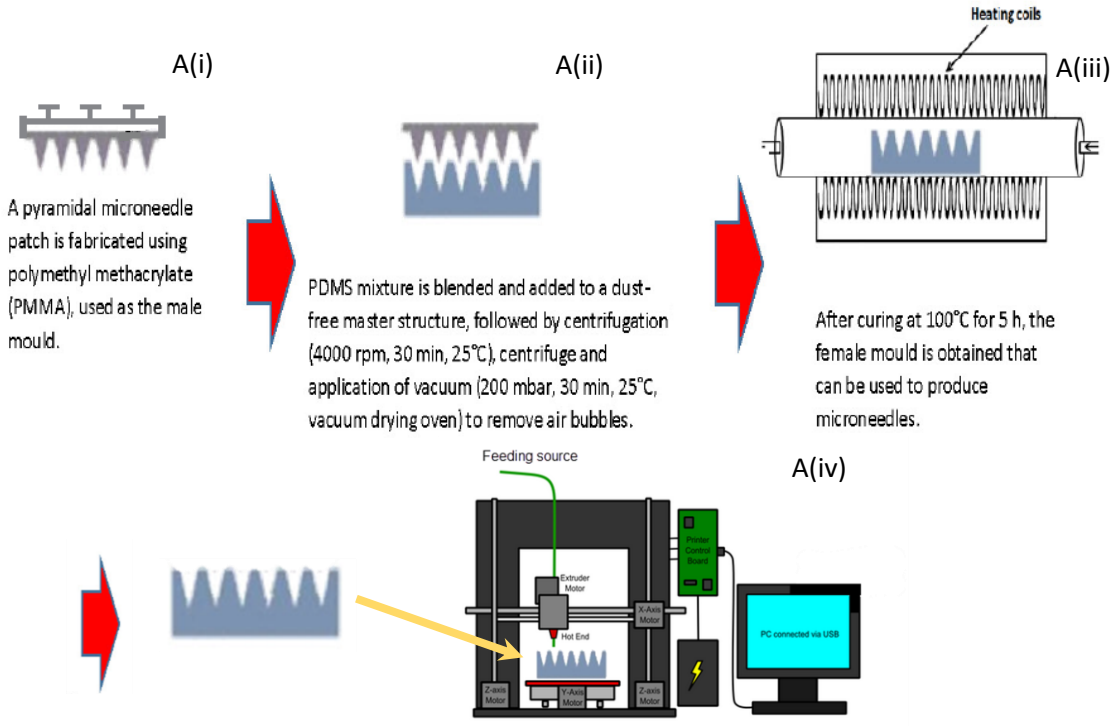


Figure 1: The microneedle composite will be prepared based on the above-mentioned steps

A mould will be fabricated based in this fabrication process. The moulds for different sizes, shape and physical scales of microneedles will be designed.

I. In the previous lab-scale fabrication, the pyramidal microneedle patch is fabricated using polymethyl methacrylate (PMMA) and used as the male mould. In this project, we will use a more solid and hard materials such as metal for the male mould.
II. Subsequently, reversed mould (which is the female mould for the microneedle fabrication) is fabricated. To do so, a PDMS mixture is blended and added to a dust-free master structure, followed by centrifugation (4000 rpm, 30 min, 25°C, centrifuge and application of vacuum (200 mbar, 30 min, 25°C, vacuum drying oven) to remove air bubbles.
III. After curing at 100°C for 5 h, the female mould is obtained, which can be used to produce microneedles made of sugar compounds (such as maltose).
IV. The above processes are to prepare reverse mould with different physical parameters to test for the suitability and conformity of the mould for fixing onto the semi-auto fabrication machine. After that, the designs have the highest aptness will be used. The parameters will be adapted into a 3D printer for small scale produce of these female moulds.



Female mould with the best parameters will be used as standard for small –scale production of the mould with different design and physical parameters used for microneedle fabrication

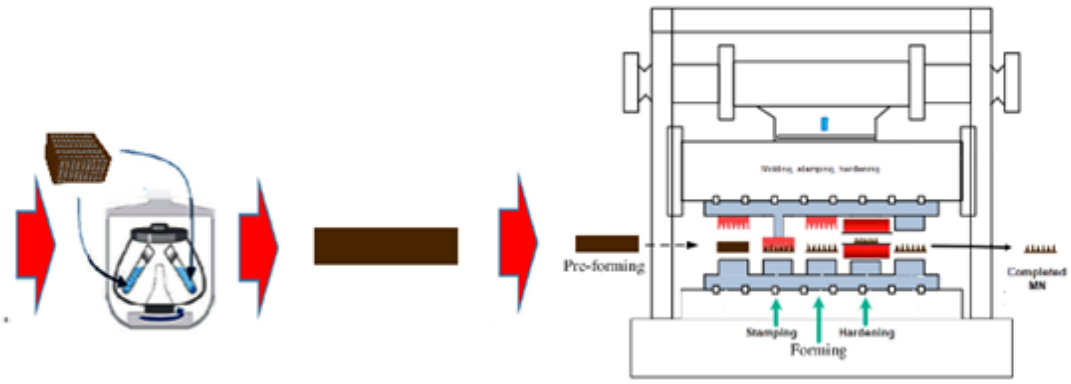
Figure 2: Moulds with different sizes, shapes and physical parameters will be designed and prepared

C. Semi-auto machine for the fabication of dissolving microneedle array patch

With the use of MAP-fabrication machine,

I. A two-step casting process will be used to fabricate Ca2+/Alg-sugar composite microneedles. Firstly, the microneedle matrix loaded with therapeutic protein or drug is poured onto the centrifuged by a refrigerated centrifuge at 10000 rpm in 20 °C for 10 min to fill up the porous container of microneedle mould to form a thicker pre-casting microneedle baseplate.

II. Then, the preformed drug (lignocaine)-loaded microneedle preforming plate will be loaded to the machine and go through stamping, forming hardening to become a final microneedle array patch as shown in a schematic diagram in Figure 3



Sugar compounds such as sucrose, trehalose, and maltose will be used to mixed to the dedicated drug/s and the mixture will go through a centrifuge process in a perforated container to form a thicker pre-casting microneedle baseplate

Preformed drug loaded microneedle preforming plate will be loaded to the machine and go through stamping, forming hardening to become a final microneedle array patch

Figure 3: The microneedle matrix composite will have filled into a porous container to form a pre-casted microneedle plate, the plate will be used to form microneedle tips and baseplate after a series of stamping, forming, hardening processes.

The microneedle patch is made in a prototype lab in Alnair Incorporated, Tokyo, Japan, which is the collaborator in this study.

The microneedles were individually heat treated at 120°C for sanitation before packaging.

4.12 Trial Conduct

4.12.1 Pharmacokinetic (PK) Study Conducts

Therefore, for this project, we will conduct a subproject as Phase 1 trial to assess the safety and tolerability of 12.5mg lignocaine-embedded microneedle on a small number of adult patients (20 patients; 10 males and 10 females) without hepatic or renal dysfunction. All participants of this phase I will be cataract patients who will be recruited from the HCTM ophthalmology outpatient clinic. Pre-treatment fasting is not required for all participants.

On the day of the study, each potential participant will be screened for study eligibility based on our pre-specified inclusion and exclusion criteria. An interim abridged medical history will be taken from each participant and their list of medications will be reviewed. Vital signs (systolic and diastolic blood pressures, oral temperature, pulse and respiratory rates) will be taken and targeted clinical examinations will be performed by the medical officers to assess the overall health of the participants.

First, an intravenous cannula will be placed at the dorsum of the hand and the routine bloods taken for investigations plus approximately 3.0 ml venous blood samples will then be withdrawn at t=0. Then the 12.5mg lignocaine-impregnated microneedles will be applied to the antecubital fossa, after which blood will be drawn from the earlier inserted intravenous cannula at t=30, 60, 90, 120, 180 minutes and collected into separate 3.5-ml of plastic blood collection tube with accelerator & separator gel (BD™, New Jersey, USA). Heparinized saline will be periodically infused to ensure that the cannula lumen remains patent throughout the sampling periods. The blood samples will then be sent to *Jabatan Kimia Malaysia* for lignocaine concentration measurements using the validated methodology of Gas Chromatography Nitrogen Phosphorus Detector (GC-NPD).

In comparing against the 12.5mg lignocaine-embedded microneedle, a similar approach as above to test the systemic absorption of EMLA will be studied in a group of another 20 patients but utilising the method employed in the previous paediatric trial, i.e. 5% EMLA cream 1 ftu enhanced by microneedle patch (JEP-2021-578; published protocol – J. Clin. Med. 2022, 11, 5291. https://doi.org/10.3390/jcm11185291).

a) Determining Serum Lignocaine Concentration using Gas Chromatography-Nitrogen Phosphorus Detector (GC-NPD): A Brief Protocol

One (1) mL of the blood will be taken from the collection tube and alkalinized with NaOH solution of pH 12. The internal standard, Methaqualone and the organic solvent, Chlorobutane will then be added to the mixture. The mixture will be subsequently mixed using a roller mixer and centrifuged to extract the organic the layer which will be then concentrated. from the partition. A clean-up solution, hexane-ethanol, will be then added to the sample mixture which will be again vortexed and centrifuged. The bottom organic layer will be then moved into another tube and it will be evaporated to complete dryness under nitrogen gas flow in room temperature. The residue will be subsequently reconstituted using absolute ethanol prior to loading into the GC-NPD system.

Lignocaine in the blood matrix will be spiked based on the level below or within the range in the therapeutic level. Besides, the response of the drug in the gas chromatography (i.e. the

resolution and the peak of in GC) will be taken into consideration. In normal practice, lignocaine at the amount of 0.5 parts per million (ppm) will be spiked and a lower amount of lignocaine (0.3 ppm) will be used for quality control, which are based on previous recommendations by Winek et al (Lignocaine: Therapeutic: 1.5-5.0 ppm; Toxic: 7-20 ppm; Lethal:>25 ppm)[62]. For calibration, a 1-point calibration to estimate serum lignocaine concentration will be used. A series of 1-point calibration will also be carried out whenever serum lignocaine concentration exceeds the therapeutic range.

b) Post-intervention Monitoring and Pharmacokinetics Data Analysis

The participants will be allowed to return home after the last blood sample is taken at t=180. The participants will be further monitored for any adverse events (AEs) such as redness, pain, itchiness, blistering, etc (local reactions) and light-headedness, euphoria, tinnitus, diplopia etc. (systemic reactions), serious adverse events (SAEs) and suspected unexpected serious adverse reactions (SUSARs) for up to 48 hours via telephone calls.

The pharmacokinetic data will be first summarized in mean / standard deviation or median / interquartile range for continuous data and count and percentage for categorical data. The pharmacokinetic parameters (AUC_{inf}, AUC_t, C_{max}, C_{min}, t_{max}, $t_{1/2}$, volume of distribution (V_d), Clearance (Cl)) of lignocaine will be evaluated using blood samples obtained at times t=0, 30, 60, 90, 120, and 180 minutes after the application of lignocaine-impregnated MN patch. The intraindividual and interindividual variations of the pharmacokinetic parameters will be evaluated using coefficient of variation (CV) and these will be classified as low (CV≤10%), moderate (CV ≈ 25%) and high (CV>40%).[63] The pharmacokinetic data will be analysed using the non-linear mixed effect models based on two-compartmental model which will be implemented on NONMEM® version VI (Icon Development Solutions, Ellicott City, Maryland, USA). The influence of clinically relevant covariates such as participant's age, gender, BMI and others on pharmacokinetic parameters will be evaluated in a stepwise fashion. First-order conditional likelihood (FOCE INTER on NONMEM) will be used to fit the data and model selection will be dependent upon the likelihood ratio test, the estimates of pharmacokinetic parameters and their 95% confidence intervals and goodness-of fit measures.

4.12.2 Pharmacodynamic (PD) Study Conduct

a) Randomization Procedure and Blinding (Masking) of Trial Participants

For random allocation, block randomization procedure with varying block size (permuted block) will be utilized to guarantee that both intervention groups will have an equal number of trial participants. This will be carried out by the trial statistician using the R package, blockrand version 1.50 which will be implemented on R platform.[64] 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 ensure the adequacy of allocation concealment, randomisation code will not be revealed until the potential trial participants have been definitively enrolled into the trial, which will be after all baseline measurements are made and all eligibility criteria are deemed fulfilled by the study recruiters.

In addition, allocation concealment is further safeguarded by ensuring identity of the allotted treatment is only revealed to the interventionist (i.e. the person who will be administering the intervention) via secured telephone calls (central randomization). Consecutive recruitments will be made until the final intended sample size is achieved.

For this study, the outcome assessors and care providers (may be the same individual) will be masked to the identity of interventions (single blinding / single masking). Only the statistician and interventionist/procedurist will be unmasked to the study interventions. Furthermore, unique ID code to indicate each treatment sequence assignment will be generated and utilised to ensure that the unintentional / intentional unmasking of one trial participant will not compromise the integrity of blinding for the rest of study participants. The primary unblinded trial persons (subjects, the statistician and the procedurist/interventionist) are instructed not to divulge the identity of the allotted treatments to other blinded trial personnel. The success of blinding will be determined by asking the blinded trial persons to guess the identity of 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 calculated to objectively assess whether blinding has been successfully achieved in this trial.[65,66]

b) Administration of Lignocaine-embedded Microneedle (intervention) and EMLA (control) patches

Prior to the administration of intervention/control, relevant clinic-demographic profiles (age, gender, ethnicity, anthropometric measurements, presence of comorbidities,) will be recorded and entered in the case report forms (CRFs) that are specifically designed for this study. This research study uses lignocaine embedded microneedles. The comparison of pharmacodynamic properties (i.e. efficacy) between 12.5 mg lignocaine delivered through direct embedment within the microneedle matrix and standard 5% EMLA dermal patch containing 1 finger-tip-unit (FTU=0.5g) of 12.5mg lignocaine and 12.5mg prilocaine will be assessed via VAS score and skin algesimeter index for the pain induced by venepuncture.

The window period given to lignocaine for it to be effective will be based on the usual clinical practice observation where it is usually applied for 30 minutes prior to venepuncture. 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 a slight anaesthetic effect. Thus, we postulate that, with the aid of microneedle, the time to onset of action for lignocaine could be greatly reduced resulting in a much more reduced pain sensation when the clinical assessment is carried out 30 minutes after treatment application.

The administrator of interventions (procedurist) will identify and draw a grid of 1cm × 1cm at the antecubital fossa, which will serve as an ideal site for venepuncture. The procedurist will then apply the lignocaine-impregnated microneedle patch. After a 30-minute application time, the attending medical officer will perform venepuncture using a 21-gauge (G) hypodermic needle inserted into the vein beneath the analgised patch.

For the participants allotted to the standard 5% EMLA dermal patch, 1 FTU of 5% EMLA cream will be applied and covered with a piece of adhesive to form a dermal patch. This will

be applied for 30 minutes similarly on the antecubital fossa. This application delivers an equivalent of 12.5mg lignocaine and 12.5mg prilocaine.

During the trial day, the participants will not be allowed to take any analgesic medications (NSAID, Opioids, Paracetamol) since they will modulate the level of pain experienced by the participants due to the received interventions. Other medications and concomitant care will be permitted during the trial.

c) Pain Assessment

The study participants will first be guided on the operating manual for a 10-points, 100mm VAS pain score by an outcome assessor. The participants 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. The study participants will then be asked to move and place the slider in the slot that accurately describes his/her pain at the following time points: 1) within 5 minutes after application of lignocaine-impregnated MN patch and before venepuncture (baseline VAS score); 2) within 5 minutes after venepuncture. The investigator/outcome assessor, who is blinded to the subject intervention arm will record the location of the slot where the slider is placed in millimetres (mm), clearly printed on the ruler's reverse side and this will be the participant's VAS score. Throughout the process, there will be a trained investigator standing by to assist the verification of the pain scale and to aid the participants who require additional assistance.

For a subset of randomly selected patients, 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 venepuncture. The procedurist will set up this machine and application before the interventions are commenced. The skin conductance peaks (in microSiemens (μS) and the skin algesimeter index (in microSiemens per second (μS/s)) will be recorded by the outcome assessor who will be blinded to the subject intervention arm. Those parameters indicate the skin's sympathetic nerve block induced by the topical anaesthetic. The measurement time points start from the point of intervention and the recordings continued for at least 15 seconds.

4.12.3 Clinical Data Collection

All study personnel (i.e. interventionist/procedurist, investigator/outcome assessors, healthcare providers) will receive training (e.g. use of open ended questions when assessing VAS pain score and adverse events related to interventions) in VAS measurement, administration of lignocaine-embedded MN patch prior to trial commencement to standardise data collection, enhance data quality and reduce data inconsistency and measurement variability.

All data collected will be checked for data quality using double data entry practice and checking for sensible data range and format (e.g. integer for the number of adverse events experienced within 24 hour of intervention). All paper-based CRFs will be stored in locked cabinets that are only accessible to the principal investigators, data manager and statistician and these will be maintained for 5 years after the trial ends. For quality control, periodic random check on a subset of CRFs will be carried out.

All data recorded on paper-based CRFs which will then be transcribed into an SPSS spreadsheet in an .sav extension at the central site conducted by statistician-trained data entry personnel. This will then be converted into a Stata-friendly file format (dta extension) to aid statistical analysis. The dataset will be password-protected and is only accessible to the principal study researchers and statistician to prevent any intended or unintended breach of patient confidentiality. Backup datasets will be stored in a password-protected thumb drives and cloud storage (GoogleDrive) that are again only accessible to the principal study investigators and statistician. The password for all datasets will be regularly changed to ensure maximum prevention against any data breach.

4.12.4 Interventional Safety Assessment

We define adverse events (AE) as "an abnormal sign, symptom, laboratory test, syndromic combination of such abnormalities, untoward or unplanned occurrence (e.g. accident), or any unexpected deterioration of concurrent illness".[67] For serious AE (SAE), this is defined as "adverse events that result in the following outcomes: 1) death; 2) life-threatening AEs; 3) inpatient hospitalization or prolongation of existing hospitalization; 4) a persistence of significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly or birth defect.[68]

We classify the likelihood of AEs / SAEs (unrelated, possible, probable, definite) based on Naranjo et al. classification.[69] All AE/SAE will be recorded and graded based on 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. All AEs or SAEs can be classified into local skin reaction (pain, erythema, ecchymosis, swelling, itchiness, tenderness) or systemic reaction (fever, irritability, tiredness, anorexia, vomiting, tachycardia, seizure, hypotension).

All AEs will be recorded on the CRFs. The detailed characteristics, the time and dates of onset and disappearance, and severity of AEs will be included in the CRFs. The study investigators will assess each participant experiencing AEs and they will receive appropriate treatments accordingly. The relationships between AEs and lignocaine-embedded microneedles will be evaluated by the investigators and classified as either unrelated, possible, probable or definite based on Naranjo et al. classification. AEs are considered as unexpected AEs when the AEs are not previously observed and not reported in the Investigator’s Brochure or standard lignocaine package insert. Any incidence of AEs or SAEs classified as possibly, probably and definitely linked to lignocaine-embedded microneedles will be monitored until the AEs/SAEs resolution is complete or the Investigator deem that the AEs or SAEs have become stable or irrevocable.

All AEs of grade 3 and above will be reported to the JEPUKM 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 JEPUKM. If AEs / SAEs occur or are still ongoing by the end of the study period, the study participants will still be continuously followed up until complete resolution of AEs / SAEs which will take the following form: 1) additional participant visit to the trial centre / hospital; 2) telephone calls to the subjects; 3) additional reporting in the form of letters from the treating physicians.

Participant enrolment, intervention allocation and administration will be stopped if one of the following occurs (study halting criteria):

a) Death related to lignocaine-impregnated MN patch

b) Any participant experiences bronchospasm, laryngospasms or anaphylaxis within 24 hours post lignocaine-impregnated MN patch

c) Any SAE related to lignocaine-impregnated MN patch

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 or erosion at the site(s) of lignocaine-impregnated MN patch

To ensure the independence of safety monitoring, all recorded safety data will be reviewed by JEPUKM which functions as an independent Data Safety Monitoring Board (DSMB) for our trial.

4.13 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 = kg / m^2. This variable will be categorised according to a widely used BMI classification[70-71]:

<18.5= underweight (SPSS code: -1)
18.5-24.9= normal BMI (SPSS code: 0; base category)
25.0-29.9= Overweight (SPSS code: +1)
≥30 = 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.

v) 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 = 5% EMLA dermal patch only group (control, base category, 30 minutes); 1 = Lignocaine-Embedded Microneedle Patch (30 minutes)

vi) 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.

vii) 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 (30-minutes post intervention application): A continuous numerical variable that will be measured during each visit; 30 minutes after lignocaine-embedded microneedle or 5% EMLA application. Missing data will be recorded as 999 in the SPSS spreadsheet.

ii) Pain score from PainMonitor™ device (30-minutes post intervention application): A continuous numerical variable that will be measured during each visit: 30 minutes after either lignocaine-embedded microneedle or 5% EMLA application. Missing data will be recorded as 999 in the SPPS spreadsheet.

4.14 Ethical Issues

Voluntary written informed consent will be obtained from each study participant. This study will be conducted in accordance with the principles of ethics in human research as stipulated by the Declaration of Helsinki (18th World Medical Association General Assembly, 1964), the Good Clinical Practice (GCP) guidelines, and ISO14155:2020 Clinical Investigation for Medical Devices for Human Subjects. Ethical approval will be obtained from the UKM Research Ethics Committee (Human) (JEPUKM).

All research participants will sign informed consent forms prior to their study participation. The participants will be made aware that their participation is completely voluntary and they can withdraw from the study at any time point. The research participants will also be notified that their decisions to withdraw from the study will not jeopardise their current or subsequent treatments and healthcare services received. To ensure the confidentiality of patient information, each participant will be assigned an anonymous research ID code that will be used for data storage and analysis. The data will solely be made available to the research team members and access to the storage may only be granted by the principal investigators.

To aid the transparency of reporting, the trial will be registered at the Clinical Trials Registry (https://clinicaltrials.gov/) and the Malaysian National Medical Research Registry (NMRR) (https://nmrr.gov.my/). The full trial protocol will be made available in the same trial registries and prepared according to the Consolidated Standards of Reporting Trials (CONSORT) guideline for parallel design and the 2013 Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) statement. Any future protocol modifications will be submitted first to the JEPUKM for approval and the list of protocol changes will be made available to the public via both the Clinical Trials and NMRR registries.

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 adherence to recommendations made by leading medical journal editors for the future publication of this research.

4.15 Statistical Analysis

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). Our primary analysis will be based on the intention-to-treat (ITT) principle by which all trial participants will be analysed according to their original intended treatment assignment. For missing observations, we will use the multiple imputation method to fill in the missing data, assuming the missing at random (MAR) mechanism. To check the robustness of the results, we will also conduct sensitivity analysis by comparing the results obtained using complete case analysis (i.e. including patients with complete observations) with full case (i.e. patients with complete and imputed observations for missing data) analysis.

The differences in terms of the outcomes measures between the groups will be statistically assessed using an independent t-test if the parametric assumption is met (i.e. the data are normally distributed) or the Mann-Whitney test if the data are non-normally distributed. To control and adjust the effects of confounding variables such as baseline pain scores, body mass index (BMI), gender of the patients, multiple linear regression analysis will be used. Variable selection will be based on a mixture of strategies: 1) stepwise regression based on Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC); 2) purposeful selection of covariates; 3) clinical domain knowledge. Effect modification will be assessed by creating and assessing the significance of the statistical interaction terms. The goodness of fit of the model will be evaluated using the coefficient of multiple determination, R2.

Model assumptions (linearity, independence, normality, and homoscedasticity of residuals) will be assessed using a studentised residual vs predicted values scatter plots and Durbin Watson statistics. Box-Cox transformation with carefully selected exponent (lambda) will be employed in the presence of skewness in the outcome variables. The presence of influential observations will be suspected in the presence of large leverage (extreme value in the x space) and residual values (extreme value in the y space) for any outlying observations. This will be further confirmed using influential diagnostic measures such as Cook's distance[72], dfFITS and dfBeta.[73] The significance threshold will be set at 0.05 and 95% confidence intervals will be presented for each effect estimate.

TRIAL WORKFLOW

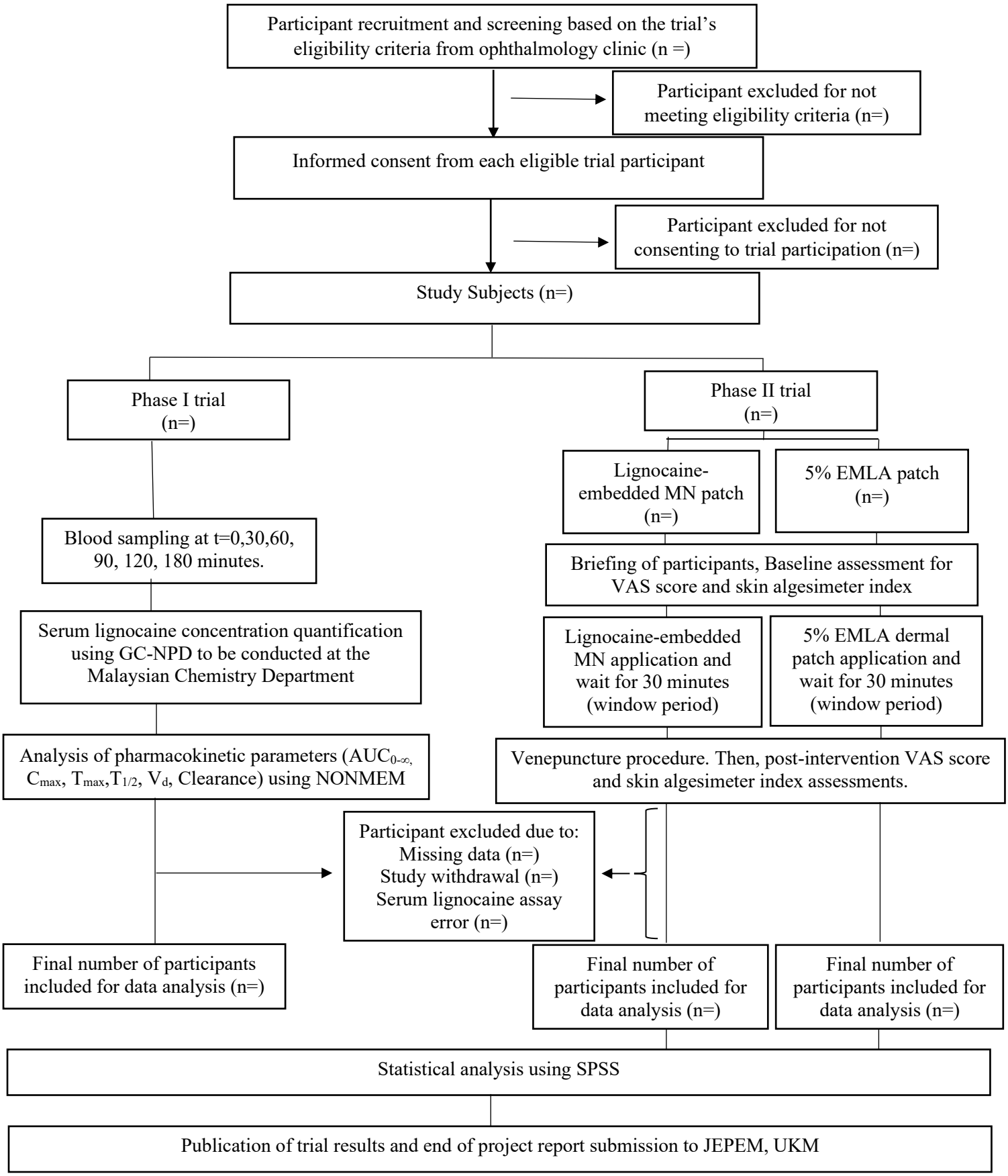


Figure 1: Anticipated trial workflow based on the 2010 CONSORT statement flowchart

GANTT CHART

Project activities	**Timeline**																						
	2022		2023												2024								
	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11
Research application for ethics approval																									
Trial subject recruitment																									
Serum assays for lignocaine levels																									
Data analyses and interpretation																									
Data presentation, Manuscript preparation, Final project report, and Publication																									
End of project																									

BUDGET

No.	Description	Unit price (RM)		Quantity	Price (RM)	
1	PROFESSIONAL SERVICE					
1.1	Lignocaine quantitation assay with GC-NPD	125	sample	120	15000	00
	Total				15000	

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APPENDIX A: PATIENT INFORMATION SHEET AND CONSENT FORM (ENGLISH VERSION)

PATIENT INFORMATION SHEET

Research Title: Transdermal microneedle lignocaine delivery versus EMLA patch for topical analgesia before venepuncture procedure to adults in a clinic setting

Introduction:
You are invited to participate in a clinical research study. Before participating in this study, it is crucial that you read thoroughly and understand the information provided in this sheet, whereby 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:
Vein-puncturing procedures elicits much pain and is regarded as the most frequently traumatic experience encountered among patients in the regular practice of the health care settings. Such pain may be reduced by applying topical anaesthetic drugs. For example, lignocaine cream is a non-invasive and convenient approach of administering anaesthesia to the body skin surface. The usual recommended duration of time taken for lignocaine cream to work is around 30 minutes. However, in a busy clinical setting, the time is often shortened to 15 minutes for a slight anaesthetic effect. As such, a transdermal drug delivery system (TDDS), the microneedle, has been introduced as an alternative to enhance the delivery of topical anaesthesia by puncturing the skin at a very superficial level to create multiple tiny tracts for faster action of anaesthetic drug absorption. The microneedle patch is a prototype device that is still being tested and not registered. It contains multiple micron-sized needles made of sugar (maltose) that will dissolve into the skin and subsequently achieve the objective of delivering the anaesthetic drugs. As such, our current research study aims to determine the safety and tolerability of lignocaine-embedded microneedle patch for patients requiring frequent venepuncture. Besides, this research also aims to obtain preliminary information on the efficacy of lignocaine-embedded microneedle in reducing pain associated with venepuncture.

What will the study involve?
For this research, you will be participating in either one of the following components of the trial: i) blood levels of lignocaine over 3hours or ii) lignocaine-microneedle versus EMLA patch. It is emphasised that you do not get to choose which group you will be in if you agree to participate. We use a selection system that the project investigators are abiding to.

i) Blood level of drug study over 3 hours

In this study, you are will undergo a venepuncture for blood collection during an outpatient visit at the Ophthalmology Clinic, HCTM. A medical doctor will perform an intravenous cannulation on the back of your right hands (dorsal side) that is intended for blood collection. Subsequently, you will be receiving a topical anaesthetic administration via lignocaine-embedded microneedle which will be applied on the skin surface of the back of your left hand. This will result in the numbness to that particular region of the hand. A small amount of blood (about 3 mLs each time) samples will then be collected from the cannula at six time points (time, t= 0, 30, 60, 90, 120, and 180 minutes) which will be sent to the laboratory for determining the level of lignocaine in your blood. In total, you are expected to be at the clinic for 3 hours, which is the average time taken for a normal visit to the eye clinic.

ii) Lignocaine-microneedle versus EMLA patch

If you are selected as a participant of this part of the research, you will first undergo a routine clinical examination on the trial day. You may be chosen to rate your baseline pain score using a ruler-styled scoring system (VAS) and a pain monitoring device. The lignocaine-embedded microneedle or EMLA patch, depending on which group you will be allotted to, will be introduced on the identified surface of the back of your hand. After 30 minutes, venepuncture will be performed by a trained medical officer. You will be then asked to evaluate the degree of your pain using the VAS score. You will also have a pain monitoring device attached to your palm for measurement purposes. You will then be monitored for one hour following the procedure to identify whether you experience any adverse effects associated with the lignocaine-microneedle application.

Risks:

This investigation possesses minimal risk to participants and is unlikely to cause side effects. Although topical skin anaesthetics are applied, you may still feel some pain from the needle/s, although this will be much reduced. Topical lignocaine is a widely used drug and little adverse reactions have been reported. Nevertheless, the possible side effects reported from the lignocaine application include:

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

With regards to EMLA patch, special precautions are advised in G6PD deficient individuals who may be at increased risk for developing symptoms as a result of methaemoglobin rise in blood causing temporarily a bluish tinge to the skin and decreased oxygen in the blood. If you are G6PD deficient, please inform us about this condition prior to the study.

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

- redness
- swelling
- itching
- blistering

Adverse side-effects reporting is part of the outcomes of this study, but If you encounter any problems or side effects experienced during the study, we will be giving you medications to reduce the side effects accordingly.

Benefits:

The information collected from this clinical study may contribute to the advancement of medical knowledge on the safety and efficacy of dissolving microneedle in delivering local anaesthetic agents, which will benefit patients in the future.

Do you have to take part?
Your participation in this study is absolute voluntarily. Your medical care will not be affected if you decide not to participate in this study. You will still 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 cannot decide which group you will be assigned to, but you are still free to withdraw from the study at any time without giving a reason or penalty. If you decide to cease from participating in this study, you must inform your study investigator and no new data will be further collected from you. The researcher may also remove your participation from the study for various reasons. In this event, you will not lose your rights as a patient and will still receive the usual standard of care.

Data & Confidentiality:
Participant’s confidentiality will be maintained throughout the investigation. Your personal data well be anonymized as your identity be always kept confidential. Data collected and entered into the Case Report Form will remain as the governed property of UKM. In the event of any publication generated from this study, your identity will be remained confidential to the public.

By signing the Informed Consent Form attached, you (or your legally acceptable representative, if relevant) are authorizing 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 Investigator: **Prof. Dr Cheah Fook Choe**
Department of Paediatrics
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Co-investigator **Prof Dr Mae-Lyn Catherine Bastion**
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Dr Lam Chenshen
Department of Ophthalmology
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No. Telefon: 03-8921 6520

Signatures
To be selected into this study, you must sign and date the signature page [ATTACHMENT A]

ATTACHMENT A

Patient/Subject Information and Consent Form

(Signature Page)

Research Title: **Transdermal microneedle lignocaine delivery versus EMLA patch for topical analgesia before venepuncture procedure to adults in a clinic setting**

Researcher's Name: Prof. Dr Cheah Fook Choe, ***Prof. Dr Mae-Lyn Catherine Bastion, Dr Lam Chen Shen***

To become a part of this study, you must sign this page. By signing this page, I amconfirming the following:

- I have read and understand all the information in this Patient Information Sheet and Consent Form, including any information regarding the risk stated in this study and I, have given sufficient time to consider about this study.
- All of my questions have been answered to my satisfaction.
- Hereby, 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 Sheet and Consent Form to keep for myself.

_____ _____

Patient Name (Print or type) Patient Initials and Number

_____

Patient I.C No. (New)

_____ _____

Signature of Patient Date (dd/MM/yy)
(Add time if applicable)

_____

Name of Individual
Conducting Consent Discussion (Print or Type)

_____ _____

Signature of Individual Date (dd/MM/yy)
Conducting Consent Discussion

_____ _____

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 C: CASE REPORT FORM (CRF)

CASE REPORT FORM

Research title: Transdermal microneedle lignocaine delivery versus EMLA patch for topical analgesia before venepuncture procedure to adults in a clinic setting

Research Investigators: Professors Dr Cheah Fook Choe, Mae-Lynn Catherine Bastion, Dr Lam CS.

Sticker Pesakit	**ID Subjek:** *Subject ID* ☐☐☐☐☐	**Tarikh:** *Date* ☐☐ / ☐☐ / ☐☐ Hari *Day* Bulan *Month* Tahun *Year*

Kod Rawak:
Randomisation code

A. MAKLUMAT SUBJEK
SUBJECT'S DETAIL

Nama Subjek : _____
Subject's name

Usia / *Age* : _____ tahun / *years* _____ bulan / *months*

No. Pend / *RN* : _____

No. telefon : Telefon bimbit : _____
Telephone no. *Handphone*

Telefon rumah : _____
Home

Tarikh lahir : _____ / _____ / _____
Date of birth

Tarikh keizinan: _____ / _____ / _____
Date of consent

Jantina : ☐ Lelaki *Male* ☐ Perempuan *Female*
Gender

Kaum : ☐ Melayu *Malay* ☐ Cina *Chinese* ☐ India *Indian*
Ethnicity

Lain-lain, silanyatakan: _____

Others, please specify

B. SEJARAH PENYAKIT KELUARGA
FAMILY HISTORY OF ILLNESS

Adakah sesiapa ahli keluarga yang dijangkit penyakit yang ketara dalam sejarah penyakit mereka:
Has any of the family members having any significant disease in their history of illness.

Darjahpertama *First degree*	**Ya**	**Tidak**
Bapa *Father*	☐	☐
Ibu *Mother*	☐	☐
Adik-beradik *Siblings*	☐	☐

Lain-lain: _____
Others

Jika Ya, sila nyatakan jenis penyakit yang terlibat dan umur ketika diagnosis: _____

If Yes, please specify the types of diseases and age at diagnosis: _____

C. KAJIAN SEJARAH PERUBATAN / PENGAMBILAN UBAT BERKAITAN
REVIEW OF MEDICAL HISTORY / MEDICATIONS CHECKLIST

Sejarah perubatan yang berkaitan:
Relevant medical history

Kencing Manis : ☐
Diabetes

Darah Tinggi : ☐
Hypertension

Penyakit Buah Pinggang : ☐
Kidney disease

Penyakit Autoimun : ☐
Autoimmune disease

Penyakit Hati : ☐
Hepatic diseases

Lain-lain : _____
Others _____
_____

Pengambilan ubat berkaitan (sila nyatakan nama ubat, tujuan perubatan, dos dan cara pengambilan)
Medications (please specify the name, indications, dosage and administration frequency)

Nama	Tujuan	Dos	Cara pengambilan

*Senarai semak ubat-ubat
Medication checklist
Erythromycin ☐
Ciprofloxacin ☐
Amiodarone ☐
Beta-adrenergic blockers ☐
(timolol eyedrops, metoprolol etc)

Adakah pesakit pernah mengalami alahan/ kesan sampingan yang teruk daripada mana-mana ubat?
Has subject ever experienced any allergy or adverse event from any medications?

Ya * ☐ Tidak ☐
Yes *No*

*****Jika '**Ya**', sila nyatakan jenis ubat yang terlibat: _____
*If **yes**, please specify the type of medication*

Sila nyatakan jenis diagnosis **MATA** dan pembedahan **MATA** yang dirancangkan:
Please specify the type of ocular diagnosis and the planned operation

Diagnosis: _____ Tarikh: _____

Mata Kiri ☐ Kanan ☐

Pembedahan / *Surgery*:

Phacoemulsification: ☐
ECCE: ☐
Trabeculectomy: ☐
Glaucoma drainage device: ☐
Pars plana vitrectomy: ☐
Corneal transplant: ☐
Lain-lain/ *Others*: _____
_____
_____

D. DATA KLINIKAL
CLINICAL DATA

Pengukuran antropometri:
Anthropometric measurement

1. Berat : kg
   Weight

2. Tinggi : cm
   Height

3. Indeks Jisim Tubuh (BMI) : kg/m^2
   Body mass index

Tanda-tanda vital
Vital signs

			Untuk kegunaan jika diperlukan *To be applied if needed*	
	Unit *Unit*	Lawatan klinik *Clinic visit*	**PK Study**	
		Tarikh/ Date: __ / __ / __	**Tarikh/Date: __ / __ / __**	**Blood Sample (Y/N) Volume (mL)**
Tekanan Sistolik *Systolic pressure*	mmHg		Time: 0 min	
Tekanan Diastolik *Diastolic pressure*	mmHg		Time: 30 mins	
Nadi *Heart rate*	bpm		Time: 60 mins	
Paras gula *Dextrostix*	mmol/L		Time: 90 mins	
Skala kesakitan (VAS) *Pain scale (VAS)*			Time: 120 mins	
Skala Kesakitan (Pain Monitor) *Pain score (Pain Monitor)*			Time: 180 mins	
			Samples sent to Jabatan Kimia	Date: Time:
Tandatangan dan nama ringkas pemeriksa *Initials and name of examiner*				

LOG PENJEJEKAN KESAN SAMPINGAN / ADVERSE EFFECTS TRACKING LOG

No.	Date reported	Adverse event description	Start date	End date	Ongoing (Yes or No)	Outcome[1]	Severity / grade[2]	Serious (Yes or No)	AE treatment[3]	Expected (Yes or No)	Intervention Attribution / Relatedness[4]

Scales:

Outcome[1]	**Severity / grade[2]**	**AE treatment[3]**	**Intervention Attribution / Relatedness[4]**
0- Fatal	0- Mild	0- None	0- Definite
1- Not recovered / Not resolved	1- Moderate	1- Medication(s)	1- Probable
2- Recovered w/sequelae	2- Severe	2- Medication TX	2- Possible
3- Recovered w/o sequelae	3- Life-threatening		3- Unrelated
4- Recovering / Resolving	4- Death / Fatal		4- Not applicable (did not receive intervention)

Verified by (Prior to data entry):

Signature:

Name:

Date: