EFFECT OF SYSTEMIC OXYGEN AS SUPPLEMENT THERAPY ON CORNEAL EPITHELIAL WOUND HEALING IN DIABETIC PATIENTS AFTER PARS PLANA VITRECTOMY

BY

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DISCLAIMER

I hereby certify that the work in this dissertation is solely my own, except for quotations and summaries which have been duly acknowledged.

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"I can no other answer make, but thanks, and thanks" – William Shakespeare

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ABSTRAK

Pengenalan: Pesakit kencing manis berisiko untuk mendapat luka di lapisan epitelium kornea, samada berpunca daripada penyakit kencing manis sendiri ataupun selepas pembedahan mata seperti vitrektomi. Jangkamasa penyembuhan luka epitelium kornea mungkin turut terjejas akibat penyakit kencing manis. Pelbagai bahan telahpun dikaji untuk menguji keberkesanannya terhadap penyembuhan luka kornea. Oksigen memainkan peranan penting sebagai nutrien untuk tisu badan dalam proses penyembuhan luka. Namun demikian, keberkesanan penggunaan oksigen untuk rawatan pemulihan luka kornea tidak diketahui.

Objektif: Kajian ini bertujuan membandingkan jangkamasa penyembuhan luka epitelium kornea antara pesakit kencing manis yang menerima atau tidak menerima rawatan oksigen selepas pembedahan vitrektomi. Ia juga untuk mengenalpasti samada faktor-faktor seperti usia, jangkamasa menghidap kencing manis, jangkamasa pembedahan, tahap pengawalan kencing manis serta paras hemoglobin mempunyai pengaruh terhadap jangkamasa penyembuhan luka epitelium kornea.

Metodologi: Kajian klinikal secara rawak ini melibatkan pesakit kencing manis yang menjalani pembedahan vitrektomi di Hospital Tuanku Ja'afar Seremban dari Oktober 2013 hingga Oktober 2014. Luka epitelium kornea berbentuk bulat dan berdiameter 6mm dibuat semasa pembedahan sekiranya berlaku kekaburan kornea. Selepas pembedahan, pesakit-pesakit dibahagikan secara rawak kepada dua kumpulan; "Standard" (rawatan lazim dengan ubat titis mata Maxitrol™ dan homatropine setiap enam jam) dan "Oxygen" (rawatan lazim beserta rawatan oxygen melalui topeng muka, sebanyak 10 liter/minit selama satu jam, setiap

12 jam sekali untuk tiga hari berturut-turut). Jangkamasa untuk luka kornea sembuh sepenuhnya dicatitkan. Parameter lain seperti usia pesakit, jangkamasa kencing manis, jangkamasa pembedahan dan paras HbA_{1C} serta hemoglobin turut direkodkan.

Keputusan: Sejumlah 32 mata daripada 32 pesakit terlibat dalam kajian ini, merangkumi 15 mata dalam kumpulan "Standard" dan 17 mata dalam kumpulan "Oxygen". Tiada perbezaan signifikan didapati antara kedua-dua kumpulan tersebut dari segi demografik pesakit dan penyakit. Purata jangkamasa penyembuhan luka epitelium kornea dalam kumpulan rawatan oksigen adalah lebih cepat berbanding kumpulan rawatan lazim (3.24 hari berbanding 4.27 hari, $p = 0.040$). Analisa regresi menunjukkan tiada hubungkait antara jangkamasa penyembuhan luka dengan faktor lain seperti usia, jangkamasa kencing manis, jangkamasa pembedahan, kawalan kencing manis dan paras hemoglobin.

Kesimpulan: Pesakit-pesakit yang menerima rawatan oksigen di samping rawatan lazim untuk luka lapisan epitelium kornea selepas pembedahan vitrektomi merekodkan masa penyembuhan yang lebih singkat. Pemberian oksigen kepada pesakit-pesakit dalam golongan ini boleh dipertimbangkan untuk membantu mempercepatkan proses penyembuhan luka.

ABSTRACT

Introduction: Diabetic patients are prone to developing corneal epithelial defects, either due to diabetic keratopathy or as a result of surgery such as pars plana vitrectomy. The healing time of corneal epithelial defects in this group of patients may also be delayed due to diabetes. Many substances have been investigated to determine their effects on corneal wound healing. Oxygen plays a key role as a nutrient for our tissues in the wound healing process. However, the effect of systemic oxygen on corneal epithelial wound healing is not known.

Objective: To compare the corneal epithelial healing time between diabetic patients receiving and not receiving supplementary oxygen after vitrectomy; and to determine whether various factors such as age, duration of diabetes, duration of surgery, glycaemic control and haemoglobin level have any influence on corneal epithelial wound healing time.

Methods: A randomised controlled trial was conducted involving diabetic patients planned for vitrectomy in Hospital Tuanku Ja'afar Seremban between October 2013 and October 2014. A 6mm circular corneal epithelial defect was created intra-operatively when there was obscuration of surgical field due to corneal haze. Post-operatively, these patients were randomised into two treatment groups; "Standard" (standard medical treatment of topical Maxitrol™ six-hourly and homatropine 2% six-hourly) or "Oxygen" (standard treatment plus systemic oxygen via simple face mask at 10 litres/min for one hour, in 12-hourly sessions for 3 days. Time taken for the corneal epithelial defect to heal completely was noted. Other parameters recorded were patients' age, duration of diabetes, duration of surgery, recent HbA_{1C} and haemoglobin level.

Results: A total of 32 eyes of 32 patients were recruited in this study, consisting of 15 eyes in the standard treatment group and 17 eyes in the oxygen group. There were no significant differences between the two groups in terms of patient and disease demographics. The mean corneal epithelial wound healing time in the oxygen group was significantly faster than those in the standard treatment group (3.24 days vs 4.27 days, $p = 0.040$). Regression analysis showed no significant linear relationship between healing time and other factors such as age, duration of diabetes, duration of surgery, glycaemic control and haemoglobin level.

Conclusion: Patients who received systemic oxygen therapy on top of the routine post-operative treatment for corneal epithelial defects after vitrectomy had a faster mean healing time. Oxygen administration may be considered in this group of patients to aid the wound healing process.

Chapter 1
Introduction

1.1 ANATOMY OF THE CORNEA

1.1.1 BASIC ANATOMY AND HISTOLOGY OF THE CORNEA

The cornea is the most anterior structure of the eyeball that plays an important role in refraction of light and protection of the eye from the external environment. Its functions are made possible by the highly specialised structural organisation which maintains transparency, and the absence of vessels. Being the principal refractive structure of the eye (accounting for about 70% of the total refractive power of the eye), the refractive requirements of the cornea are met by the regular anterior curvature and optically smooth quality of the overlying tear film.

Histologically, the cornea consists of five layers (from anterior to posterior): epithelium, Bowman's layer, stroma, Descemet membrane and endothelium. As recently as 2013, however, a novel pre-Descemet layer has been discovered in the human cornea and was named Dua's layer, in honour of the eye researcher who discovered it (Dua *et al.*, 2013).

1.1.2 CORNEAL EPITHELIUM

The corneal epithelium is composed of five to seven layers of stratified non-keratinised squamous cells that make up a thickness of about 50 – 60µm (about 10% of the total corneal thickness). The layers are made up of distinct cells such as the superficial cells, wing cells and basal cells.

The corneal epithelium's most important function is to serve as a barrier between the volatile external environment and the internal milieu (Suzuki, 2003). In doing so, the corneal epithelium is also crucial for the maintenance of corneal transparency, as any interruption or damage to the corneal epithelium will trigger a cascade of events which may result in a temporary or even permanent loss of corneal transparency and integrity. Thus, it is important to repair any damage to the corneal epithelium as soon as possible.

1.1.3 INNERVATION OF THE CORNEA

The cornea also holds the distinction of being one of the most densely innervated mammalian tissues in the body. Richly supplied by sensory and autonomic nerve fibres, the corneal innervation is anatomically organised in four levels from the penetrating stromal nerve bundles up to the intraepithelial nerve terminals (Müller *et al.*, 2003). The integrity of these nerve fibres is crucial in maintaining the refractive and protective functions of the cornea. Many ocular and systemic diseases can adversely affect corneal sensory nerves and consequently impair their function (Lambiase *et al.*, 1999).

1.2 CORNEAL WOUND HEALING

Under normal circumstances of tissue growth and maintenance, the process requires interplay of a number of factors including hormonal, paracrine, neural, vascular, and cellular factors. When tissue injury occurs the interaction of these factors, on top of additional factors from other sources, become crucial in achieving a normal repair and healing process. (Dayhaw-Barker, 1995a).

The cornea presents an additional challenge to this already complex process. As it is an avascular structure that must maintain a highly organised architecture in order to uphold its clarity, the wound healing process significantly differs from the normal tissue repair process. The nature of the wound, whether is it infectious, chemical, thermal, mechanical or others, also greatly influences the outcome of the healing response (Dayhaw-Barker, 1995a).

The corneal wound healing response is a complex process involving a cascade of cytokine-mediated interactions between the epithelial cells, keratocytes of the stroma, corneal nerves, lacrimal glands, tear film, and cells of the immune system (Wilson *et al.*, 2001). These interactions differ greatly between the three main tissues of the cornea, i.e. epithelium, stroma and endothelium. Nevertheless, they share a common feature in that the healing process is initiated by a loss of communication between cells (Dayhaw-Barker, 1995b).

1.3 CORNEAL EPITHELIAL WOUND HEALING

1.3.1 ROLES OF BASAL CELLS AND LIMBAL STEM CELLS

Basal cells and limbal stem cells play crucial roles in the healing process of any epithelial wounds (Suzuki, 2003). The mitotically-active basal cells produce daughter cells that migrate anteriorly and differentiate into the wing cells and superficial cells (Beebe and Masters, 1996). The '*X, Y, Z*' hypothesis outlines the three processes involved in maintenance of corneal epithelium stability: *X*, the proliferation of basal epithelial cells; *Y*, the centripetal movement of peripheral cells; and *Z*, the epithelial cell loss from the cornea surface (Thoft and Friend, 1983).

The replenishment of corneal epithelial cells is also contributed by the presence of stem cells located in the limbal region (Dua and Azuara-Blanco, 2000; Lavker *et al.*, 2004; Schlötzer-Schrehardt and Kruse, 2005). These stems cells, however, enter the cell cycle slowly to preserve their proliferative potential and to minimise deoxyribonucleic acid (DNA) replication-related errors (Lavker and Sun, 2000). In response to epithelial injury, however, as much as 50% of the limbal stem cells may be induced to undergo proliferation (Lehrer *et al.*, 1998).

The corneal epithelial stem cells derived from the limbus give rise to transient amplifying (TA) cells. These cells are capable of multiple instances of cell division. The efficiency is amplified by their ability to shorten their cell cycling time from 60 hours under normal physiological conditions to 24 hours when there is epithelial injury.

1.3.2 PHASES OF CORNEAL EPITHELIAL WOUND HEALING

The healing of corneal epithelial defects occurs under three distinct but continuous phases: (1) migration of neighbouring intact epithelial cells over the defect until the denuded surface is covered by a cell monolayer; (2) cell proliferation and stratification resulting in the restoration of normal epithelial layer thickness; and (3) differentiation of epithelial cells to restore the structured organisation and smooth surface of the corneal epithelium (Steele, 1999).

The three phases are preceded by a lag phase, in which there is cellular reorganisation and synthesis of cytoskeletal proteins (Agrawal and Tsai, 2003).

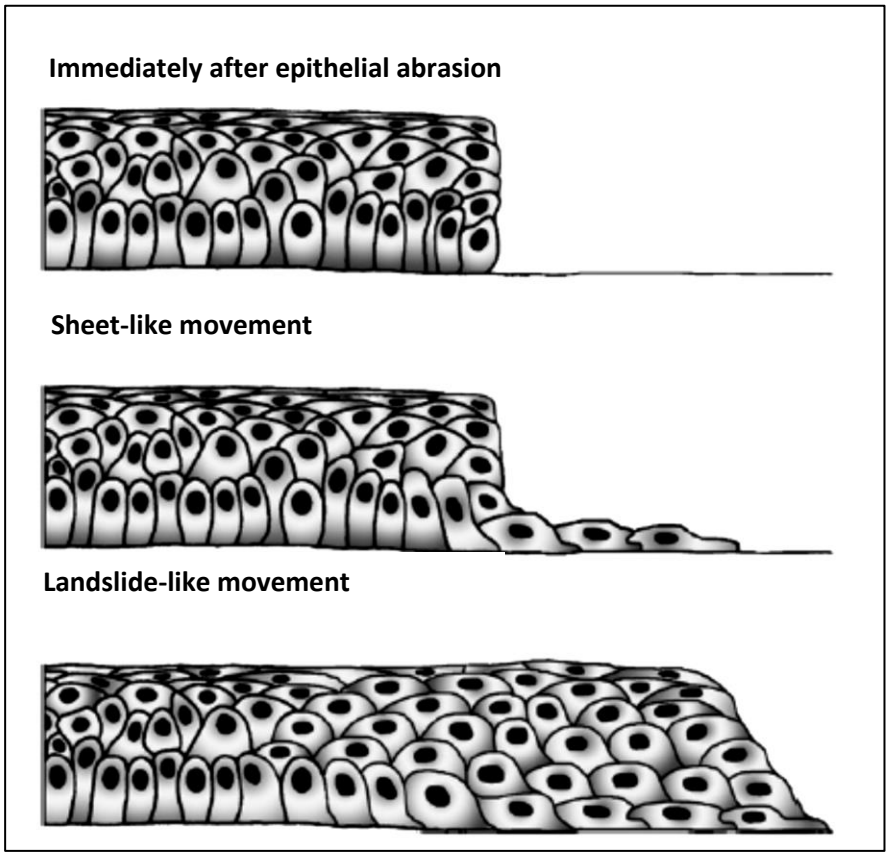

Figure 1.1: Phases of corneal epithelial wound healing (Adapted from Suzuki, 2003).

1.3.3 ROLES OF GROWTH FACTORS IN EPITHELIAL WOUND HEALING

The important phase of cell proliferation in corneal epithelial wound healing is mediated by a multitude of growth factors, including epidermal growth factor (EGF), hepatocyte growth factor (HGF), keratinocyte growth factor (KGF) and insulin-like growth factor (IGF) (Wilson *et al.*, 1994; Imanishi *et al.*, 2000; Zelenka and Arpitha, 2008). These growth factors bind to their specific receptors and are subsequently activated or phosphorylated to induce release of reactive oxygen species (ROS).

1.3.4 REACTIVE OXYGEN SPECIES (ROS)

Reactive oxygen species such as superoxide anion ($O^{\cdot}{}_2^-$), hydroxyl radical ($^{\cdot}OH$) and hydrogen peroxide (H_2O_2) have been implicated in the oxidative damages that occur in aging (Finkel and Holbrook, 2000), malignancies (Hanausek *et al.*, 2003) and degenerative diseases such as cataract (Spector, 1995). However, ROS also have other physiological functions which are beneficial to the cells generating them. In phagocytic cells of the immune system, ROS are essential in the elimination of harmful pathogens (Chanock *et al.*, 1994). In non-phagocytic cells they have been associated with the stimulatory action of growth factors during cell replication (Burdon, 1995; Chao-Wei Chen *et al.*, 2004).

In the corneal epithelium, epidermal growth factor has been demonstrated to induce the production of intracellular ROS which in turn was associated with cell proliferation. In addition to that, EGF-induced ROS was also found to profoundly affect corneal epithelial cell adhesion, migration and surface wound healing (Huo *et al.*, 2009).

1.4 NEUROTROPHIC KERATOPATHY

Neurotropic keratopathy is a degenerative corneal disease characterised by the impairment of corneal sensitivity associated with epithelial breakdown, a deficiency in the healing process leading to corneal ulceration, and subsequent vision loss. It has been classified into three stages (Mackie, 2000):

Stage 1: characterised by Rose Bengal staining of the inferior palpebral conjunctival surface, a decrease in tear breakup time, increased mucous viscosity and punctate epithelial keratopathy.

Stage 2: characterised by epithelial breakdown, which is usually oval or circular in shape and often localised in the superior half of the cornea. The defect is surrounded by a rim of loose epithelium which become smooth and rolled over time. There may be an associated stromal swelling and rarely, anterior chamber inflammatory reaction.

Stage 3: characterised by stromal involvement with a corneal ulcer that may progress to melting and perforation.

There are many ocular and systemic diseases that can cause neurotropic keratopathy. They share the same pathogenetic mechanism, namely the involvement of the trigeminal nerve (Lambiase *et al.*, 1999).

1.5 DIABETIC KERATOPATHY

Diabetes mellitus, as one of the leading systemic causes of neurotropic keratopathy, has gained the attention of many as the global burden of the disease has been profound. While abnormalities of the retina and lens secondary to diabetes have been extensively studied, corneal disorders due to diabetes (also known as diabetic keratopathy) are increasingly being recognised as a significant cause of morbidity associated with diabetes (McNamara, 1997; Cisarik-Fredenburg, 2001; Kaji, 2005). It has been estimated that diabetic keratopathy occurred in 47 – 64% of diabetic patients during the course of their disease (Schultz *et al.*, 1981).

The corneal epithelial changes in diabetic patients are increasingly being documented. Their corneal epithelial cells are larger in size, more heterogenous in morphology, and are irregularly arranged as compared to the corneas of non-diabetics (Tsubota *et al.*, 1991a; Tsubota *et al.*, 1991b; Meller *et al.*, 1996). There is also alteration in the thickness and interaction between the epithelial basement membrane cells (Taylor and Kimsey, 1981; Azar *et al.*, 1992). Hemidesmosomal attachments onto the epithelial basement membrane is also reduced (Tabatabay *et al.*, 1988).

As such, it is not surprising that diabetic patients have an increased risk of developing keratopathies such as epithelial fragility corneal epithelial defects, recurrent epithelial erosions, decreased sensitivity, abnormal wound healing, increased susceptibility to injury and non-healing or infected corneal ulceration (Fowler, 1980; Kabosova *et al.*, 2003; Chikama *et al.*, 2007).

Corneal sensitivity is also significantly reduced in diabetic patients (Schwartz, 1974). In fact, the reduction in corneal sensitivity is a strong predictor of the presence of diabetic peripheral neuropathy. The severity of keratopathy has been found to be directly related to the degree of peripheral sensation loss (Schultz *et al.*, 1983).

Metabolic and physiological differences also exist between corneas of diabetic and non-diabetic patients, including altered expression of growth factors (Saghizadeh *et al.*, 2001), reduced oxygen uptake (Rubinstein *et al.*, 1990) and increased aldose reductase enzyme activity (Akagi *et al.*, 1984).

While the exact mechanism remains elusive, experimental studies have identified that the enzyme aldose reductase may be involved in both diabetic keratopathy and reduced corneal sensitivity. During hyperglycaemia, accumulation of sorbitol within epithelial cells occurs due to the activation of the polyol pathway and increased aldose reductase activity (Akagi *et al.*, 1984). Sorbitol causes osmotic stress, which may lead to cellular oedema and damage of the corneal epithelial cells and nerves.

There is little doubt that there is a myriad of corneal complications which can potentially occur in a diabetic patient. If left unrecognised or untreated early, they may ultimately lead to permanent visual loss.

1.6 VITREORETINAL SURGERY IN DIABETIC PATIENTS

The complications of diabetes on the eye such as keratopathy and retinopathy inevitably lead to an increased incidence of interventions and surgery. The altered corneal sensation plus structural changes of the cornea increases the risk of corneal diseases such as infection, ulceration and perforation. Meanwhile, diabetic retinopathy is one of the commonest indications for vitreoretinal surgery.

It has been established that vitreoretinal surgery can be associated with intraoperative corneal epithelial oedema and even late corneal decompensation (Brightbill *et al.*, 1978; Foulks *et al.*, 1979; Friberg *et al.*, 1984). During surgery, corneal epithelium debridement is sometimes necessary to ensure a clear and unobstructed view of the posterior segment. In a survey involving 44 active vitreoretinal surgeons with high volume of surgeries in Japan and the United States, the average overall debridement rate was 17.4% ± 19.0% (mean ± standard deviation) with a range of 0% to 90% (Friberg *et al.*, 2003). However, the survey was not designed to include data regarding the surgical complexity of the cases or the duration of surgery.

Nevertheless, it is safe to assume that the risk of corneal epithelial defects in diabetic patients undergoing pars plana vitrectomy is particularly high and presents significant morbidity issues in terms of management and further complications.

1.7 TREATMENT OF CORNEAL EPITHELIAL DEFECTS

The standard treatment strategy for a non-infected corneal epithelial defect includes infection prevention by instillation of prophylactic topical antibiotics, pain relief by oral or topical analgesics, and promotion of epithelial wound healing either by patching or therapeutic ('bandage') contact lenses.

While there are no randomised clinical trials to demonstrate the advantage of prophylactic antibiotics use in non-infected epithelial defects, eye surgeons generally do prescribe topical antibiotics due to the higher risk of infection in a denuded cornea epithelium, particularly if the eye if patched or a bandage contact lens is applied (Dohlman *et al.*, 1973). There are also controversies surrounding the use of patching, as some studies and meta-analyses have shown that patching does not promote healing and may even have deleterious effects as compared to non-patched eyes (Campanile *et al.*, 1997; Flynn *et al.*, 1998; Turner and Rabiu, 2006).

While there are also concerns regarding the use of topical steroids in corneal epithelial defects, it has been shown in one study that there is no significant difference in the healing time between patients receiving topical steroid (i.e. dexamethasone) and those without postoperative topical steroid treatment after vitreoretinal surgery (Yülek *et al.*, 2006).

1.7.1 AGENTS USED FOR ACCELERATION OF CORNEAL EPITHELIAL WOUND HEALING

There have been an increasing number of studies done to evaluate the safety and efficacy of various substances which can be used in the treatment of corneal epithelial defects in diabetic patients or animal models. These include the use of topical insulin (Zagon *et al.*, 2007; Bastion and Ling, 2013), autologous serum (Schulze *et al.*, 2006), opioid antagonist i.e. naltrexone (Zagon *et al.*, 1998) and substance P (Nishida *et al.*, 1996).

1.8 USE OF OXYGEN IN OPHTHALMOLOGY

The use of oxygen in ophthalmology is largely confined to the hyperbaric oxygen therapy (HBOT) delivery method. Nevertheless, a growing body of evidence have demonstrated the beneficial effects of HBOT on a wide range of ocular conditions (Butler Jr, 1995; Oguz and Sobaci, 2008). Among the uses of hyperbaric oxygen therapy in ophthalmic practice include occlusive vasculopathies such as central retinal or vein occlusion (Kiryu and Ogura, 1996; Krott *et al.*, 1999), diabetic retinopathy (Dumitru, 1992), scleral melting and necrosis after antimetabolite or beta irradiation in pterygium surgery (Green and Brannen, 1995; Bayer *et al.*, 2001), corneal oedema and anterior segment ischaemia (De Smet *et al.*, 1987; Recupero *et al.*, 1992) and ocular infections (Yohai *et al.*, 1994).

The physiological basis of hyperbaric oxygen therapy is essentially hyperoxygenation (or hyperoxia), which confers many biochemical, cellular and physiological benefits (Tibbles and Edelsberg, 1996; Leach *et al.*, 1998). The rise in the level of oxidants may also act as cell messengers in the stimulation of growth factor release in wound healing (Sen *et al.*, 2002).

The secondary effects of hyperbaric oxygen include vasoconstriction, fibroblast proliferation, immune cell-mediated oxidative killing of pathogens, toxin (clostridial) inhibition, and antibiotic (fluoroquinolones and aminoglycosides) synergy (Leach *et al.*, 1998).

1.8.1 OXYGEN IN CORNEAL WOUND HEALING

Oxygen benefits wound healing by several mechanisms, including upregulation of various growth factors, downregulation of inflammatory cytokines, improvement in leukocyte function and reduction of oedema. Furthermore, it demonstrates antibacterial effects and at the same time supports angiogenesis and new tissue ingrowth (Sheikh *et al.*, 2000; Wright, 2001).

While there are more studies examining the use of hyperbaric oxygen therapy (HBOT) in ophthalmology, there have been no studies utilising other methods of oxygen therapy until recently. A pilot study done in Iran (Sharifipour *et al.*, 2011), in which systemic oxygen therapy was delivered via face mask at 10 litres per minute twice daily in acute ocular chemical or thermal injury. The eyes of subjects receiving systemic oxygen therapy showed improvement in limbal ischemia, acceleration of epithelialisation, increase in corneal transparency, and decrease in corneal vascularisation. The likely end-result of these effects is improvement in visual acuity and reduction in complications.

There is no evidence of direct risk from administration of systemic oxygen via a face mask to the ocular structures. The likely risks of this route of oxygen administration are towards the other structures such as drying of nasal and pharyngeal mucosa, and skin irritation. It may also pose a fire hazard risk.

This study aims to examine the effects of systemic oxygen as a supplementary therapy on the healing of corneal epithelial wounds in post-vitrectomy diabetic patients. Due to the ease of administration of oxygen and the potential effect of oxygen therapy, the use of supplemental oxygen therapy for the treatment of corneal epithelial wound post-vitrectomy will perhaps accelerate the healing process in diabetic patients. It is hoped that by accelerating the closure of an epithelial wound in this group of patients, the risk of complications such as recurrent erosions, non-healing wounds and infective keratitis may be further reduced in these already at-risk corneas.

Chapter 2
Objectives

2.1 GENERAL OBJECTIVE

To study the effects of systemic oxygen supplementary therapy on corneal epithelial wound healing time after pars plana vitrectomy in diabetic patients.

2.2 SPECIFIC OBJECTIVES

2.2.1 To compare the corneal epithelial wound healing time between diabetic patients receiving and not receiving supplementary oxygen after vitrectomy.

2.2.2 To determine the confounding factors affecting corneal epithelial wound healing time in diabetic patients post-vitrectomy.

Chapter 3
Methodology

3.1 STUDY DESIGN

Prospective interventional randomised clinical trial

3.2 POPULATION, TIME AND PLACE OF STUDY

Study sample:	Diabetic patients who underwent pars plana vitrectomy in Hospital Tuanku Ja'afar, Seremban who met the inclusion and exclusion criteria.
Study subjects:	Diabetic patients who underwent pars plana vitrectomy in Hospital Tuanku Ja'afar, Seremban and consented to take part in the study.
Period of study:	31 October 2013 – 31 October 2014.
Place of study:	Department of Ophthalmology, Hospital Tuanku Ja'afar, Seremban, Negeri Sembilan Darul Khusus.

3.3 SAMPLING AND SAMPLE SIZE

3.3.1 SAMPLING METHOD AND RANDOMISATION

A purposive sampling method was used in this study. Patients were allocated a number determined from a computer software-generated (*QuickCalc*, GraphPad Software Inc., California) table of random numbers and were divided into two treatment groups:

- "Standard" group (odd numbers) – Standard medical treatment: topical Maxitrol$^{(R)}$ (Alcon Labs, USA) consisting of an antibiotic-steroids sterile suspension of Dexamethasone 0.1%, Neomycin Sulfate and Polymyxin B Sulfate), and topical Isopto® Homatropine 2% (Alcon Labs, USA)

- "Oxygen" group (even numbers): Standard medical treatment plus systemic oxygen via face mask given at 10 litres/min, one hour per session and twice daily (12 hours apart).

3.3.2 SAMPLE SIZE CALCULATION

Sample size calculation was done using the *PS Power and Sample Size Calculation* software version 3.1.2 (January 2009, Tennessee, USA; Vanderbilt University). The power of the study was set at 90%, while the probability of error (α) was set at 0.05.

As the main objective of this study was to compare the mean healing time between two treatment arms, calculation of sample size using the software was done under the t-test mode.

To the best of our knowledge, there had not been any similar studies conducted to evaluate the effects of systemic oxygen on corneal epithelial wound healing.

Therefore, the difference in mean (δ) and the standard deviation (σ) of 2.8 days and 2 days respectively were taken from a study that had an almost similar design but used autologous serum as its treatment under study (Schulze *et al.*, 2006).

To account for the possibility of a 20% drop-out rate in this study, the sample size for each treatment arm was therefore:

$$12 + \left(\frac{20}{100}\% \text{ x } 12\right) = 12 + 2.4 = 14$$

A minimum sample size of 14 patients were required in each treatment group to obtain a study power of 90% at an alpha of 0.05.

3.4 SELECTION CRITERIA

3.4.1 INCLUSION CRITERIA

1. Diabetic patients indicated for pars plana vitrectomy
2. Epithelial debridement performed during pars plana vitrectomy
3. Age 18 years and above

3.4.2 EXCLUSION CRITERIA

1. Known case of ocular surface or corneal disease.
2. History of eye surgery within one month from the date of pars plana vitrectomy
3. Pre-existing primary or secondary glaucoma e.g. open angle or angle closure glaucoma, ghost cell glaucoma
4. Patients with chronic obstructive airway disease

3.5 ETHICAL BOARD APPROVAL

The study received approval from the Human Research Ethics Committee, Universiti Sains Malaysia on 17th October 2013 (Reference number: USM/JEPeM/269.3 (2)) and the Medical Research and Ethics Committee, Ministry of Health Malaysia on 8th October 2013 (Reference: (6) dlm. KKM/NIHSEC/800-2/2/2 Jld 3 P13-368).

The study was also registered with the National Medical Research Registry (http://www.nmrr.gov.my) (Reference: NMRR-13-449-15617) and also with ClinicalTrials.gov (http://www.clinicaltrials.gov) (ID: NCT02344732).

3.6 DEFINITION OF TERMS

3.6.1 CORNEAL EPITHELIAL DEFECT

It is a defect in the corneal epithelium that, in this study, was induced intra-operatively by means of an iatrogenic corneal epithelial debridement. The area of debridement was marked using a 6mm corneal trephine imprint, and debridement was performed using the Kimura spatula (McGrath and Lee, 2014).

3.6.2 CORNEAL EPITHELIAL WOUND HEALING

Corneal epithelial wound healing is a complex process involving three distinct components of cell migration, cell proliferation and cell adhesion (Dua *et al.*, 1994).

3.6.3 SYSTEMIC OXYGEN THERAPY

Systemic oxygen therapy is the administration of oxygen to the patients delivered via nasal prongs, face mask, high-flow mask or endotracheal tube (Bateman and Leach, 1998). In this study, the systemic oxygen therapy was administered using a face mask.

3.6.4 PARS PLANA VITRECTOMY

Pars plana vitrectomy is a surgical procedure involving the removal of the vitreous humour approached via the pars plana (Young and Shea, 1977).