Comparative Clinical Performance of Scleral Lenses with Varying Limbal Clearance in a Keratoconic Population

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

PURPOSE

The purpose of this study is to investigate the impact varying the limbal clearance (LC) has on the clinical performance of scleral lenses and the levels of inflammatory mediators in the tear film of keratoconic patients. The specific aims of each chapter are outlined.

In Chapter, 3 a group of keratoconic patients were fitted with a scleral lens of both high and low LC, with a constant CCC.

In Chapter 4, the subjective response, ocular response and clinical performance of scleral lenses having varying LC were investigated.

In Chapter 5, the changes in the level of inflammatory mediators in the tear film of keratoconic patients with scleral lenses wear with varying LC were determined.

In Chapter 6, the association between clinical performance and tear cytokine changes that varied with scleral lens wear with low and high LC was discussed.

METHODS

For Chapter 3: based on the corneal sagittal depth at a chord of 15.0mm, participants were fitted with two sets of scleral lenses with the same central sagittal depth and varying limbal clearances (LC) which differed by 50µm. Lenses were worn in a randomly assigned order for a two-week period. Lens fitting parameters including central and limbal clearances were measured with the Visante™ OCT and compared.

In Chapter 4: visual analog scales were completed concerning vision, comfort, burning and dryness. Corneal and conjunctival responses to lens wear, such as limbal and bulbar hyperemia, corneal swelling based on pachymetric values, and corneal staining as seen on biomicroscopy were observed and compared.

In Chapter 5: tear samples were collected from both the inferior tear meniscus using the flush tear method, and from the pool of tears in the bowl of the inverted scleral lens using a microcapillary tube. Subsequently, tear cytokine analysis was performed using a multiplex electro-chemi-luminescent array (Meso Scale Discovery, Rockville, MD) instrument. Levels of IL-1, -6, -8, TNF-α, MMP-1 and -9 were compared using a Student t-test statistical analysis.

In Chapter 6: correlations between cytokine levels and clinical parameters using the Pearson correlation coefficient (r) were performed. P values of less than 0.05 and p less than 0.10 for tear data were considered to be statistically different.

RESULTS

In Chapter 3, 11 subjects (22 eyes) were fitted with scleral lenses of a sagittal depth of 4.539±0.240mm for low LC and 4.550±0.243mm for high LC (p=0.877). There was no difference in CCC between low and high LC (p=0.671 for initial CCC, and p=0.475 for final CCC). The initial limbal clearances, before lens settling were 159.9 ±45.02μm for low the low LC lenses, and 194.07±66.10μm for high LC lenses (p<0.05). The final limbal clearances, after lens settling, were123.74±56.68μm and 167.31±69.75μm for the low LC and high LC lenses, respectively (p=0.006). There

were significant differences between the nasal and temporal limbal clearances, with more clearance found temporally (p<0.001).

In Chapter 4, compared to baseline, both low and high LC lenses resulted in improved subjective responses (p=0.07 low LC, p<0.01 high LC for overall comfort). Greater comfort was reported with scleral lenses with high LC (p=0.013 for comfort, p<0.01 for dryness, p=0.08 for burning) compared to low LC. There was no difference in limbal and bulbar hyperemia between high and low LC lenses. Corneal swelling was noted in all corneal locations and especially at the 6mm zone where there were significant differences for both low and high LC lenses compared to baseline (p=0.004, and p=0.039, respectively). Corneal response to scleral lens wear with either low or high LC appears to result in either peri-limbal staining or negative corneal staining.

In Chapter 5 the median volume of tears collected from the flush tear collection was 1.0 µL (Range 0.2 to 6.0 µL). The median volume of tears collected from the post-lens tear film was 5.0 µL (Range 0.2 to 10.0 µL). A statistically significant difference was noted between sample volumes from either collection method (both, p<0.05). Significant differences at the p<0.10 levels were found comparing low and high LC with - TNF-α, MMP-1 and MMP-9 (all p<0.10) from the samples taken from the lens bowl. Scleral lenses with high LC were associated with increased levels of IL-1β, TNF-α, and MMP-1 and decreased levels of MMP-9.

In Chapter 6, there was a statistically significant correlation between changes in LC and peripheral corneal thickness in the inferior quadrants. With high LC, a correlation was noted between IL-6 and IL-8 levels.

CONCLUSIONS

This study illustrated how low and high LC can vary the clinical performance of scleral lenses and physiological responses of the ocular surface in a keratoconic population. The clinical changes, such as hyperemia, corneal thickness, and corneal staining, and subclinical responses in tear cytokine levels are associated with hypoxic and mechanical etiologies. Eye care practitioners must take into consideration individual patients' ocular condition when determining the ideal limbal zone fitting parameter.

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List of Abbreviations

CCC	CCC
GP	Gas permeable
IL	Interleukin
KC	KC
LC	Limbal clearance
LSCD	Limbal stem cell deficiency
MMP	Metalloproteinase protease
OCT	Optical coherence tomography
RGP	Rigid Gas Permeable
SD	Standard deviation
TNF	Tumor necrosis factor

Chapter 1
Literature Review on Keratoconus and Scleral Lens

1.1 Keratoconus

The term, keratoconus (KC), is derived from two Greek words, *kerato* as in cornea and *konos* as in cone, which describe the cornea taking on a conical shape.[1] This condition has been reported to affect 2 to 5.4 people in every 10,000.[2-4] However, with new understanding and redefined diagnostic thresholds, the prevalence and incidence might be significantly greater.[5] Clinically, KC is recognized as a bilateral, but asymmetric, non-inflammatory corneal ectatic disease.[1,3,6-9]

1.1.1 Signs and Symptoms

Abnormal corneal thickness distribution is a key criterion in the diagnosis of KC.[9] As the cornea thins irreversibly, the biomechanical stability of the cornea is challenged and both the anterior and posterior corneal surfaces steepen in curvature (i.e. the radius of curvature reduces), taking on a conical shape.[4] This altered corneal thickness profile and abnormal corneal topography are detectable, particularly in early stages, by corneal tomography[10-12] and placido-ring-based topography. [4] Topographic elevation maps may reveal changes in the posterior cornea prior to that in the anterior cornea in early KC, as seen in Figure 1-1.[9] The pattern of corneal thinning also allows for the differential diagnosis of KC with other corneal ectatic diseases.[9]

In refractive terms, the corneal protrusion or ectasia results in an increase in myopia and the development of corneal astigmatism. The astigmatism is typically with-the-rule or oblique, and is non-orthogonal or irregular.[2,9] Due to the bilateral, but

asymmetric, nature of the condition, the change in the refractive astigmatism, both in axis orientation and amount, will be different between right eye and left eye, with one eye leading and the other lagging behind in progression. Changes in the corneal thickness and curvature profile of the tissue produce higher order aberrations, resulting in a reduced best-corrected visual acuity, and symptoms of glare, haloes, monocular diplopia, and photophobia.[9,13]
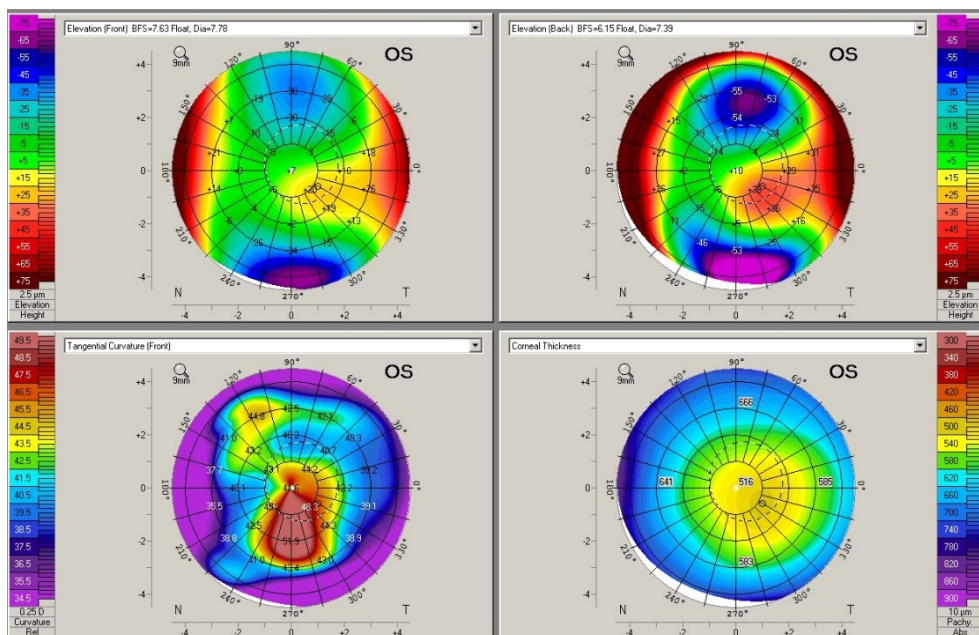
Figure 1-1: Topography of a Keratoconic Cornea. In the top two maps, elevation of anterior (top left) and posterior (top right) corneae are shown where red and warmer colors represent increased elevation compared to a best-fit sphere. In the bottom left map, a tangential curve reveals an inferior oval cone where steepened curvature is represented by red and warmer colors.

The presence of clinical signs may be observable through slit-lamp biomicroscopy. KC results in tissue changes at all levels of the cornea,[14] and will vary depending on

the severity of the condition. With increasing severity, the following are the changes that are observed:

1. In the earliest stages of KC, mild asymptotic tissue and topographical changes will present, such as a shift in the corneal apex and slight corneal steepening with a change in astigmatism.[15,16] This subclinical stage of the ectatic disease is known as *forme fruste* KC.

2. As the cornea begins to steepen and take on the shape of a cone, iron (or hemosiderin) deposits from the tear film will accumulate in the corneal epithelium at the base of the cone (that is at the junction of the thinned and thicker cornea) to form the diagnostic feature known as a Fleischer's Ring, as seen in Figure 1-2A.[4,17-19]

3. Vogt's striae (Figure 1-2B) is indicative of KC of moderate severity. This occurs in 8.3% of KC cases due to stromal thinning caused by a significant loss of collagen fibrils, an increase in extracellular matrix (proteoglycans), and a decrease in keratocytes in the anterior stroma.[14,19-21] This causes vertical creases, or wrinkling, of Descemet's membrane as the layer collapses on itself.[22,23] This clinical sign is observed to occur in an orientation which is parallel to the axis of the cone.[4] Furthermore, loss in collagen fibrils occurs also at the level of Bowman's layer resulting in breaks.[19] At this moderate stage of KC, corneal hysteresis (the ability of the tissue to absorb and dissipate force applied onto it), decreases as the condition advances.[19,24]

4. In later stages, when the KC is more obvious due to the cornea is protruding, corneal apical scarring occurs at the sub-epithelial level as a result of

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biomechanical stress in the stromal layer, as seen in Figure 1-2C.[4] The scarring presents as a wispy, fibrous appearance is noted mostly at the apex of cone.[14,20]

5. As the cone advances some researchers have also reported the presence of Charleux oil droplet[4] sign in the tissue. This phenomenon is more easily observed on retro illumination with biomicroscopy and mydriatic pupils.

6. In advanced cases of KC, Munson's sign describes the V-shaped deformation of the lower eyelid margin which develops on downgaze when the corneal protrusion distorts the lower eyelid.[14]

7. In 3% of severe keratoconic cases, as the cornea continues to thin and weaken, Descemet's layer may rupture, resulting in a breakdown of the stroma/aqueous barrier, leading to corneal hydrops.[4,14,25,26] The break in the barrier between the aqueous chamber and the stromal tissue allows for the leakage of aqueous fluid into the posterior corneal layers, resulting in edema and, in severe cases, bullous keratopathy. Symptoms of corneal hydrops include a sudden onset of vision loss and a high level of pain.[27] As the hydrops resolve, the swelling subsides, but the disruption in the tissue may result in vision-compromising scarring of stromal tissue.
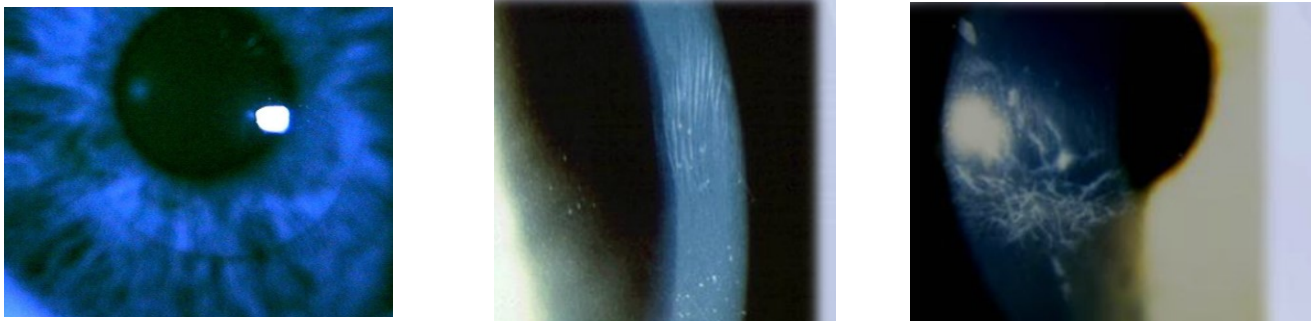
Figure 1-2A-C: Biomicroscopic signs of KC. These include Fleischer's Ring (Left), Vogt's Striae (Center), and apical scarring at the apex of the cone (Right).

1.1.2 Pathophysiology

Currently, the pathogenesis of KC is still not completely understood. Gomes et al. have categorized the multitude of pathogenic factors in KC into four major components: genetics, biochemical, biomechanical, and environmental.[9]

1.1.2.1. Genetics and Biochemistry

A positive family history is considered a relative risk factor for KC.[4,28] The prevalence of KC in first-degree relatives is 3.34%, which increases the risk by 15-67 fold.[29] Kriszt et al.[30], have indicated that KC is a non-Mendelian disease while Hauser et al.[31] have suggested the inheritance pattern of KC may resemble that of autosomal dominant.

KC has been linked to a range of genetic loci developing from single-nucleotide polymorphisms and mutations in chromosomes 13 through 18.[23,32-34] Most of these genetic changes alter the formation and function of components in the cytoskeleton and extracellular matrix, as well as the signaling necessary for cell-cell and/or cell-matrix interactions.[33] For example, altered expression of Lysyl oxidase (LOX), a necessary enzyme in the natural cross-linking of collagen and elastin, contributes to the weakening of the bio-stability of the stromal tissue.[35] Down-regulation of aquaporin-5(APQ5)[36] affects the water-channels at the level of the corneal epithelium and can impact corneal wound healing. This was the first molecular defect identified in KC and suggests that water transport and wound healing in the human corneal epithelium are defective.[36]

Contrary to clinical categorization as a non-inflammatory disorder[1], recent tear analysis studies have provided a better insight on the involvement of inflammatory

mediators in keratoconic subjects. For example, in 1996, Pouliquen et al.[37] suggested the involvement of inflammatory cytokines in the degradation of extracellular matrix.

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These developments have led to the current understanding of the pathogenesis of KC: an abnormal expressions of cytokines and proteolytic enzymes resulting in an imbalance of the formation and removal collagen in the extracellular matrix and increased apoptotic activity in corneal stroma.[38] This suppressed ability for wound healing against reactive oxygen species and reactive nitrogen species leads to cytotoxicity, cell damage, and damage to mitochondrial DNA.[38,39] The inflammatory cascade has been identified as an upregulation (by nearly two-fold) in proteolytic activity in keratoconic eyes and a decrease in the inhibition of their proteolytic activity.[40]   In addition, some researchers have tied the increased presence of inflammatory mediators with the symptoms associated with dry eye reported by patients with KC.[41]

1.1.2.1.1.   Cytokines

Cytokines are glycoprotein molecules that are secreted by inflammatory cells that modulate the downstream inflammatory response of the tissue either directly or by modulating other cytokines activity.[42] In normal corneal physiology, cytokines are responsible for regulating activation, differentiation and proliferation of inflammatory cells and mediators.[43,44] Tear and corneal cytokines, as seen in Figure

1-3, change to adapt and protect tissues against exogenous factors.[45][46] However, prolonged inflammatory activity that deviates from homeostasis, such as in the event of chronic oxidative stress, tissue damage and pathology will occur.[47] As such, tear film analysis of changes in levels of proteases and cytokines have helped researchers gain a better understanding of the pathophysiology of corneal diseases.[48]
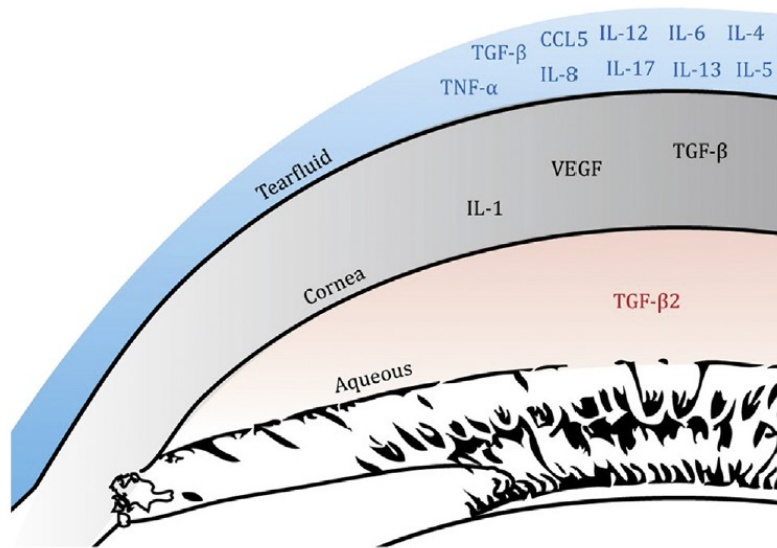
Figure 1-3: Adapted by Wisse et al[49], an illustrated representation of cytokines in tear fluid, cornea tissue, and aqueous humor of those with KC.

In KC, cytokine changes have been reported as the pathogenesis of KC.[41,50-53] Some cytokines that have been associated with KC are listed in Table 1-1. Higher presence of inflammatory markers and cell adhesion molecules (e.g. ICAM-1, VCAM-1) has also been reported in those with KC.[50]

Table 1-1: Cytokines associated with KC.

Cytokines	Implicated Processes
IL-1	IL-1 exists in two pro-inflammatory forms, IL-1 α and β.[54] It is a potent inducer for other cytokines, IL-6, IL-8, and TNF-α[55,56], and stimulates production of MMP enzymes by epithelial and inflammatory cells.[57] In addition, IL-1β plays a role in corneal wound healing by regulating of keratocyte apoptosis and corneal tissue organization.[58] On the ocular surface, IL-1β can normally be found in its inactive form, until activated by proteolytic activity, such as that by MMP-9.[59] IL-1 has been reported to trigger apoptosis of keratocytes. [86, 151, 152, 160] Elevated IL-1 levels have been found in those with dry eye[54], and has also been implicated in the pathogenesis of ocular surface disease, bullous keratopathy [60], KC[61], and sterile corneal ulcer.
IL-6	IL-6 is a pro-inflammatory mediator that is produced by corneal epithelial cells.[62] IL-6 is upregulated during corneal injury that might occur with dry eye[63-66], ocular allergy[62,67], contact lens wear[68], and KC[52,69-71]. IL-6 plays an important role in the natural defense mechanism against microbial infection[62] and corneal healing, including the stimulation of migration of corneal epithelial cells[72,73] and angiogenesis[74].
IL-8	IL-8 is a pro-inflammatory chemokine which has been associated with angiogenic activities by attracting neutrophils along the vascular wall.[56,75] Mechanical stimulation by rigid corneal contact lenses has been linked with increased tear levels of IL-8.[76] Il-8 is, also, involved in proteolytic cascade that results in breakdown of ECM in KC.[37] Over-expression of IL-8 has been linked with neovascularization, corneal ulcer formations, and formation of diffuse lamellar keratitis (DLK) status-post-refractive surgery. [5]
TNF-α	Tumor necrosis factor-alpha (TNF-α) is a pro-inflammatory mediator that has been reported at elevated levels in patients with KC[50-52,65,70] and dry eye disease[64,66]. Produced by corneal epithelial cells, TNF-α acts on vascular endothelium to promoting vasodilation, edema, and leukocyte recruitment.[77] Along with IL-6, TNF-α has hemangiogenic and lymphangiogenic functions and is involved in the inflammatory process that leads to corneal neovascularization.[77,78] Finally, TNF-α is associated with decreased collagen synthesis, increased corneal degeneration, and increased apoptosis of keratocytes in KC.[37,79]

1.1.2.1.2. Matrix metalloproteinases

Matrix metalloproteinases (MMPs) are a family of collagen degrading enzymes that play an important role in normal corneal physiology.[80] In the cornea, MMPs are released by epithelial cells, stromal keratocytes, and neutrophils[81] and they function to regulate cell differentiation, apoptosis, wound healing, and host defense.[82,83] For example, regulation of MMP by another protein, called tissue inhibitor of MMP (TIMP-1), is needed for biostability of the corneal epithelial basement membrane,[84–87] and a normal cornea will exhibit complete inhibition of MMP-9 by TIMP-1 during an open-eye environment.[86] Interestingly, Fini et al.[88] and Kenney et al.[89] have reported no changes in MMP-2 and MMP-9 concentration in normal and KC corneae, but found decreased levels of TIMP-1 and their inhibitory regulation of MMP can explain the increased proteolytic activities.[40,88] Some MMPs that have been associated with KC are listed in Table 1-2. In particular, higher concentrations of MMP-1[90] and MMP-9[51,87] have been found in KC.

Table 1-2: MMPs associated with KC.

MMPs	Implicated Processes
MMP-1 (Collagnase)	Upregulation of MMP-1 in the corneal stroma has been linked with corneal wound healing[91] and KC[90,92]. It is also reported to be involved in the degradation of, collagen types I, II, and III.[57,93]
MMP-9 (Gelatinase B)	MMP-9 is the primary, extracellular matrix-degrading enzyme produced by basal corneal epithelial cells and neutrophils.[86] MMP-9 plays an important role in corneal wound healing.[94] Moreover, it is involved in the initiation of the neutrophil-associated inflammatory cascade. In the turnover of extracellular matrix, MMP-9 is responsible for the degradation of collagen Type VII, a major component of the epithelial basement membrane.[80] Up-regulation of MMP-9 has been linked with recurrent corneal erosion.[86]

1.1.2.2. Biomechanics

Biomechanically, KC leads to ectatic effects in all layers of the cornea, as seen in Figure 1-4.[14] Ectasia at the most superficial layer of the cornea, the epithelium, occurs as a result of apoptosis, as well as the degeneration of the basal cells of the epithelium.[4,95,96] This oxidative stress also leads to mechanical instability which further leads to abnormal regulation of healing, excessive inflammation, and, in later stages, stromal haze.[97] While the overall thinning is correlated to some extent with the severity of the condition,[98,99] small areas of the epithelium thicken, due to hyperplasia, as a compensatory mechanism against the overall thinning of the stromal tissue. Furthermore, there is a loss of anchoring fibrils at Bowman's layer, leading to breaks in the basement membrane and epithelial hyperplasia and/or thickening.[14,100] At the anterior stroma, corneal nerve fibres have been observed to thicken.[14] An impaired corneal sensory nerve activity may lead to dry eye symptoms that can present with KC.[101]
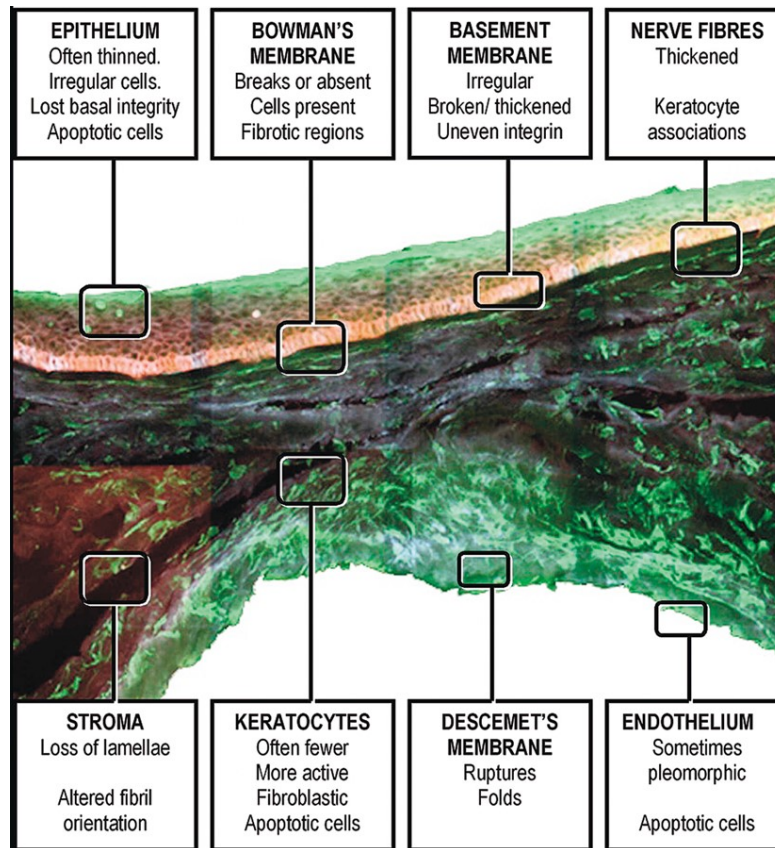
Figure 1-4: Morphological changes in all corneal layers by Sherwin et al.[14]

Stromal thinning accounts for most of the thinning in KC. The stroma, which comprises of approximately 85% of the corneal thickness, is primarily comprises collagen type I. The mechanism behind the thinning is attributed to a reduction in keratocytes secondary to increased degradation and structural alteration in collagen lamellae[4,102–104], as a result of upregulation of proteases mentioned in Table 1-2, and increased keratocyte apoptosis.[4,14,58,95] Furthermore, the formation of constituents in the extracellular matrix is also defective.[4]

Vogt's striae, or folds in Descemet's membrane, also occur due to biomechanical instability.[22] The structural changes at Descemet's membrane have been linked to pleomorphism and/or polymegathism of endothelial cells.[22] In advanced KC,

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Descemet's membrane may weaken and eventually rupture, causing corneal hydrops.[4,14,25,26] The influx of aqueous humor into the posterior corneal layers may further the separation between the corneal layers.[4]

There have been controversial reports on the effects of KC on the corneal endothelium. While some have noted there is a decrease in cell count due to apoptosis,[14,41] others have noted an increase in cell density.[22,105] Altered endothelial layer including pleomorphism and polymegathism have also been observed.[22]

1.1.2.3. Environmental

Many environmental factors that may exacerbate the pathophysiology of KC have been identified, in particular an increased exposure to ultraviolet radiation associated with an increase in oxidative stress.[38,106] KC has also been linked to ocular allergy and atopic diseases, which commonly present with a habit of vigorous eye rubbing.[14]

Although genetics play a major role in the pathogenesis of the disease, eye rubbing may contribute to the expression of the disease and contributes to the biomechanical instability of the KC cornea. The mechanical trauma to the cornea, as a result of eye rubbing, has been known to contribute to the expression of rep. The association has been attributed to increases in hydrostatic tissue pressure, corneal temperature, and protease activity[71] in the corneal tissue, although the habit, alone, is not the major etiological cause of the condition.

Furthermore, keratoconic patients often display dry eye symptoms and signs, including reduced tear quality and corneal staining.[107] Carracedo et al have reported on the association between the inflammatory nature of dry eye diseases and chronic

corneal insult and pro-inflammatory molecules that exacerbate the pathophysiology of KC, such as matrix-metalloproteinase 9 (MMP-9).[50,108] Cumulatively, these environmental factors may induce chronic epithelial injury that can activate a cascade of events involving apoptotic cytokines.[58,95,96]

1.1.3. Management

In the management of KC, the first line of action is to minimize the progression of the condition. This includes extensive patient education on how eye rubbing is detrimental to their condition.[9] Younger patients may also be candidates for corneal cross-linking (CXL).[109] CXL involves the photo-sensitization of riboflavin with ultraviolet A (UVA) light to induce the polymerization of collagen fibers found in the anterior 200 to 300 microns of the corneal stroma.[109-113] CXL effectively increases the corneal rigidity and the biomechanical strength by three-fold.[113-115] CXL also results in the simultaneous flattening of the steepest area and steepening of adjacent flatter areas around the cone, which decreases corneal asymmetry and spherical aberration.[115]

Visual rehabilitation is a major focus in the management of KC. Since there is a significant degree of higher order aberrations with KC, most patients will not be able to obtain satisfactory vision with spectacles and regular soft contact lenses. Corneal rigid gas permeable (RGP) lenses are the main mode of visual correction in keratoconic patients.[116] These lenses provide excellent vision correction as the fitting of the lens traps a tear film layer between the front surface of the cornea and the back surface of the lens to form a post-lens tear film. It allows the masking of higher-order aberrations arising from the irregular corneal surface. There are several fitting

challenges in the use of corneal RGP lenses in the management of KC. Firstly, not all patients are successful in wearing corneal RGP lenses as the interaction between the lens and the palpebral conjunctiva of the eyelid and the eyelid margin causes significant lens awareness and discomfort. Secondly, the irregular corneal surface may increase the likelihood of the lenses becoming decentred or dislodged. Other modalities, including hybrid contact lenses, which a central RGP surrounded by peripheral skirt made of soft lens material skirt, and piggy-back systems with a corneal RGP lens fitted over a soft contact lens, may be able to overcome some of these fitting challenges. However, they present other challenges such as a difficult contact lens handling experience and more complex storage and care routine. In recent years, the use of scleral contact lenses has become a more popular non-surgical management for corneal ectasia and ocular surface disease.[117–126]

In 20-25% of keratoconic cases, functional vision is not attainable by means of vision correction. A significant reduction in best corrected visual acuities due to corneal scarring is the primary cause for consideration of surgical management, namely penetrating keratoplasty or deep anterior lamellar keratoplasty.[127] Intra-corneal rings segments have also been used in the management of KC.[127,128] These segments of polymethylmethacrylate plastic are inserted into stromal tunnels with the intention to reshape and flatten the corneal surface and improve visual acuity.

Management of KC is summarized in Figure 1-5.
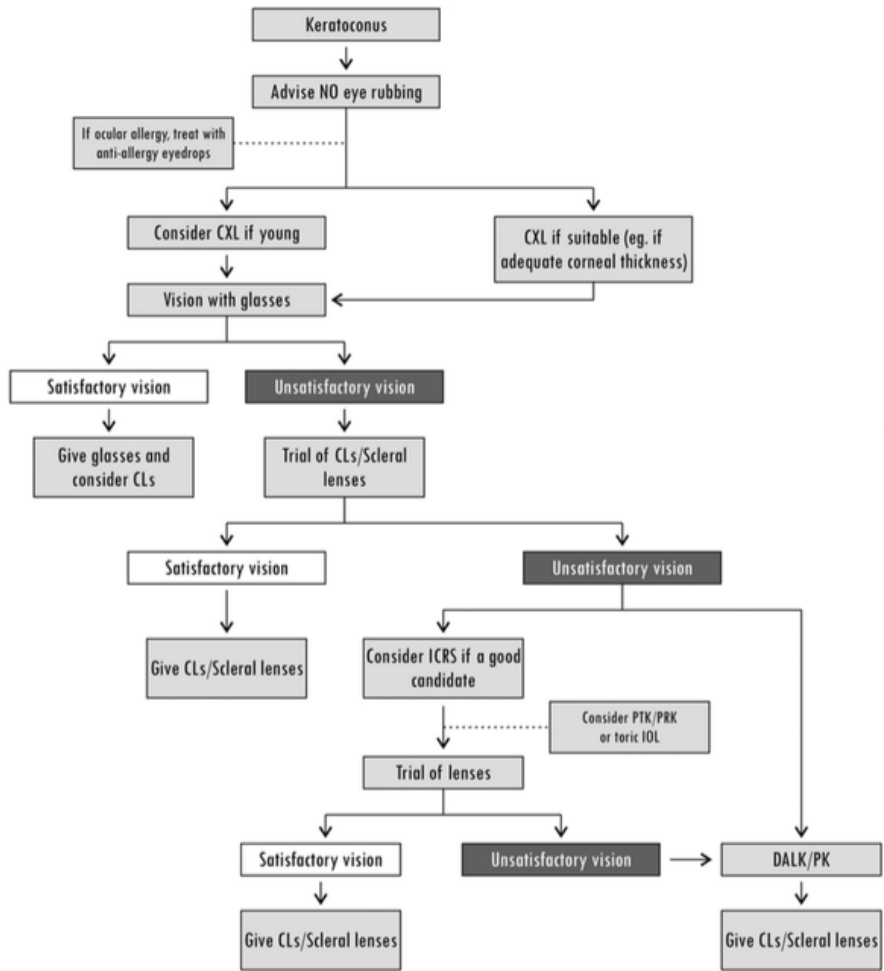
Figure 1-5: Management of KC summarized by by Gomes et al.[9]

1.2 Scleral Lenses

Modern scleral contact lenses are RGP lenses with an overall diameter that is approximately 6mm larger than the horizontal visible iris diameter.[118,126,129] These lenses are designed to rest on the sclera while vaulting over the cornea and create a fluid reservoir.[120,126] The use of scleral contact lenses is becoming a more popular non-surgical management technique for corneal ectasia, as well as, other ocular surface diseases.[117-126] The post-lens fluid reservoir can provide patients with irregular corneae and a likely history of poor tolerance to soft contact lenses and corneal RGP lenses), with visual rehabilitation, corneal protection and tear conservation.[118,130] In practice, these lenses may also be indicated in the correction of refractive error for healthy corneae.[129] Compared to corneal RGP lenses that have a diameter of 10mm or smaller; scleral lenses can provide increased comfort by minimizing lid interaction on blinks with lens diameters from 14.3 to 18.2mm. Scleral lenses also provide similar or superior optics, with reduced higher order aberrations, more stable vision, and minimal mechanical impact on the central cornea.[120,124,126,131-133]

1.2.1 Anatomy of a Scleral Lens

The overall shape and anatomy of a scleral lens is divided into three zones: the central optic zone, the mid-peripheral or intermediate zone (that typically aligns with the limbal area), and the scleral landing zone. (Figure 1-6)
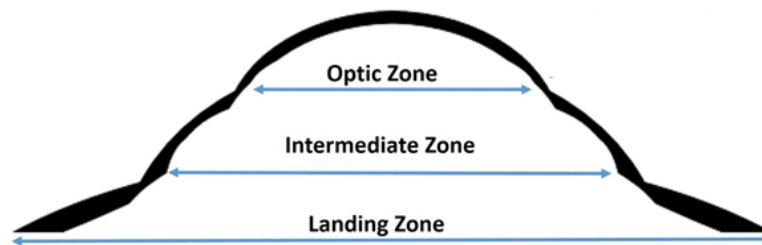
Figure 1-6: Anatomy of Scleral lens zones.

The central optic zone maintains its position over the pupil with an underlying layer of fluid reservoir, also referred to as the post-lens tear film. This post-lens tear film is key in the clinical performance of scleral lenses. It masks the irregularities of the ocular surface, found with KC and other irregular corneae, allowing for superior visual rehabilitation for patients wearing scleral lenses.[121,122,133,134] The post-lens tear film also provides protective and therapeutic benefits to those patients with severe ocular surface disease over other contact lens modalities, since it continuously bathes the compromised ocular surface with fluid.[135-137] Finally, this post-lens tear film enables the scleral lenses to maintain its apposition relative to the ocular surface with a balance between the hydrostatic pressure to support the weight of the lens and negative pressure within the reservoir for lens stability.[120]

An ideal post-lens tear film thickness is the primary goal when fitting a scleral lens. According to theoretical models, based on the oxygen tension of the post-lens tear film, excessive central corneal clearance may result in reduced oxygen transmissibility despite the use of highly oxygen permeable (Dk) materials and, thereby, induce corneal hypoxia.[138-140] On the other hand, inadequate central corneal clearance may result in mechanical insult to the central corneal epithelium, as has been shown with flat fitting corneal RGP lenses.[141-143] Minimal central corneal

clearance is necessary to provide sufficient hydrostatic pressure to maintain the position of the lens relative to the cornea during lens wear.[120]

Between the central optic zone and the peripheral scleral landing zone, the limbal zone of the scleral lens is intended to align with and vault over the limbal area. This is an important area of the cornea as it houses the limbal vasculature, responsible for supplying oxygen and nutrients to the avascular peripheral limbal cornea and the limbal stem cells necessary for corneal epithelial regeneration.[144-146] There is currently a lack of consensus amongst eye care practitioners as to what the ideal fitting relationship should be for the limbal curvature.

Finally, the scleral landing zone is the most peripheral zone. The purpose of this zone is to rest on the conjunctival tissue over the sclera for support of the other lens zones. It should conform to the overall scleral shape for an optimal fit of perfect scleral alignment.
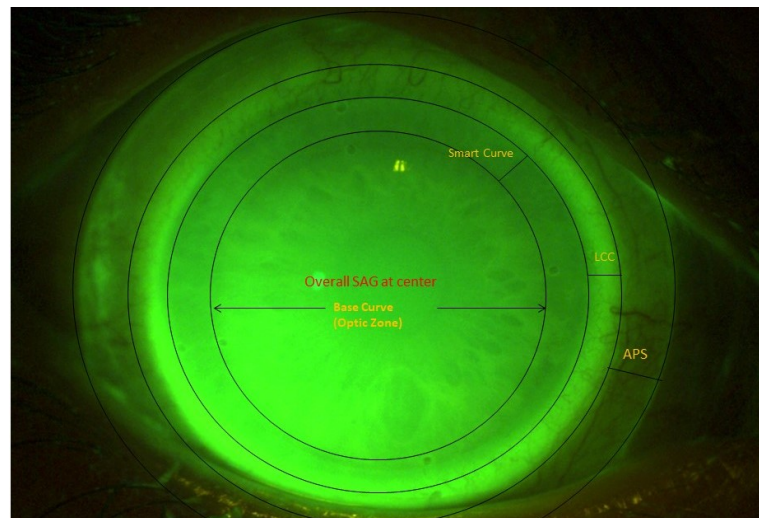
Figure 1-7: A scleral lens observed with fluorescein using slit-lamp biomicroscopy.
Central optic zone connected to the limbal clearance zone (LCC) with a "Smart Curve" and scleral landing zone (APS) outlined. (Photo credit: Alden Optical.)

1.2.2 Scleral Lenses and Indications

The primary indication for scleral lenses is to offer an optical correction for an irregular cornea.[120,123,147,148] Management with scleral lenses for irregular corneae is on an upward trajectory.[148] While the scleral lens and GP lens are similar in having a post-lens tear reservoir between the lens and the corneal surface that is able to neutralize irregular astigmatism and mask optical aberrations that present with distorted corneal topography,[121,122,133,134] Unlike corneal RGP lenses that rests entirely on the cornea, scleral lens rests on the scleral conjunctiva. Thus, the apposition and centration of a scleral lens are not affected by a displaced corneal apex in cases of irregular corneae. For this reason, scleral lenses provide keratoconic patients, who are intolerant of other contact lens modalities, with an alternative optical solution to surgical interventions.[122] For example, the number of corneal transplants performed in Norway for the management of KC reduced by 25% over a 3 year span following the adoption of scleral lens fitting.[149] In addition, scleral lenses are utilized in the management of secondary corneal ectasia which includes, but is not limited to, corneae that have undergone penetrating keratoplasty and refractive surgery such as, radial keratotomy, photorefractive keratectomy, laser-assisted in situ keratomileusis (LASIK), and cases of corneal scarring secondary to trauma or infectious keratitis, provided that the endothelia of these conditions are sound.[117,119,148]

The management of ocular surface diseases is another common indication for the use of scleral lenses.[117,134,147,148,150-152] During scleral lens wear, the post-lens tear film provides continuous hydration for the corneal surface and is able to facilitate the rehabilitation of the ocular surface and protect the ocular surface from desiccation.[135-

[137,152] As a result, these lenses are effective in therapy for conditions such as keratitis sicca and exposure keratopathy, cicatrizing conjunctivitis (e.g. Stevens-Johnson syndrome), and ocular cicatrical pemphigoid.[121,148] Furthermore, the lens surface is able to provide protection for the ocular surface against mechanical irritation caused by the shear force applied to the corneal surface by the upper eyelid during blinking. Scleral lenses have proven to be effective in the management of exposure keratopathy and promote corneal re-epithelization in cases of persistent corneal epithelial defects[150,152] secondary to trauma or corneal dystrophies (epithelial basement membrane dystrophy) [152,153]

Scleral lenses may also be used in sports vision and for cosmetic purposes.[129] Finally, prosthetic scleral lens have been used in the management of a disfigured ocular surface, such as aniridia and albinism.[154]

1.2.3 Scleral Lenses and Complications

Similar to other contact lens modalities, a good scleral lens fit must not have a negative impact on the ocular surface. Walker et al.[119] categorized complications associated with scleral lens wear into 1) physiological responses and 2) complications resulting from fitting challenges. Physiological responses include infection-related, inflammatory, and hypoxic issues. Injection in the limbal and bulbar conjunctival areas are two of the most common complications associated with scleral lens wear.[119,126] Injection is indicative of the presence of distress to the ocular surface.[155] It has been suggested that poor central lens-cornea and lens-limbus fitting relationships, combined with the greater thickness of these scleral lenses, may result in a negative impact on the corneal and limbal physiology.[117-119] Others have

20

suggested the presence of sub-clinical corneal hypoxia as a result of scleral lens wear, despite the use of lens materials with high oxygen transmissibility.[139,156,157] On the other hand, mechanical etiologies of complications may arise from poor lens-ocular surface fitting relationships. These include conjunctival prolapse, limbal staining, conjunctival staining, and lens edge awareness.[119] In all, continual efforts must be devoted to better understand the effect of scleral lenses on the corneal physiology, with the aim of developing the ideal scleral lens fitting characteristics that provide good comfort, good vision, and good fit.

Chapter 2
Study Rationale

2.1 Study Background

All lines of treatment must be analyzed and assessed for their effectiveness, safety, and tolerability before they are utilized and recommended by medical professionals.[158]

The effectiveness of scleral lenses in the management of KC is well documented. Scleral lenses can provide superior optics with reduced glare by creating a post-lens tear reservoir that masks the irregular astigmatism and higher order optical aberrations that present with distorted corneal topography.[121,122,133,134] As this post-lens tear film continuously bathes the corneal epithelium during scleral lens wear, this contact lens modality is also able to provide relief from some of the dry eye symptoms associated with KC.[41,101,135-137] Furthermore, scleral lens are associated with reduced lens awareness and greater lens comfort as they are fitted to result in minimal lid interaction on blink and minimal mechanical impact on the central cornea.[120,124,131-133] Extensive research has focused on determining the ideal lens-corneal relationship for the central optic zone. The central zone must be fitted to ensure there is adequate post-lens tear film thickness, taking into consideration lens settling, to avoid mechanical insult by the posterior lens surface on the central corneal epithelial surface.[141-143159-161] On the other hand, an excessive post-lens tear film thickness, combined with the lens thickness, may result in reduced oxygen transmission to the anterior cornea despite the use of highly oxygen permeable (Dk) materials, and thereby induce corneal hypoxia.[138-140]

Despite a suitable central fitting relationship, adverse events and ocular surface changes are still noted with scleral lens wear. Cases of scleral lens wearers exhibiting adverse limbal findings and conjunctival injection have been reported anecdotally.[119,126,162] These ocular findings, as seen in Figure 2-1, include limbal epithelial hypertrophy, which appears as negative corneal staining in the mid-peripheral cornea, limbal injection, and corneal edema, suggesting ocular sequelae of hypoxic or mechanical etiologies. This suggests that other fitting parameters, namely the limbal zone, may play a role in how scleral lenses interact with the ocular surface.
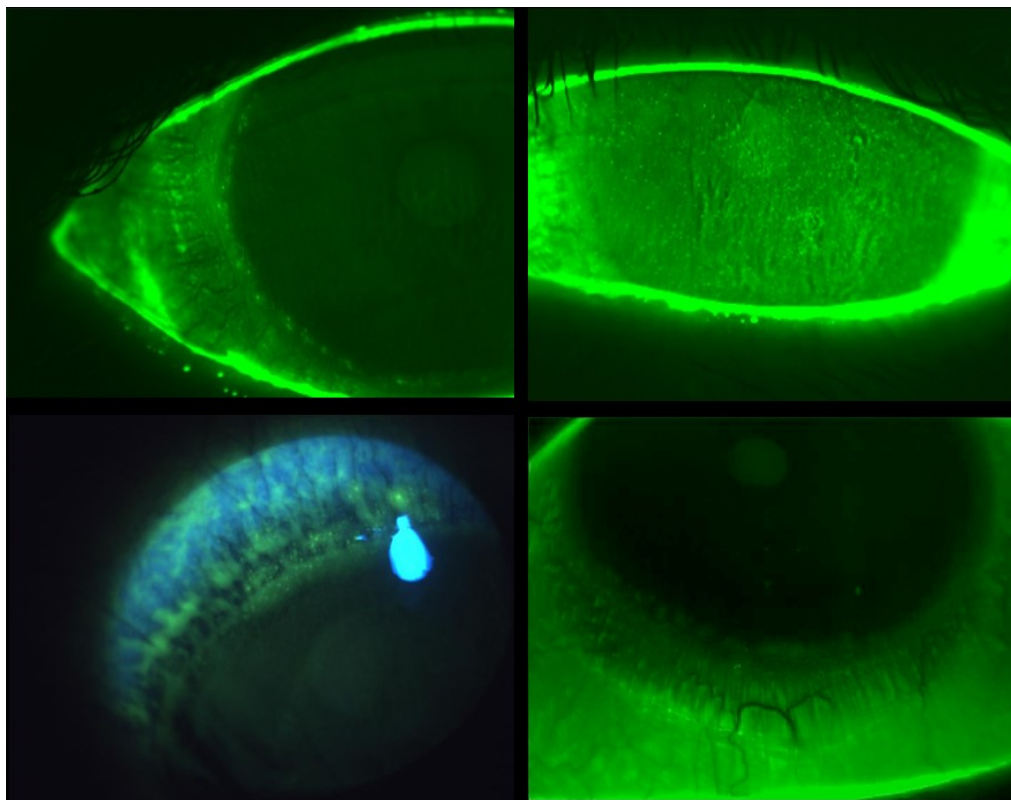
Figure 2-1A-F: Fluorescein images highlight conjunctival staining (A, top left) diffuse epithelial irregularity (B, top right), negative staining at limbus (bottom left), and limbal injection (bottom right).

The limbal zone of the scleral lens is intended to align with and vault over the limbal area, an important area of the cornea which houses all the corneal stem cells necessary for the re-population of epithelial cells within the cornea.[146] A poor-fitting relationship in the limbal zone can result in mechanical and hypoxic effects to the ocular environment. At this time, however, there is a lack of literature focusing on how the limbal zone should be fitted to provide optimal lens performance while minimizing the ocular sequelae.

2.2 Scleral Lens Prescribing Trends and Complications

To gain a better understanding on of the impact the scleral lens may have on the corneal physiology. A 2016 survey investigated scleral lens parameter prescribing trends and the frequency of various ocular health findings associated with scleral lens wear.[163] The results were presented at the 2017 Global Specialty Lens Symposium. The following is a summary of the survey.

2.2.1 Scleral Lenses Prescribing Trends and Complications Survey

2.2.1.1 Purpose

The purpose of this study was to survey scleral contact lens fitters on their prescribing habits and to sample the frequency of various ocular health findings associated with scleral lens wear in their patient population.

2.2.1.2 Methods

The web-based survey was self-administered electronically. Participants were asked about their scleral contact lens prescribing preference for overall diameter, ideal CCC post lens-settling and fitting goals for limbal clearance post-settling. In addition,

participants were asked about the proportions of their scleral lens wearing population encountering six corneal and conjunctival complications: midday fogging, conjunctival staining, limbal hyperemia, limbal staining, corneal staining and corneal edema, on a scale of never, then approximately 25%, 50%, 75%, or 100% of the time. The survey provided options for which participants had to respond based on forced choice.

2.2.1.3 Results

Nearly 200 scleral lens practitioners across North America were invited to participate in a web-based survey with a response rate of 47.2% (93/197). Of the respondents, thirteen participants did not complete the survey; and results from one respondent was excluded due to inadequate scleral lens fitting experience. Of the seventy-nine participants with a minimum of five scleral lens fitting experiences who responded, the following data were compiled and summarized in Figures 2-2 A-C and Table 2-1.
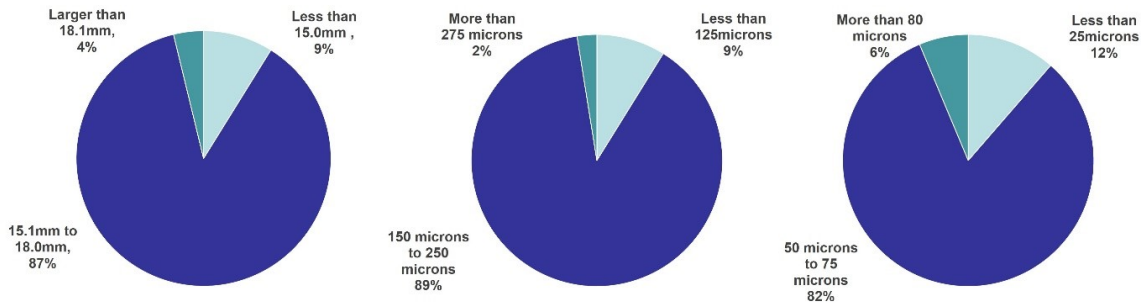
Figure 2-2A-C: Percentage of scleral lens practitioners prescribing trends for overall diameter (A), ideal CCC (B) and, ideal limbal clearance (C)

Table 2-1: Respondent reports of percentage of patient population exhibiting various ocular findings subsequent to scleral lens wear.

Ocular Findings	Mean
Midday Fogging	26.8%
Conjunctival Staining	26.7%
Limbal Hyperemia	23.7%
Limbal Staining	18.9%
Corneal Staining	13.3%

2.2.1.4 Discussion

For the scleral lens practitioners surveyed, 15.1mm to 18mm was the most commonly prescribed scleral lens overall diameter. There also appears to be a good agreement between the scleral lens fitters on the ideal central lens-corneal fitting relationship, where the most common fitting goal was to achieve between 150μm and 250μm centrally and between 50μm to 75μm post-lens settling in the limbal zone.

Similar to the findings in this survey, midday fogging has been reported in 20-33% of scleral lens wearers.[119] This accumulation of debris in the post-lens tear film may or may not be visually significant. It has been attributed to excessive lift at the scleral landing zone.[119]

2.2.2 Study Interpretation

Overall, there is a good consensus amongst scleral lens fitters on ideal fitting characteristics for scleral lens of 15.1mm to 18mm in diameter in diameter and the ideal fitting characteristic for the limbal zone. The survey indicates that in both the central corneal zone and the limbal clearance zone, the scleral lens system should have a post-lens tear film to allow the posterior surface of the lens to clear over the ocular tissue. Despite a similar lens-ocular surface fitting relationship, limbal

hyperemia and staining is more commonly noted as a result of scleral lens wear than corneal findings.

2.3 Protease and Cytokine Analysis

Tear cytokines change in response to biological, pathogenic, or environmental factors.[48] As such, they are biomarkers for subclinical inflammatory changes.[43,48] Analysis of inflammatory mediators present in the tear film has helped scientists gain a better understanding of the pathophysiology of complications in soft contact lens wearers,[164,165] dry eye,[166] and KC.[41,87] Understanding how specific tear proteins change with scleral contact lens wear can provide a more in depth insight on its impact on corneal physiology.

Prior to this thesis study, a pilot study was performed at the lab of Dr. Maria Markoulli at the University of New South Wales. This collaboration aimed to replicate and optimise a method of tear collection verified by Dr. Markoulli in earlier papers, the flush tear method[167], for scleral lens wearers. The pilot study focused on those with normal corneae. From the tears collected, the concentration of MMP-9 and TIMP-1 was determined using an enzyme linked immunosorbent assay (ELISA), as they are important in normal corneal re-epithelialization. An increase in MMP-9 has been linked with recurrent corneal erosions and mechanical trauma secondary to eye-rubbing and contact lens wear.[80,84,87]

2.4 Study Aims

As eye care practitioners continue to fit more patients in scleral lenses, it is becoming increasingly important to gain a more in-depth understanding of the impact of scleral

lens wear has on the physiology of the cornea. This thesis study aims to elucidate how varying the limbal clearances of a scleral lens may impact ocular health, both clinically and sub-clinically. The goal of the study is to simulate a scleral lens system with an overall lens diameter (between 15.1mm and 18mm) and central corneal clearance (between 150µm and 250µm) consistent with that preferred by most eye care practitioners, while isolating the limbal zone as the only changing variable. The clinical trial aims to evaluate how varying limbal clearance can affect the clinical performance subjectively and objectively; focusing on comfort, vision, hyperemia, and mechanical and hypoxic related complications. In addition, using the flush tear methods for tear collection mentioned in Section 2.3, tear cytokine analysis can reveal subclinical inflammatory changes in association with scleral lens wear designed with high and low limbal clearance. Ultimately, results from this study will help clinicians determine the safety profile of scleral lens wear for keratoconic patients.

Chapter 3
Scleral lens Fitting Procedures and Parameters

3.1 Overview

Purpose: To fit a group of keratoconic patients with a scleral lens of both high and low limbal clearance, with a constant central corneal clearance. A secondary purpose is to observe the impact of lens settling on limbal clearance.

Methods: A group of keratoconic participants were enrolled according to the Tenets of Helsinki and with written consent. Corneal sagittal height was measured using the Visante OCT at a 15mm chord. The sagittal depth of the custom scleral lens used for each subject (Zenlens, Alden, NY) was determined by adding 0.35mm to the sagittal height measured. From the central sagittal depth, two sets of study lenses were designed with varying limbal clearances (LC) which differed by 50µm. Lenses were worn in a randomly assigned order for a two-week period. Lens fitting parameters including central and limbal clearances were measured with the Visante OCT and compared.

Results: 11 subjects (22 eyes) were enrolled in the study and all were male participants. The sagittal height for the subjects was 3.71± 0.25mm. This produced a sagittal depth of 4.539±0.240mm in scleral lenses with low LC and 4.550±0.243mm in scleral lenses with high LC (p=0.877). There was no difference in central corneal clearance between low and high LC (p=0.671) for initial central corneal clearance, and p=0.475 for final central corneal clearance). The initial limbal clearances, before lens settling, were 159.9 ±45.02µm for low LC lenses, and

194.07±66.10μm for high LC lenses (p<0.05). The final limbal clearance, after lens settling, were 123.74±56.68μm and 167.31±69.75μm for the low and high LC lenses, respectively (p=0.006). A significant change in initial and final LC was expected and found in both group of study lenses due to lens settling (p<0.001, both). Finally, there were significant differences between the nasal and temporal LC, with more LC found temporally (p<0.001).

Discussion: The use of an anterior segment OCT, such as the Visante, was instrumental in scleral lens fitting. This aided the fitting of scleral lenses with varying limbal zone designs. Limbal clearance ranges from 106.40μm (low LC) to 185.78 μm (high LC) at the final visit using two lens designs that had with a 50μm difference in the LC parameter of the scleral lens designs.

3.2 Introduction

The use of scleral lenses in the management of KC is well-documented.[120-123,131,147,168-171] This mode of vision correction can facilitate visual rehabilitation for individuals with KC.[121,122,130,131] Similar to corneal GPs, scleral lenses are fitted with a post-lens tear film which masks the optical aberrations caused by an irregular corneal surface. It has been shown that for those who are intolerant of corneal GPs, scleral lenses are successful in deferring the need for keratoplasty.[122] In comparison to corneal GPs, Schornack and Patel[122] noted an ease of fitting scleral lenses, with their success less dependent on the severity of changes in topographic corneal curvature and irregular astigmatism. With scleral lenses, greater centration and stability can be achieved even in severe KC.

Scleral lenses are supported by the conjunctival tissue over the sclera.[120,126,172] The eccentricity and rate of change in curvature drastically changes in the peripheral cornea towards the sclera in an aspheric and rotationally non-symmetric fashion.[173,174] Current corneal topographers have a limited ability to accurately acquire data on the topographic shape of the sclera. As the correlation between corneal topographic data with scleral back optic zone radius is poor[175], the use of diagnostic lenses in conjunction with sagittal height information is the preferred fitting method.[118,122,176,177] The sagittal depth of the scleral lens is defined as the distance from the central back surface of the lens to the plane of the peripheral edges of the scleral lens. In an ideal scleral lens fitting, the sagittal depth is the sum of the sagittal height of the eye at the chord diameter of the overall diameter of the scleral lens plus the central thickness of the post-lens tear film or central corneal clearance (CCC). Figure 3-1

shows an OCT image of the sagittal height of the cornea at a chord of 15.79 mm. The scleral lens has a greater sagittal depth compared to the anterior eye as indicated by the clearance of the lens from the cornea.
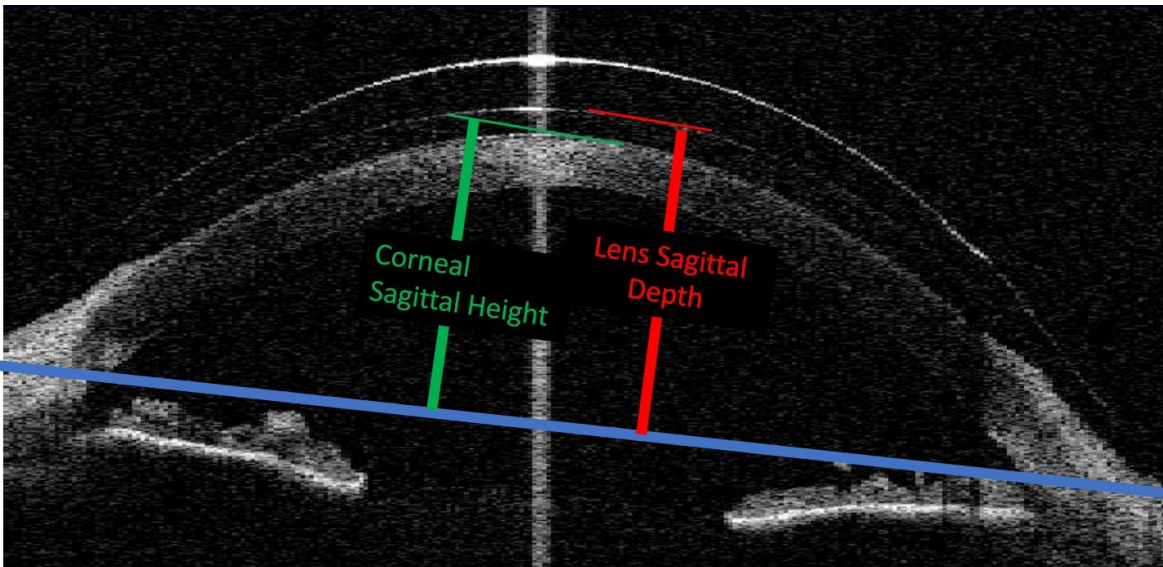
Figure 3-1 The apposition of a scleral lens relative to the ocular surface with corneal sagittal height and scleral lens sagittal depth demarcated.

Compared to corneal RGPs, in which the primary fitting parameter is determined based on the central corneal curvature, Schornack and Patel[122] discussed that an appropriate sagittal depth, in scleral lens fitting, is a more important fitting criterion than aligning the back radius curve of the scleral lens to the curvature of central cornea. In this chapter of the thesis, the fitting process used to determine an appropriate scleral lens for the keratoconic participants having the sagittal depth as the primary fitting parameter will be discussed.

A secondary purpose of this chapter is to demonstrate the impact of lens settling has on limbal clearance in two sets of scleral lens design that varies in the limbal zone while keeping the central lens-ocular surface fitting relationship the same.

3.3 Methods and Materials

3.3.1 Participant Recruitment

The protocol for this study was approved by the Office of Research Ethics of University of Waterloo (ORE#21364) and followed the Tenets of Helsinki. Participants were recruited from the University of Waterloo, Contact Lens Clinic (UW-CLC), by their attending doctor. Informed consent was obtained from all participants.

3.3.1.1 Inclusion and Exclusion Criteria

The requirements for participation was that the participants had been previously diagnosed with KC in at least one eye. In addition, the participants must be male, over 18 years of age and able to provide consent. Individuals with any ocular pathology or severe insufficiency of lacrimal secretion or had undergone any corneal surgery were excluded from the study.

3.3.2 Study Materials

3.3.2.1 Study Lenses

The ZenLens™ semi-scleral lenses used in this study are approved by Health Canada, with Health Canada license #96602, and are commercially available. They are manufactured using the Boston XO material (Health Canada license #71386) by Alden Optical (Lancaster, NY, USA). This study lens was chosen because the sagittal depth and limbal curve can be adjusted independently from one another. Lenses were fitted

according to the manufacturer's fitting guidelines, and participants proceeded into the study only if a suitable fit with the lenses could be obtained.

3.3.2.2 Anterior Segment Optical Coherence Tomography

The Visante™ OCT (Carl Zeiss Meditec, Dublin, CA) is a time-domain, anterior segment optical coherence tomographer.[178] (Figure 3-2) The instrument uses a 1,310nm super-luminescent light source to acquire single images of the anterior segment of the eye. Using the high-resolution mode (10mm wide beam[179]), images can be obtained across a chord up to 16mm in diameter, and to a 6mm depth within the eye, without contact.[178] Optical tissues are detected and imaged based on the strength of reflectance of the return signal from that tissue.[179] By combining several A scans, the Visante™ software is able to construct a 2-dimensional cross-sectional model with an axial resolution of up to 18μm and a transverse resolution of up to 60μm.[178] Furthermore, the Visante™ software allows analysis using built-in calipers, angle tools, and a flap tool in its high resolution mode. With these functions, manual measurements of any distance of interest along the axial or tangential direction of ocular surface can be obtained. For example, the anterior segment OCT allows for measurement of sagittal heights and chord values, which is helpful in the fitting of contact lenses.

Figure 3-2 The Visante™ Anterior Segment OCT.

3.3.2.3 Corneal Tomography

The Oculus Pentacam HR® (Wetzlar, Germany) is a corneal tomographer which can provide keratometric and pachymetric measurements of the cornea. (Figure 3-3) It uses a Scheimpflug (1.45 megapixel) camera, which rotates through 360 degrees to collect up to 50 slit images of the anterior segment. This takes approximately two seconds to complete.[10,12] The light source of this instrument is a custom-designed, ultraviolet radiation free, cobalt-blue, LED with a wavelength of 475nm. The Pentacam HR® software stitches together the slit images to construct a 3D model of the cornea with 25,000 true elevation points and up to 138,000 data points. Unlike other corneal topographers, the Oculus Pentacam HR® primarily measures height or elevation points of the corneal surfaces and other optical tissues with respect to a best-fit sphere. From the elevation data, simulated keratometric values or curvature values of the anterior and posterior cornea is calculated by the Pentacam HR® software. Furthermore, corneal pachymetric data can be determined as the difference between the front and back surface elevation measurements of the cornea. Other parameters

that can be calculated include the estimated corneal diameter, corneal wavefront aberrations, densitometry and anterior chamber volume.

Figure 3-3 The Oculus Pentacam HR® Corneal Tomographer.

3.3.3 Study Procedure

A total of five study visits were required to complete the study. At the initial screening and lens fitting appointment, eligibility based on inclusion and exclusion criteria (Section 3.3.1.1.) was determined. If informed consent was received, baseline measurements were obtained. For each participant, simulated keratometric values and estimated corneal diameter were obtained with the Oculus Pentacam HR®; sagittal height at a chord of 15.0mm was determined using the Visante™ OCT.

The fitting protocol first decides on an approximate lens diameter based on the size of the cornea. Larger lens diameters are chosen for larger eyes.[180] Subsequent to choosing the appropriate lens diameter, sagittal depth of the lens is chosen based on the sagittal height of the eye by adding 0.35mm, to account for the CCC, to the sagittal height measured at 15.0mm.[161,177,181] During the lens fitting process, a

ZenLens™ scleral lens diagnostic, prolate design (Alden Optics, Lancaster, NY, USA) would be chosen with the appropriate sagittal depth in accordance with the manufacturer's fitting guide. (Figure 3-4) The diagnostic lens was inserted with the bowl of the lens filled with non-preserved saline solution (0.9% Sodium Chloride injection solution, Addipak (3mL), Stevens Inc.). Once the lens was inserted, the scleral lens-to-cornea fitting relationship was evaluated using the Visante™ OCT. The fitting goals included adequate central corneal clearance of 250-300µm based on the fitting guide and published literature[161], complete limbal clearance, and alignment in the scleral landing zone. An alternative diagnostic lens was chosen if these fitting goals were not met. Once the appropriate diagnostic lens was chosen, an over-refraction was performed to determine the contact lens power for each eye.

Diagnostic Set Configuration

PROLATE		Z-1	Z-2	Z-3	Z-4	Z-5	Z-6
	16MM	4200 SAG 8.20 BC	4500 SAG 7.60 BC	4800 SAG 7.10 BC	5100 SAG 6.70 BC	5400 SAG 6.40 BC	5700 SAG 6.10 BC
		Z-7	Z-8	Z-9	Z-10	Z-11	Z-12
	17MM	4300 SAG 9.20 BC	4600 SAG 8.40 BC	4900 SAG 7.80 BC	5200 SAG 7.30 BC	5500 SAG 6.90 BC	5800 SAG 6.60 BC

Figure 3-4 The Zenlens® Trial Set by Alden Optics, Lancaster, NY, USA.

For every eye, two sets of lenses were ordered. The central sagittal depth and contact lens power was determined based on the diagnostic fitting. The limbal zone of one set of study lenses has its limbal zone was adjusted to provide adequate clearance of approximately 25-30µm in accordance to the fitting guide, and was labeled as low LC. For the second set of study lenses labeled as high LC, the limbal zone was

adjusted to increase the LC by 50μm. Each set of study lenses were to be delivered to the participants in a randomized order and double-masked to the participant and researcher. Participants were asked to wear each scleral lens design for a duration of two weeks between a delivery appointment and a follow-up appointment, based on a daily wear schedule of a minimum of six hours. A wash-out period of minimum of seventy-two hours was required between the first follow-up visit and the second lens delivery visit.

At each study visit, visual acuities were measured. In addition, clearance was observed and measured in three zones, central corneal clearance (CCC), LC in nasal and temporal quadrant, using the caliper function in the Visante™ OCT. The measurements were categorized as initial clearance, measured approximately twenty minutes after lens application at the lens delivery appointment, and final clearance, measured after minimum of two-hour wear time at the follow-up appointment.

The summary of the five study visits is illustrated in Figure 3-5.
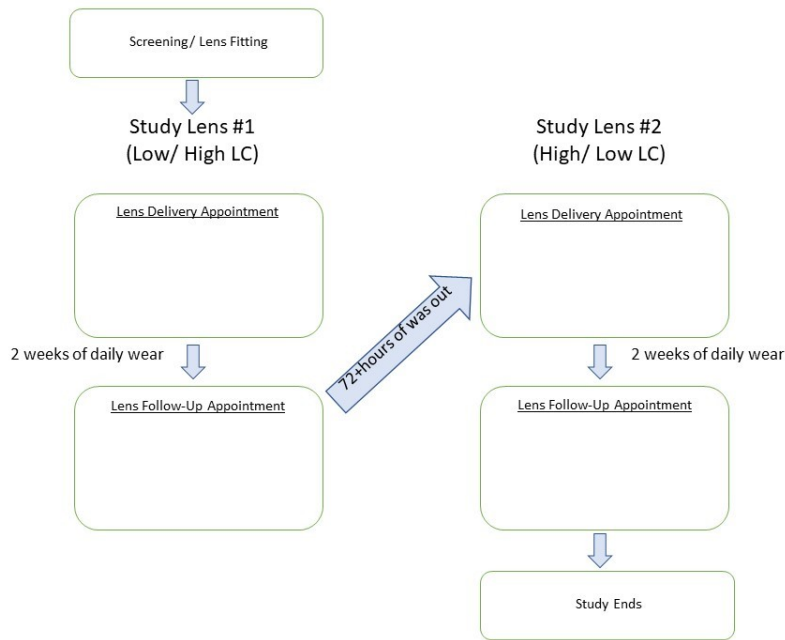
Figure 3-5: Flow chart of study visits.

3.4 Results

3.4.1 Participant Demographics

Fourteen male individuals (27 eyes, 1 participant was monocular; mean age: 38.5±13.5 years; range: 24 to 67 years) were recruited. Eleven patients (22 eyes; mean age: 39.2±12.8 years; range: 24 to 67 years) completed the study. Of the three participants who discontinued, reasons included poor adaptation compared to their habitual corneal GP, difficulty handling lens application and removal (habitual correction was also corneal GP), and one was lost to follow-up (habitual correction = spectacles). Only data for the twenty-two eyes that completed the study were included in the analysis.

The habitual correction for the twenty-two eyes were as follows: 11 (50.0%) were in corneal GPs, 6 (27.3%) of whom were wearing a piggyback system, 4 (18.2%) were in

other scleral lens designs, and 4 (18.2%) were wearing silicone hydrogel toric lenses. Baseline measurements for simulated keratometry, sagittal height at 15mm chord diameter, and estimated corneal diameter for all twenty-two eyes are listed in Table 3-1 The mean sagittal height at a chord diameter of 15.0mm was measured to be 3.71± 0.23mm.

Table 3-1: Participant details and baseline measurements.

PATIENT ID	AGE	EYE	HABITUAL CORRECTION	SIMULATED KERATOMETRY	SAGITTAL HEIGHT AT 15MM	ESTIMATED CORNEAL DIAMETER
1	24.0	OD	Soft Lens (SiHy)	50.3D @ 41.0˚/ 53.6D	3.76mm	11.8mm
		OS	Soft Lens (SiHy)	45.5D @ 142.0˚/ 48.3D	3.48mm	11.9mm
2	24.9	OD	Soft Lens (SiHy)	45.8D @ 59.9˚/ 47.6D	3.71mm	11.7mm
		OS	Soft Lens (SiHy)	48.5D @ 112.6˚/ 52.3D	3.88mm	11.9mm
3	28.1	OD	Corneal GP	44.7D @ 145.7˚/ 47.3D	4.18mm	11.8mm
		OS	Corneal GP	44.7 @ 137.5˚/ 48.0D	4.14mm	11.7mm
4	29.2	OD	Corneal GP	47.1D @ 18.8˚/ 50.0D	3.55mm	11.9mm
		OS	Corneal GP	49.2D @ 171.1˚/ 54.8D	3.78mm	12.0mm
5	35.4	OD	Corneal GP	42.5D @ 180.0˚/ 44.5D	3.49mm	12.1mm
		OS	Corneal GP	42.7D @ 161.6˚/ 47.3D	3.47mm	12.2mm
6	38.4	OD	Piggyback System	44.2D @ 35.9˚/ 46.9D	3.62mm	11.8mm
		OS	Piggyback System	43.3D @ 142.1˚/ 46.4D	3.73mm	12.0mm
7	39.4	OD	Corneal GP	44.9D @ 66.1˚/ 47.2D	3.16mm	11.8mm
		OS	Corneal GP	47.2D @ 121.6˚/ 48.2D	3.55mm	11.8mm
8	42.2	OD	Scleral Lens	47.6D @ 46.3˚/ 48.7D	3.61mm	11.9mm
		OS	Scleral Lens	46.3D @ 141.8˚/ 47.7D	3.80mm	11.9mm
9	42.8	OD	Scleral Lens	43.4D @ 43.1˚/ 47.2D	3.71mm	12.5mm
		OS	Scleral Lens	56.7 @ 180.0˚/ 60.0D	3.88mm	12.9mm
10	52.7	OD	Piggyback System	49.1D @ 22.4˚/ 52.1D	3.61mm	10.9mm
		OS	Piggyback System	42.9D @ 141.1˚/ 46.5D	3.61mm	11.0mm
11	67.0	OD	Piggyback System	48.2D @ 42.3˚/ 48.6D	3.94mm	11.4mm
		OS	Piggyback System	55.9D @ 164.7˚/ 58.1D	4.00mm	11.4mm

3.4.2 Study Lens Parameters and Fitting Characteristics

The final lens parameters for all scleral lens fittings are listed in Tables 3-2 and 3-3, along with the best corrected visual acuities found with the study lenses. It is noted that, based on the average corneal diameter of 11.83±0.43mm, a 16.0mm overall diameter was chosen for all study lenses in accordance to the fitting guide. The median visual acuity was 6/7.5 for both low and high LC. A mean initial central corneal clearance, measured at approximately 20 minutes post-lens application at the lens delivery study visit, was 273.77±91.44μm and 262.07±74.20μm (p=0.671) for the study lenses with low and high LC, respectively. These can be compared to a CCC of 196.95±75.84μm and 216.90±101.55μm (p=0.475) for fittings with low and high LC measured at the follow-up study visit, after a minimum of two hours wear time. There was a significant change in CCC, as would be expected, due to lens settling between baseline to the final visit for both the low and high LC lenses (p<0.001, both). Finally, the mean sagittal depths for study lenses with low and high LC were 4.539±0.240mm and 4.550±0.243mm (p=0.877).

Table 3-2: Lens parameters and Visual acuities for all study lenses with low LC.

PATIENT ID	Sagittal Depth	Base Curve	Limbal Clearance	Periphery	CL power	VA
1	4.50mm	8.2mm	Std	Std	+2.75D	6/7.5
	4.35mm	8.2mm	Std	Standard H/ Steep 2 V	+1.5D	6/7.5
2	4.5mm	7.6mm	-50	Std H/ Steep #5 V	+0.50D	6/6
	4.50mm	7.6mm	-50	Std H/ Steep #5V	+1.25D+	6/7.5
3	5.00mm	6.7mm	-50	Std H/ Steep #3 V	-11.75D	6/7.5
	4.90mm	7.1mm	-50	Std H/ Steep #5V	-9.00D	6/7.5
4	4.80mm	8.0mm	-125	Std	-1.25D	6/6
	5.15mm	7.10mm	-125	Flat #3 H/ Std V	-7.62D	6/7.5
5	4.30mm	8.20mm	Standard	Steep #1 H/ Standard V	-1.75D	6/6
	4.30mm	8.20mm	Standard	Steep #1	-1.00D	6/6
6	4.50mm	7.60mm	Standard	Standard	-4.75D	6/7.5
	4.50mm	8.40mm	Standard	Standard	-1.00D	6/7.5
7	4.95mm	7.80mm	+25	Standard	-2.00D	6/6
	5.10mm	7.30mm	Standard	Standard	-6.00D	6/6
8	4.40mm	8.20mm	-50	Standard H/ Steep #2 V	+2.00D	6/7.5
	4.40mm	8.20mm	-50	Standard H/ Steep #3 V	+3.00D	6/7.5
9	4.40mm	7.60mm	Standard	Standard H/ Flat #3 V	-5.50D	6/7.5
	4.55mm	7.10mm	Standard	Standard H/ Flat #2 V	-9.25D	6/6
10	4.35mm	8.20mm	-50	Steep #1	-1.75D	6/7.5
	4.30mm	8.20mm	-50	Flat #2 H/ Steep #2 V	-2.25D	6/6
11	4.45mm	7.60mm	-50	Steep #1	-0.75D	6/6
	4.45mm	7.60mm	-50	Steep #2 H/ Standard V	+0.50D	6/7.5

Table 3-3: Lens parameters and Visual acuities for all study lenses with high LC.

PATIENT ID	Sagittal Depth	Base Curve	Limbal Clearance	Periphery	CL power	VA
1	4.50mm	8.2mm	+50	Std	+2.50	6/7.5
	4.40mm	8.2mm	+50	Std H/ Flat #3 V	+1.25	6/7.5
2	4.50mm	7.6mm	Std	Std H/ Steep #7 V	+0.75D	6/7.5
	4.50mm	7.6mm	Std	Steep #1 H/ Steep #6V	+2.00D	6/7.5
3	5.0mm	6.7mm	Std	Std H/ Steep #3 V	-11.75D	6/7.5
	4.90mm	7.1mm	Std	Std H/ Steep #5V	-9.00D	6/7.5
4	4.80mm	8.0mm	-75	Standard	-1.25D	6/6
	5.20mm	7.10mm	-75	Flat #3 H/ Std V	-7.62D	6/7.5
5	4.30mm	8.20mm	+50	Steep #1 H/ Standard V	-1.50D	6/6
	4.30mm	8.20mm	+50	Steep #1	-0.75D	6/6
6	4.55mm	7.60mm	+50	Standard	-4.75D	6/7.5
	4.55mm	8.40mm	+50	Standard	-1.00D	6/7.5
7	4.95mm	7.80mm	+75	Flat #2	-2.00D	6/6
	5.10mm	7.30mm	+50	Flat #2	-6.00D	6/6
8	4.40mm	8.20mm	Standard	Standard H/ Steep #2 V	+2.50D	6/7.5
	4.40mm	8.20mm	Standard	Standard H/ Steep #3 V	+3.00D	6/7.5
9	4.40mm	7.60mm	+50	Standard H/ Flat #3 V	-4.25D	6/7.5
	4.55mm	7.10mm	+50	Standard H/ Flat #2 V	-9.25D	6/6
10	4.35mm	8.20mm	Standard	Steep #1	-1.75D	6/7.5
	4.30mm	8.20mm	Standard	Flat #2 H/ Steep #2 V	-2.25D	6/6
11	4.50mm	7.60mm	Standard	Steep #1	plano	6/7.5
	4.55mm	7.60mm	Standard	Steep #2 H/ Standard V	+1.00D	6/9

The initial limbal clearance was measured, before lens settling at the delivery appointment, to be 159.93±68.32µm for low LC lenses and 186.83±58.08µm for high LC lenses with the Visante™ OCT (p<0.001). This is compared to the final limbal clearance measured after minimum of 2 hours of wear time (i.e. after lens settling) at the follow-up appointment, which were 123.74±62.42µm for low LC lenses and 167.31±57.31µm for high LC lenses (p=0.006). There was also a significant change in low and high LC, as would be expected, due to lens settling comparing baseline to the final visit (p<0.001, both). The limbal clearances in nasal and temporal quadrants are reported in Table 3-3 and Figures 3-6 and 3-7. Across all 22 lens fittings with low and high LCs, there is a significant difference for the LC between the temporal and nasal quadrants by +78.09±67.98µm (low LC) and +82.33±61.96µm (high LC) (greater in the temporal quadrants) (p<0.001) at the initial fitting. This difference was still found at the 2 week visit but had reduced to +34.67±55.41µm (low LC) and +36.94±50.60µm (high LC) (greater in the temporal quadrants) (p<0.001). Furthermore, Figures 3-7A and 3-7B illustrates how the study lenses with low and high LC designs fit on one study participant. Note that the CCC remained constant.

Table 3-4: Quadrant specific limbal clearance for all study lenses.

	Low LC		High LC	
	Nasal	Temporal	Nasal	Temporal
Initial LC	120.89 ± 37.87	198.98 ± 70.21	145.67 ± 51.12	252.43 ± 104.71
Final LC	106.40 ± 35.09	141.08 ± 83.01	148.83 ± 50.59	185.78 ± 69.75
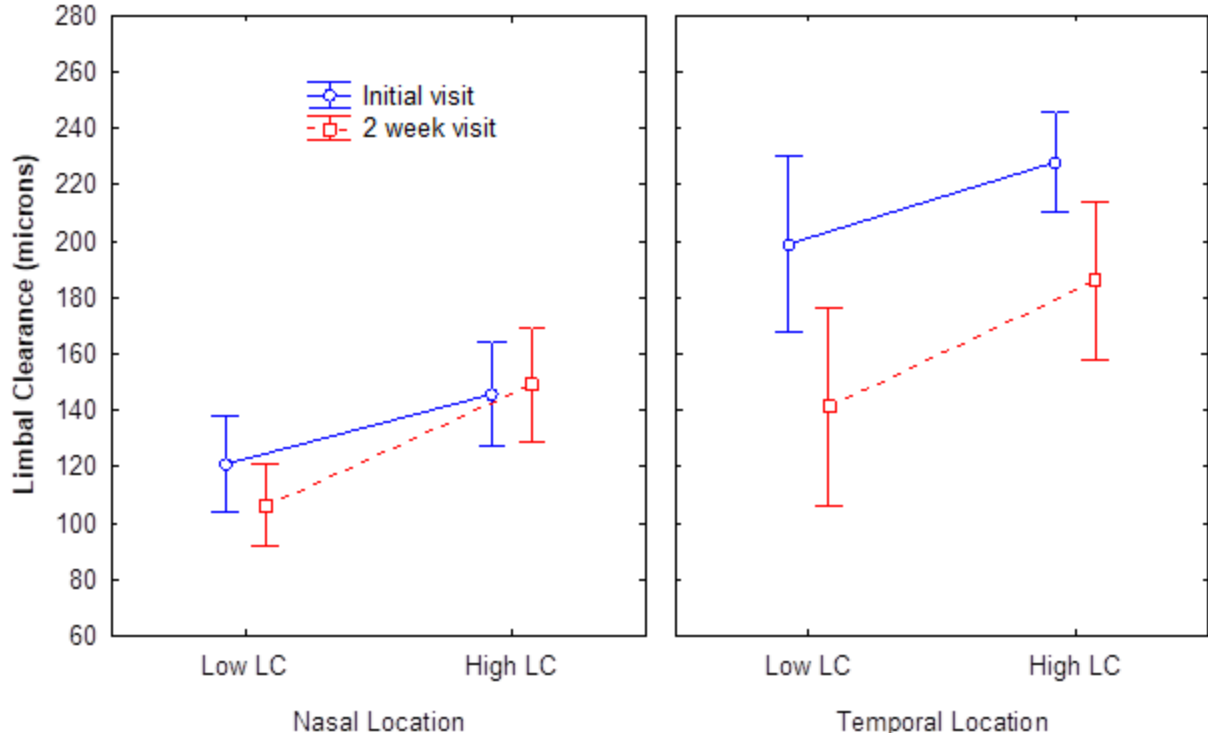
Figure 3-6: Comparison of Nasal and Temporal Limbal Clearance at lens delivery and follow-up visit, that is, before and after lens settling.
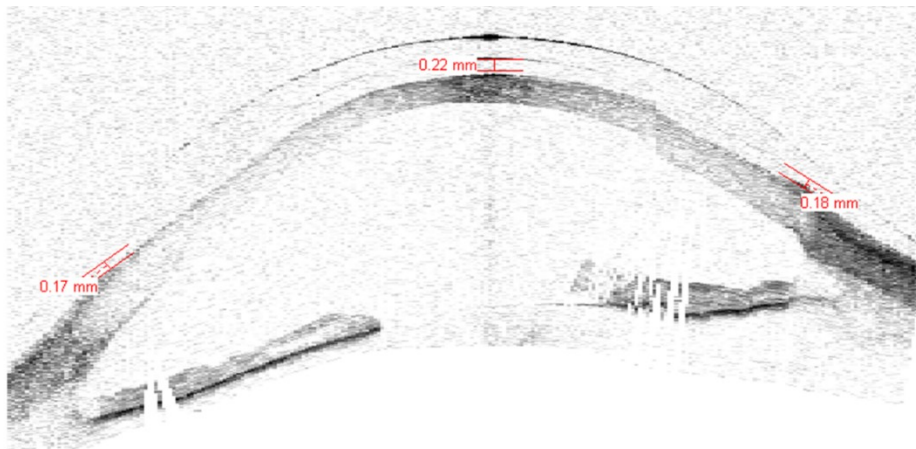
Figure 3-7: Visante™ Anterior segment OCT of Scleral Lens with low LC
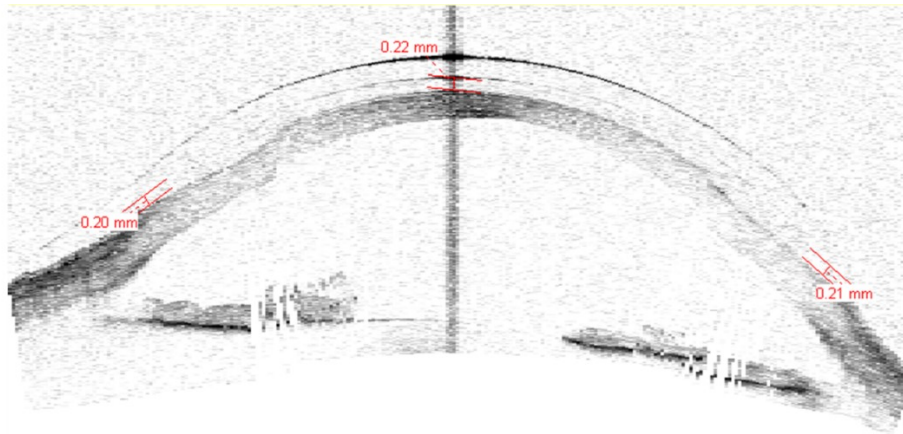
Figure 3-8: Visante™ Anterior segment OCT of Scleral Lens with high LC

3.5 Discussion

Of the fourteen participants enrolled in the study, eleven participants were successfully fitted bilaterally with scleral lenses. Three individuals discontinued citing issues with difficulty with insertion or removal and poor adaptation as the primary reasons. Other studies have also reported handling and lens care, as well as irritation from lens awareness as common reasons for unsuccessful scleral lens fits.[124,133,152]

For the twenty-two eyes that completed the study, a median visual acuity of greater than 6/9 were achieved with all the study lenses. The visual outcome is comparable to those Segal et al.[121], Schornack and Patel[122], and Severinsky and Millodot[131] reported with 91%, 87%, and 84%, respectively, of scleral lens wearers achieving 6/12 or better visual acuities. It was noted that the visual acuities could theoretically be further improved by incorporating a front surface toric contact lens power to the fitted scleral lens to correct any residual cylinder. This modification was eliminated from the fitting protocol in this thesis project as vision was not one of the primary outcomes of this

study and the change in lens thickness would vary according to the amount of cylinder resulting in a non-constant center thickness.

In this study, the overall diameter used across all study lenses was 16.0mm. This falls within the range of lens diameter of 15.0 to 17.0mm that was shown to be preferred by the majority of scleral lens practitioners.[163,180] The sagittal height in the twenty-two eyes in this study is similar to that reported by Sorbara et al.[178] and Otchere et al.[177] Furthermore, the mean sagittal depth of all study lenses exceeded the sum of mean initial CCC and mean sagittal height. This is likely due to the sagittal height differential at a 15.0 mm chord diameter being under-estimated compared to a 16.0 mm lens diameter.

Central corneal clearance is a primary focus when assessing scleral lens fits. Clinically, the anterior segment OCT is a preferred method to observe the central corneal-lens fitting relationship since observation with the slit lamp can grossly over-estimate the central corneal clearance due to the oblique orientation of the slit lamp.[182] In this study, mean central corneal clearance measured with the OCT at the follow-up visit was 194.00 ± 67.54 μm. This is comparable to the results from a 2016 survey where 89% of scleral lens practitioners surveyed indicated the ideal central corneal clearance after lens settling should be between 150 to 250 μm.[163] Schornack et al.[175] also indicated that the ideal central corneal clearance should range between 125 and 250 μm. The mean change in central corneal clearance measured at lens delivery appointment and that at the follow-up visit of an average of 64.56 ± 155.17μm can be attributed to lens settling. This is consistent with a mean lens settling of 146 μm over a span of 1 month of lens wear that Mountford[183] reported.

The amount of limbal clearance that was found cannot be easily compared to standard values as this measurement is rarely reported on. It is noted that there is a trend for the temporal limbal clearance to be greater than that in the nasal quadrant. This is likely due to the decentration of the scleral lens, typically in the infero-temporal direction. Visser et al.[130] report on ensuring adequate limbal clearance but did not give a number to aim for. The role limbal clearance plays in the overall clinical performance of the scleral lens fit will be further analyzed and discussed in the subsequent chapters.

In the scleral landing zone designs, 26 of the 44 study lenses (59.1%) used had a toric back surface landing zone to achieve scleral alignment. Visser et al.[130] was the first group to start using toric back surface scleral landing zones to achieve better fit and comfort for wearers. Toric and quadrant specific modifications allows a more even weight distribution circumferentially.[173] Scleral alignment is key in stability of the lens as it can allow for improved rotational stabilization[173] and to mitigate other complications such as midday fogging.[126,184]

Consistent with the community of contact lens practitioners[121-123,133,180], this study found great overall success fitting individuals with KC with scleral lenses using the lens sagittal depth as the primary fitting parameter. Using an anterior segment OCT, an appropriate sagittal depth was determined based on the sagittal height of the individual eye and an aim of central corneal clearance of 250-300μm.[161,178] The instrument further allows for detailed and accurate monitoring of fitting characteristics, such as post-lens tear film thickness at central and limbal zone of the

scleral lens.[182] Overall, this chapter summarized a systematic scleral lens fitting protocol that was straightforward and accurate.

3.6 Conclusion

The use of an OCT such as the Visante™ has again proven to give a good estimate of the central sagittal depth of the scleral lens that results in an adequate amount of central clearance. A final limbal clearance of 123.74μm (low LC) to 167.31μm (high LC) was achieved after lens settling with scleral lens sets designed with a 50μm difference in the LC parameter.

Chapter 4

Objective and Subjective Evaluation of Clinical Performance of Scleral Lenses with Varying Limbal Clearance in KC

4.1 Overview

Purpose: To investigate the clinical performance, ocular response and subjective responses to the wear of scleral lenses having varying limbal clearance.

Methods: Lenses with varying limbal clearances, one with low limbal clearance (LC) and one with high LC were fitted on a group of keratoconic participants. The lenses were worn over a two-week period and the ocular response to lens wear was examined. Visual analog scales were completed concerning vision, comfort, burning and dryness. Corneal and conjunctival responses to lens wear such as were observed and compared. These included: limbal and bulbar hyperemia, as seen on the Oculus Keratograph 5®; corneal swelling, based on pachymetric values measured by the Oculus Pentacam HR®; and corneal staining, as seen with biomicroscopy.

Results: Compared to baseline both low and high LC lenses resulted in improved subjective responses (p=0.07 low LC, p<0.01 high LC for overall comfort). Participants reported greater comfort with scleral lenses with high LC (p=0.013 for comfort, p<0.01 for dryness, p=0.08 for burning). There was a slight decrease in limbal and bulbar hyperemia with low LC lenses compared to baseline but no difference between high and low LC lenses was found (p=0.733). Corneal swelling was noted in all corneal locations and especially at the 6mm zone where there were significant differences for both low and high LC lenses compared to baseline (p=0.004, and p=0.039, respectively). Quadrant specific analysis indicated that

peripheral corneal thickness in four quadrants, at both 6 and 8 mm, increased with scleral lens wear with low LC (all p<0.05) and only the temporal region was significantly increased for the high LC lenses (p=0.018). Corneal response to scleral lens wears with either low or high LC appears to result in similar peri-limbal staining and negative corneal staining.

Conclusions: Limbal clearance may play an important role in subjective performance in scleral lenses, but does not impact the degree of hyperemia in either the limbal and bulbar regions measured objectively. A significant increase in peripheral corneal thickness was found with low LC lens wear likely due to mechanical irritation. The increase in corneal thickness with scleral lens wear of approximately 4% is consistent with the literature.

4.2 Introduction

The limbal zone of a scleral lens connects the central corneal/back optic zone of the lens to the peripheral scleral landing zone. In an ideal scleral lens fit, the limbal zone should clear, or vault, over the peripheral cornea and limbus. The limbal area plays an important role in maintaining healthy corneal physiology. Firstly, it houses the limbal vasculature, which supplies oxygen and nutrients to the avascular cornea. It contains the minor arterial circle of the iris. Insults to the cornea and ocular surface can result in inflammatory responses through the limbal vasculature.[144] Secondly, limbal stem cells, necessary for corneal epithelial regeneration also reside in the limbal area.[145,146] Located in the palisades of Vogt, limbal stem cells have a high proliferative potential (least differentiated) which is necessary for corneal cell proliferation, turnover, and wound healing ability in the central corneal tissue.[146,185] This population of cells also plays a role in preventing conjunctivalization of the cornea.[186]

Limbal clearance (LC) refers to the amount of clearance or the thickness of the post-lens tear film between the lens and the ocular surface over the limbus. In Chapter 2, we reviewed a case series of scleral lens wearers exhibiting adverse corneal findings and conjunctival injection despite having an ideal central lens-cornea fitting relationship.[119,126,162] As such, it can be argued that limbal clearance plays an important role in the clinical performance of a scleral lens fit.

Similar to central lens-cornea fitting relationship, excessive or inadequate clearance in the limbal zone can result in a negative impact on the ocular health. In cases of excessive clearance, it is hypothesized that the post-lens tear film reduces the

amount of oxygen delivery to the cornea, resulting in peripheral corneal edema. Inadequate clearance over the limbal area will result in the lens mechanically rubbing on the ocular surface, which can present as superficial punctate keratopathy or negative staining. Most fitting guides recommend an initial peripheral corneal/ limbal clearance of 50 – 100 microns. However, there are inconsistencies in the recommended limbal clearance depending on lens design and overall lens diameters and how much of a change in LC should be make once it is determined that it is either excessive or insufficient. In a 2016 survey, 93 scleral lens fitters reported an ideal limbal clearance after lens settling ranging from less than 25 microns (6%) to somewhere between 50 and 75 microns (82%) to over 80 microns (12%).[163] While there are numerous reports on the effect of the lens settling on central corneal clearance[120,138–140], little has been reported on that of limbal clearance.

The clinical performance of a contact lens fit is determined by a summation of comfort, vision, and physiological response. Comfort is generally assessed subjectively from the patient's standpoint, with improvement in quality of life as a key factor in the subjective satisfaction.[117,133,187] On the other hand, visual satisfaction can be determined both subjectively and objectively. Data on visual acuity has been reported in Chapter 3. Finally, an appropriate corneal-scleral lens fitting relationship is necessary to ensure that scleral lens wear does not negatively impact the health of the ocular surface. In this chapter, the impact of limbal clearance on the clinical performance of scleral lens fit will be investigated, based on comfort, vision, hyperemia, mechanical and hypoxia-related complications.

4.3 Methods and Materials

4.3.1 Participants

This study was a continuation of the study presented in Chapter 3. As with Chapter 3, this study was approved by the Office of Research Ethics of University of Waterloo (ORE#21364) and performed under the Tenets of Helsinki. Informed consent was obtained from the same participants as in Chapter 3.

4.3.1.1 Inclusion and Exclusion Criteria

The same inclusion and exclusion criteria were followed in Chapter 3, Section 3.3.1.1.

4.3.2 Study Materials

4.3.2.1 Study Lenses

The study lenses were the same as described in Chapter 3. The final lens parameters for the pair of lenses with low and high LC are listed in Tables 3-2A and 3-2B and the lenses were worn for 2±1weeks.

4.3.2.2 Corneal Tomography

Description of the Oculus Pentacam HR® (Wetzlar, Germany) can be found in Chapter 3, Section 3.3.2.3. This instrument was used to measure pachymetric changes (CT) in the cornea.

4.3.2.3 Corneal Topography

The Oculus Keratograph® 5 is a non-contact, placido-ring based corneal topographer.24 (Figure 4-1) The placido-disc or concentric ring pattern is projected onto the tear film anterior to the cornea. Keratometric measurements of the anterior

cornea can be determined by Keratograph® software based on the distance between the concentric ring. In addition, it is equipped with an on-board camera that can capture both video and still images.

Figure 4-1: The Oculus Keratograph 5® Corneal Topographer.

By instilling sodium fluorescein, the tear film can be highlighted and imaged. This feature is helpful in the photo-documentation in dry eye assessment and contact lens fitting. This instrument was used to photograph any corneal staining responses to the scleral lenses.

In addition, the Oculus Keratograph 5® has a built-in *R-Scan* function (Figure 4-2). The feature determine the bulbar and limbal redness based on the analysis of surface area occupied by sclera-to-blood vessel and sclera-to-thin blood vessel ratios, respectively.[188] Limbal and bulbar injection is graded in accordance the validated JENVIS classification (Figure 4-3) The R-Scan was preferred over subjective grading scales as the latter method are tiered with uneven spacing between each level.[189]

Bulbar and Limbal Redness
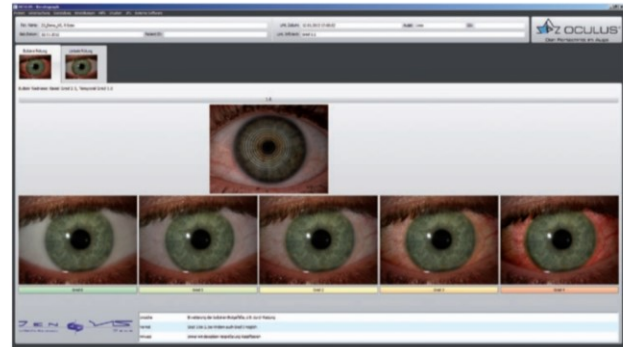
JENVIS Grading Scale

Figure 4-2: The R-Scan Function by the The Oculus Keratograph 5® Corneal Topographer.
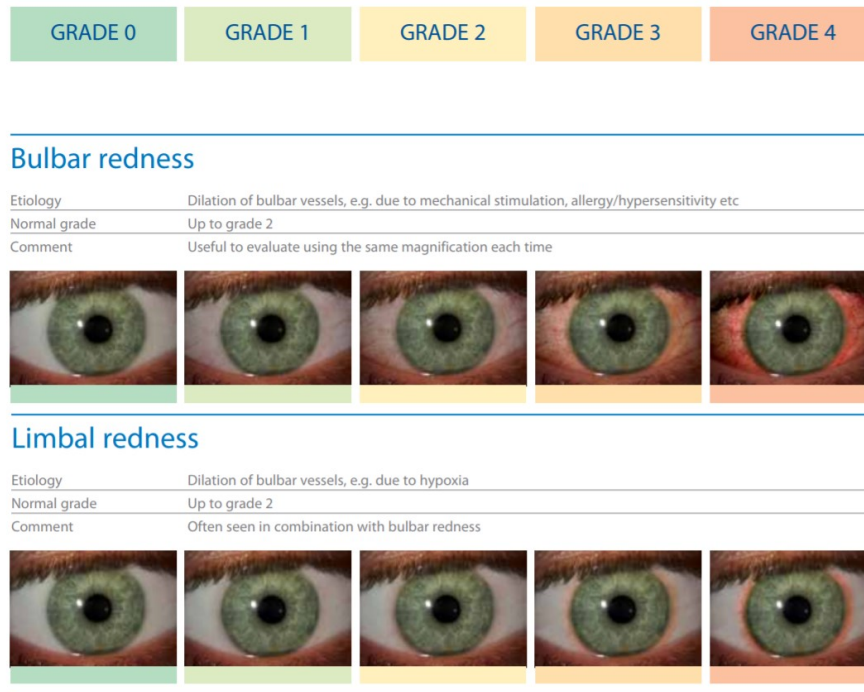
Figure 4-3: The JENVIS Classification for bulbar and limbal redness.

4.3.3 Study Procedure

As a continuation of the study from Chapter 3, the schedule and protocol for study visits was the same in that described in Section 3.3.3. Each participant was fitted with the two scleral lens designs bilaterally with high and low limbal clearance differing

by 50μm. A wash-out period of a minimum of 72 hours was required between wearing the first and the second set of study lenses. The order of which lenses were worn was randomized and double masked to the participants and researcher. At each lens delivery and follow-up appointment, comfort, vision, hyperemia, mechanical and hypoxic related complications were assessed subjectively and objectively (Figure 4-4).
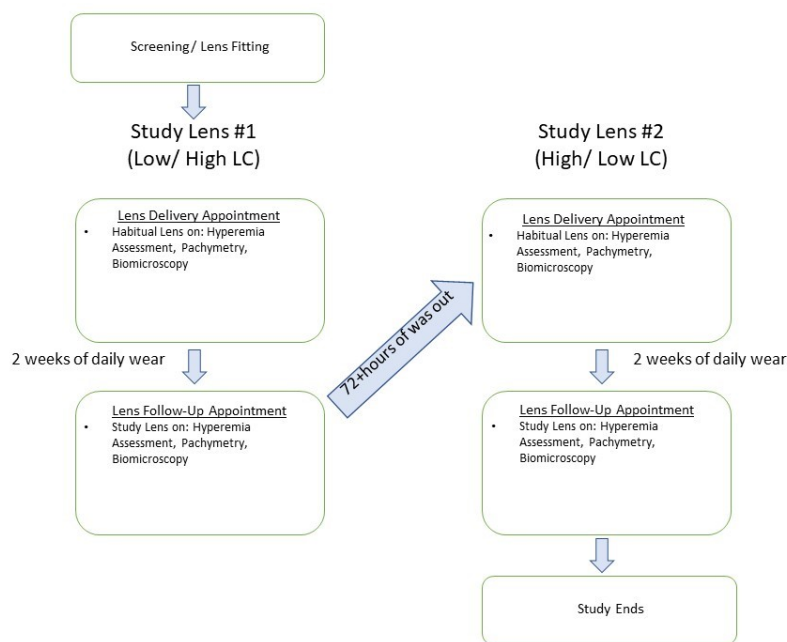
Figure 4-4: Flow chart of study visits.

4.3.3.1 Subjective Comfort

All participants completed a questionnaire on lens comfort, presence of dryness symptoms, and presence of burning sensation, and clarity of vision with their study lenses at each of the lens delivery and follow-up visits. Scores were obtained on a

visual analog scale (VAS); the scores ranged from 0 (unacceptable performance) to 100 (excellent performance). A copy of questionnaire is included in Appendix A.

4.3.3.2 Hyperemia

Biomicroscopy was performed at each lens delivery and follow-up appointment. The bulbar and limbal injection in each nasal and temporal quadrants were recorded according to the Validated Bulbar Redness (VBR) scales. The subjective assessments were compared against the Bulbar Redness (BR) and Limbal Redness (LR) scores generated by the Oculus Keratograph 5®, which generated a hyperemia grading score based on the analysis of surface area occupied by sclera-to-blood vessel and sclera-to-thin blood vessel ratios on a linear scale according to the JENVIS classification system.

4.3.3.3 Corneal Swelling

Biomicroscopy was performed at each lens delivery and follow-up appointment. The researcher noted and graded the presence of any corneal microcystic edema, stromal haze, and/or corneal striae. In addition, corneal pachymetry maps were obtained using Scheimpflug imaging (the Oculus Pentacam HR®), before and after each set of study lenses were worn. Pachymetric values at central and peripheral cornea (transverse section chords at 6mm and 8mm; that is, at the 3mm ring and 4mm ring from the central point) in four quadrants: superior, nasal, inferior and temporal, were compared and analyzed by lens design.

4.3.3.4 Corneal Staining

Biomicroscopy was performed at each lens delivery and follow-up appointment. The researcher noted the location and the severity of positive and/or negative fluorescein staining in the limbal area.

4.4 Results

As reported in Chapter 3, the mean initial LC for the nasal and temporal LC's were 159.93±68.32µm for low LC lenses and 186.83±58.08µm for high LC lenses (p<0.001). The mean final limbal clearance were 123.74±62.42µm for low LC lenses and 167.31±57.31µm for high LC lenses. LC was measured to be greater in high LC lenses than low LC lenses by 26.90±57.72µm initially, and 43.57±45.63µm after lens settling (p<0.001). Finally, the lens settling in the limbal zone was measured as the difference between the initial and final LC, which were noted to be 36.19±63.52µm for low LC lenses and 19.52±65.43µm for the high LC lenses (p<0.001). Table 3-4 reports the differences between the nasal and temporal LC's. Consistently, more LC found temporally compared to nasally (p<0.001).

4.4.1 Subjective Comfort

In this study, 81.8% (9 of 11) participants reported greater comfort with study lenses with either low or high LC than their habitual contact lenses. Lens awareness and poor adaptation were the main reasons the two participants cited less-than-ideal subjective performance with scleral lenses.

By lens design, the subjective reports for comfort, absence of dryness, absence of burning sensation and subjective vision are represented in Table 4-1 and Figures 4-5A to 4-5D. The median VAS outcomes for the lenses with low LC were: 80 for

comfort, 82.5 for dryness, 85 for burning and 84 for vision; while that with high LC were: 90 for comfort, 90 for dryness, 95 for burning and 89 for vision. Compared to the participants' habitual lenses (27.3% in corneal GPs, 50.0% in a piggyback system, 18.2% in other scleral lens designs, and 18.2% in soft silicone hydrogel toric lenses), there was statistically significant change in subjective assessment in comfort and vision for high LC, and dryness for both low and high LC (all p<0.05). Scleral lenses with higher LC was generally graded with higher scores compared to low LC for subjective assessment (77.8% of 22 eyes for comfort, 44.4% for no dryness, 66.7% for no burning and 59.1% for vision) (p<0.01, p=0.181, p=0.232, p=0.028).

Table 4-1: Mean and range of subjective response reported with habitual optical correction (Baseline), and with study scleral lenses with low and high LC.

	Comfort		*Dryness*		*Burning*		*Vision*	
Baseline	80 (55-98)		61.25 (41.7-80)		90 (50-100)		83 (65-90)	
≥2 weeks After Low LC	80 (55-95)	p=0.491	82.5 (50-100)	**p<0.01**	85 (65-100)	p=0.439	90 (60-100)	p=0.29
≥2 weeks After High LC	90 (70-100)	**p=0.013**	90 (60-100)	**p<0.01**	95 (70-100)	p=0.08	92.5 (70-100)	**p=0.026**
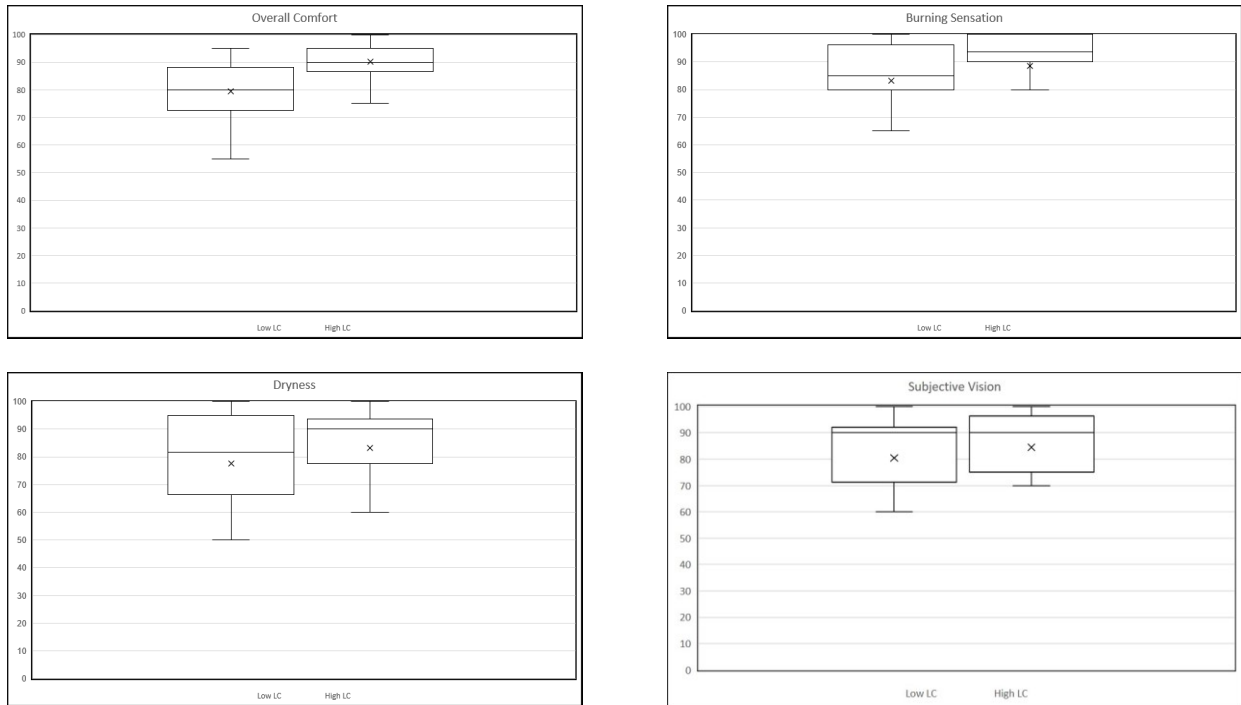
Figure 4-5A-D: Subjective overall comfort (A, top left), dryness sensation (B, bottom left), burning sensation (C, top right), and subjective vision (D, bottom left) reported with scleral lenses fitted for low and high LC.

4.4.2 Hyperemia

Results from conjunctival hyperemia are represented in Table 4-2 and Figures 4-6A and 4-6B according to JENVIS scale. Although there was an increase in nasal redness with the high LC lenses, it was not significant compared to baseline and to the low LC lenses (p>0.05, both). There was a significant statistical decrease comparing baseline to the temporal limbal redness (p=0.012) and bulbar redness (p=0.009) with the low LC. Overall, there was no statistically significant difference in hyperemia in the limbal and bulbar regions observed subsequent to scleral lens wear, comparing low and high LC. There was a significant difference comparing nasal and temporal

bulbar redness for both the low and high LC lenses (p=0.013 and p=0.004, respectively) where there was more nasal redness.

Table 4-2: Mean and range of hyperemia with habitual optical correction (Baseline), and with study scleral lenses with low and high LC.

	Limbal Nasal		Limbal Temporal		Bulbar Nasal		Bulbar Temporal	
Baseline Median (Range)	0.93±0.37 (0.17-2.00)		1.03±0.39 (0.36-1.90)		1.51±0.39 (0.91-2.43)		1.50±0.48 (0.75-2.93)	
≥2 weeks After Low LC	0.92±0.62 (0.17-2.57)	p=0.91	0.77±0.34 (0.20-1.30)	**p=0.012**	1.60±0.52 (0.77-2.63)	p=0.51	1.30±0.34 (0.73-2.17)	**p=0.009**
≥2 weeks After High LC	1.03±0.83 (0.13-3.2)	p=0.57	0.95±0.52 (0.13-2.33)	p=0.49	1.63±0.57 (0.60-2.83)	p=0.38	1.40±0.46 (0.70-2.63)	p=0.33
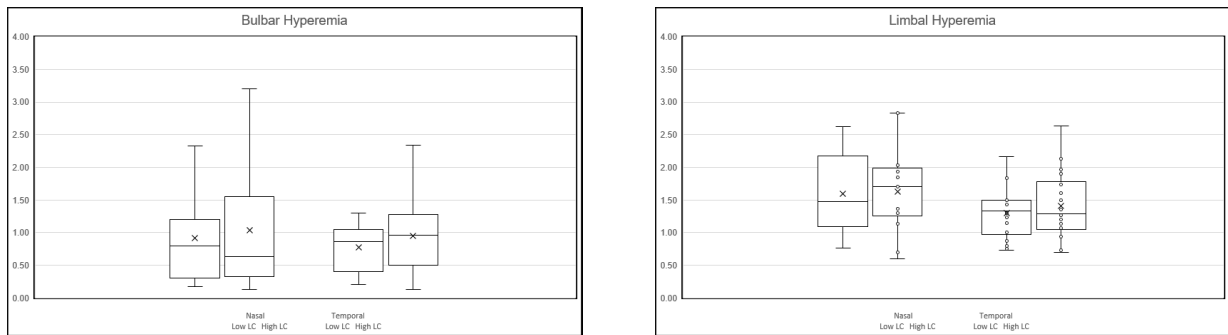
Figure 4-6A and B: Bulbar hyperemia (A, left) and limbal hyperemia (B, right) in nasal and temporal quadrant associated with scleral lens wear with low and high LC.

4.4.3 Corneal Swelling

Examination of the eyes with biomicroscopy revealed no striae and no stromal haze at any visit for all twenty-two eyes.

Pachymetric results are reported in Table 4-3. Comparison of corneal thickness from baseline to post-low and high LC lens wear showed an increase, which was not statistically significant centrally (p>0.10), for low LC, but significantly different centrally for the high LC lenses (p=0.002). There were significant differences at the 6mm chord across the cornea for both the low and high LC lenses (p=0.004 and p=0.039, respectively) compared to baseline. After ≥2 weeks of lens wear, CT increased centrally by +3.97±3.29% and +4.09±3.78% for low and high LC, respectively (p=0.0004). CT increased by +4.92±3.17% at 6mm and +4.87±3.23% at 8mm for low LC (p=0.004 and p>0.05, respectively); and +3.05±4.78% at 6mm and +3.23±4.86% at 8mm for high LC (p=0.039 and p>0.05, respectively), when all meridians were averaged together. Comparing the 6mm and 8mm changes for low and high LC there was no significant differences at either the 6 or the 8mm chord (both p>0.05) when all quadrants were averaged together.

Table 4-3: Mean and range of corneal pachymetry in central and peripheral cornea with habitual optical correction (Baseline), and with study scleral lenses with low and high LC.

	Centre		*6mm chord*		*8mm chord*	
Baseline	458.9±42.0μm (382-554μm)		571.8±46.5μm (406-661μm)		635.8±51.3μm (502-749μm)	
≥2 weeks After Low LC	469.7±37.7μm (400-540μm)	p>0.10	590.3±42.7μm (490-670μm)	**p=0.004**	650.7±56.7μm (530-843μm)	p=0.065
≥2 weeks After High LC	485.6±32.7μm (434-557μm)	**p=0.002**	578.9±51.3μm (465-660μm)	**p=0.039**	651.9±54.7μm (531-816μm)	p=0.060

Quadrant specific analysis of peripheral corneal pachymetry values can be seen in Figure 4-7. The peripheral corneal thickness is also represented as a percent change compared to baseline, which is illustrated in Figure 4-8A for low LC and Figure 4-8B for high LC. A significant increase in thickness was noted in CT in all quadrants at both the 6mm and 8mm for low LC from baseline (all $p<0.05$). For the high LC, a significant change was found only in the temporal quadrant at 8mm chord ($p=0.0179$), where a non-significant decrease, compared to the low LC lens, was also found ($p=0.469$). Comparing peripheral corneal thickness associated with low and high LC, only the superior quadrant demonstrated a significant ($p=0.042$ at 6mm and $p=0.0003$ at 8mm), and an overall trend of higher pachymetry values was noted with high LC.
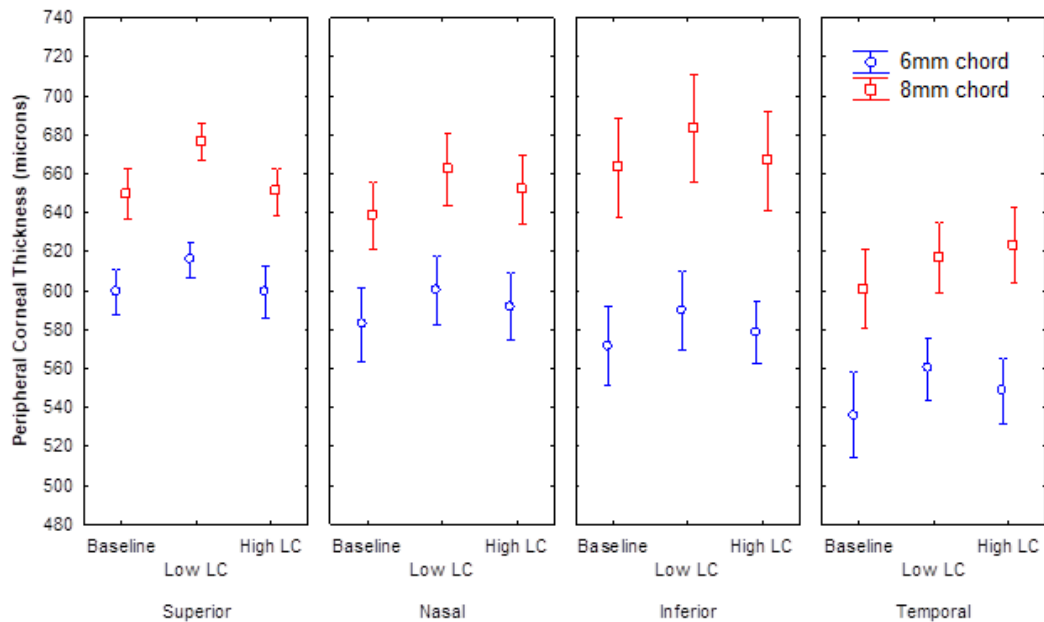
Figure 4-7: Peripheral Corneal Thickness (microns) in each quadrant associated with habitual optical correction (Baseline), and with study scleral lenses with low and high LC.

65
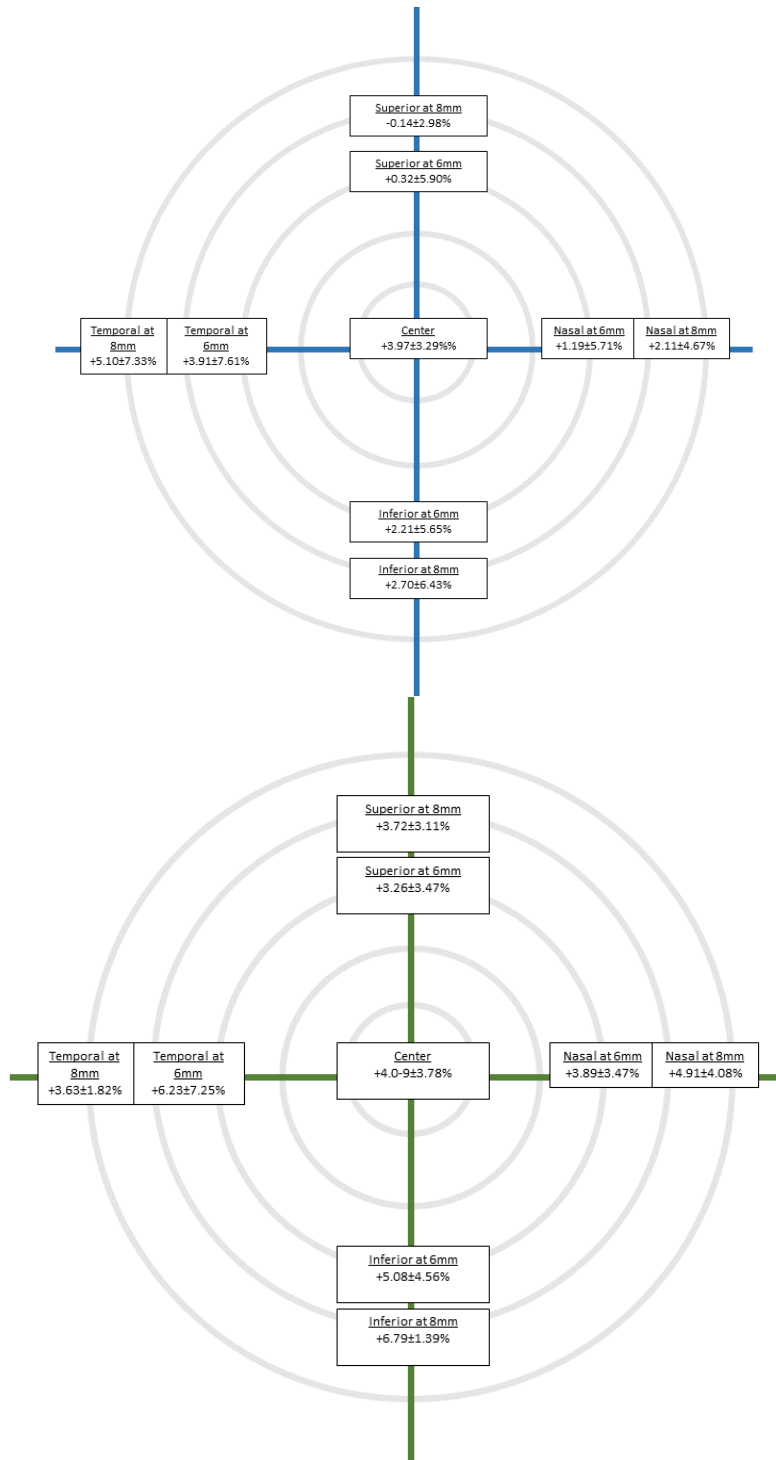
Figure 4-8A and B: Percentage change in corneal thickness after 2 weeks wear of scleral lens with low LC (A, blue, top) and high LC (B, green, bottom).

4.4.4 Corneal Staining

In biomicroscopic exams, 4 (18.2%) cases of limbal staining were noted after two weeks of wearing lenses with low LC, 3 of which were graded as trace (0.5) on a 0 to 4 scale and 1 case was graded 1. In high LC cases, 5 (22.7%) eyes were noted with limbal staining, all were graded 1 or less. Negative staining or limbal imprint was noted in 5 (22.7%) cases after wearing low LC, and 6 (27.3%) cases after wearing high LC. All cases of negative staining was graded 1 or less. No statistical significance was found between severity of positive and negative staining in low and high LC cases (p=0.351 and p=0.841, respectively). Examples of limbal staining and negative staining are shown in Figures 4-9 and 4-10.
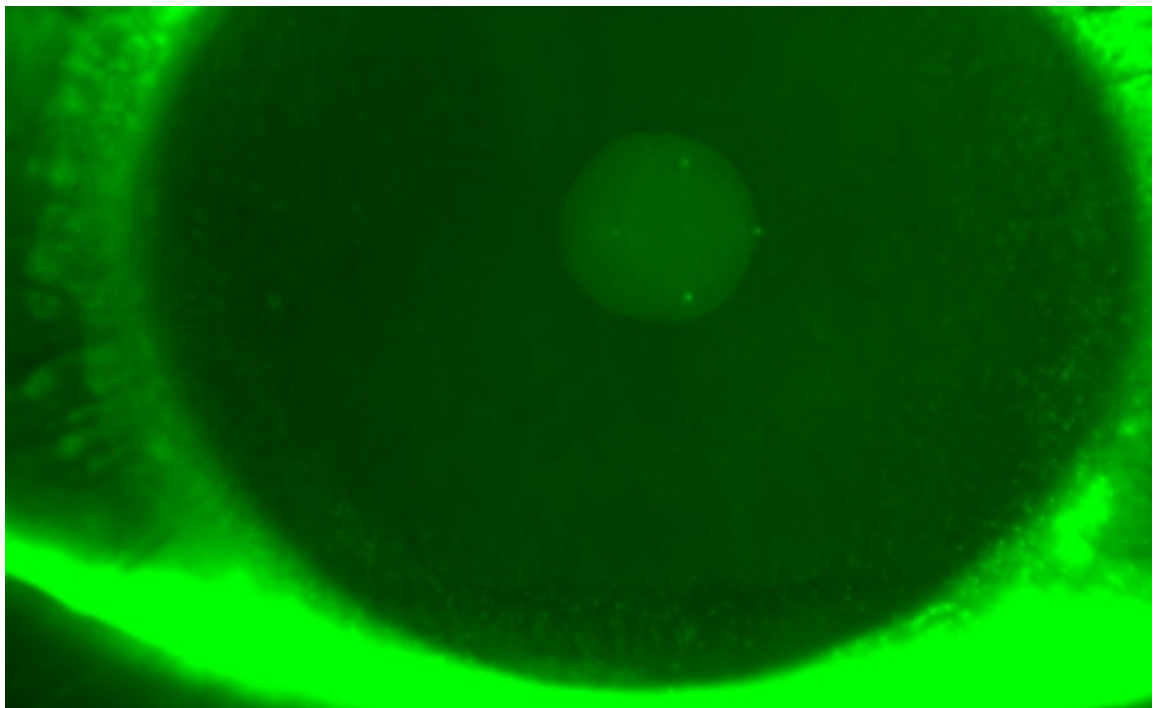
Figure 4-9: Fluorescein imaging of positive limbal staining. Fluorescein pools at the center of the superficial epithelial defects, highlight the entire surface area.
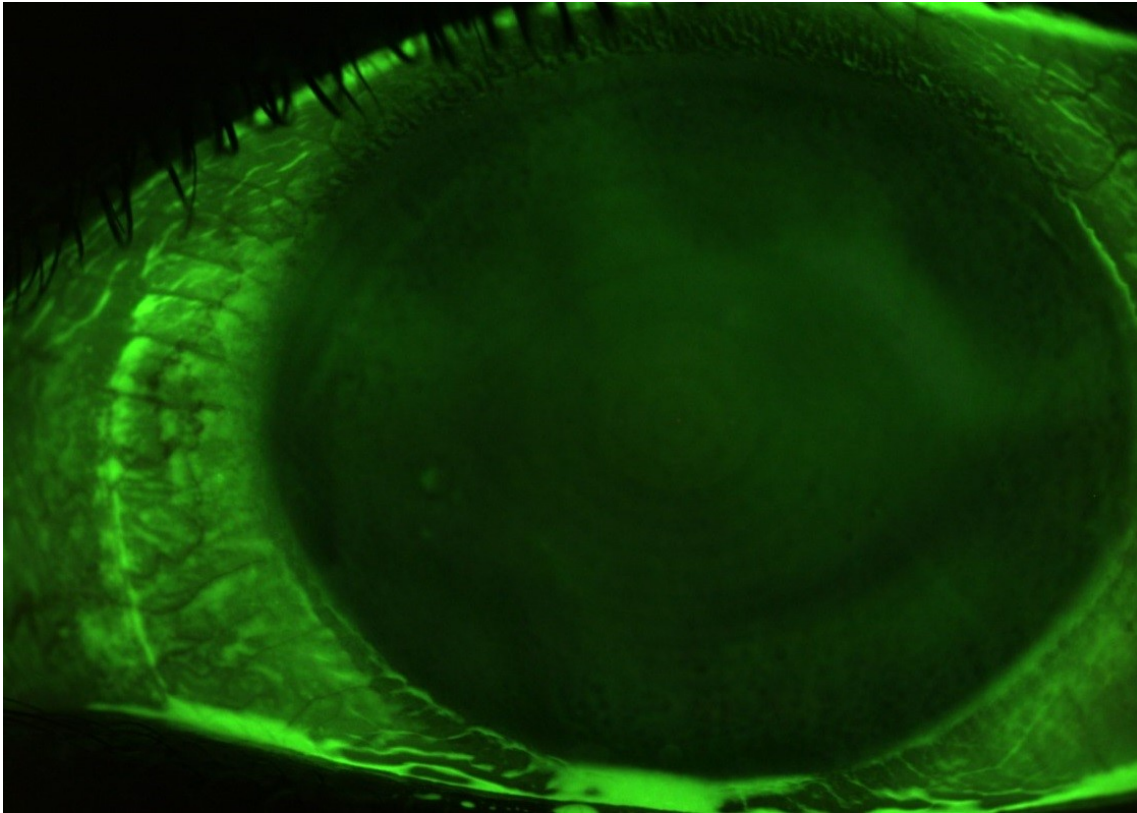
Figure 4-10: Fluorescein imaging of negative limbal staining. Fluorescein pools at the edge of epithelial defects, highlight the border of the lesion.

4.5 Discussion

The clinical performance of scleral lenses with low and high limbal clearance was examined in this chapter. The ZenLens™ semi-scleral lenses was chosen for the purpose of this study because it allowed for adjustment in the limbal zone independently from the sagittal depth in the optical zone. The two sets of study lenses were designed to differ in limbal clearance by 50μm. The final difference in limbal clearance between low and high LC was 43.6 microns close to the intended difference of 50 μm. Similar to the central optic zone fitting parameters, adjustments in sagittal depth may not always be reflected in changes in central corneal clearance;

as this study found that there was a difference in lens settling in the limbal zone between low and high LC. This reinforces the importance for eye care practitioners to re-examine the scleral lens fits at progress check visits after lens settling.

Similar to other reports[117,133], scleral lenses with both low and high LC in this study yielded a higher subjective performance score for most questions asked. Only the high LC lenses were significantly rated better than baseline for comfort, dryness and vision. The low LC was significantly less dry than baseline. One of the reasons scleral lenses are considered more comfortable compared to corneal GPs is the overall diameter of scleral lenses ranges between 14.3 to 18.2mm, which place the lens edge outside the limbus. As a result, it is expected to reduce lens awareness by minimizing lid interaction[122]. In addition, scleral lenses can achieve greater lens stability, therefore, greater comfort, compared to other modes of contact lenses.[122] This is made possible by the alignment of the lens over the regular scleral topography allowing for greater centration; as well as the hydrostatic forces that minimize lens movement on blinking.[120] Visser et al. indicated that a daily wear time of greater than six to eight hours is a good indication of high subjective performance of the scleral lenses.[133] In this study, 81.8% (9 of 11) participants reported great comfort with both study lens design based on a full-time wear schedule of daily wear time exceeding six hours per day. For the two participants who reported less than ideal subjective performance due to lens awareness and poor adaptation, their habitual mode of vision correction was soft toric silicone hydrogel lenses. Furthermore, these two participants exhibited only mild keratoconic signs. Wu et al recommended that contact lens fitters must take into consideration the severity of KC when prescribing

appropriate contact lens modality.[190] In cases where patient is able to achieve adequate functional vision with habitual soft toric silicone hydrogel and/or spectacles, they may not find scleral lenses with either low or high LC to be significantly more comfortable. Comparing low and high LC, scleral lens fits with higher LC were reported to provide greater comfort for keratoconic scleral lens wearers. This may be due to the association between low LC and tight lens syndrome, and a greater negative pressure in post-lens tear film, which can result in photophobia, epiphora, or ocular irritation.[130,191]

Hyperemia in the limbal and bulbar conjunctival areas are persistently two of the most common complications associated with scleral lens wear.[119,126] Injection is indicative of the presence of distress to the ocular surface.[155] In the presence of a foreign object causing mechanical irritation, the limbal capillaries dilate to allow increased blood flow pulsing through arterioles of the limbal loop.[155] Bulbar and limbal hyperemia has also been reported in contact lens wearers using soft lenses with low oxygen transmissibility lenses.[192] In this study, scleral fittings with high LC were associated with greater degree of limbal and bulbar hyperemia. High LC creates a thicker tear meniscus over the limbal area, which may be associated with hypoxia or hypercapnia. Papas et al. have found a strong relationship between peripheral lens oxygen transmissibility and induced limbal redness, as graded using a decimalized scale.[193] When assessing scleral lenses, the eye care practitioner must pay close attention to any limbal or bulbar injection and identify the source. Diffuse injection might indicate hypersensitivity to a foreign object, while localized injection might

indicate specific mechanical irritation, such as an ill-fitting quadrant within the scleral landing zone.

Oxygen permeability of a contact lens plays a key role maintaining healthy corneal physiology. Corneal swelling and an increase in corneal thickness has been studied extensively as the primary measurement to indicate the presence of corneal hypoxia.[194] In other studies investigating short-term scleral lens wears, Pullum and Stapleton reported less than 3% of corneal thickness change in scleral lens wearers with normal cornea using material of Dk=115.[156] Compan et al. reported, in normal corneae, that there was corneal swelling of 1.6% and 3.9% for thinner and thicker fluid reservoirs, respectively, ranging from central clearance of 150-350 after 3 hours of wear time and using material of Dk=100.[139] Vincent et al has reported an average of less than 2% of corneal swelling after 8 hours of scleral lens wear fabricated with lens material of Dk=90 in young individuals with normal corneae.[157]

From theoretical and practical studies, it has been demonstrated consistently that key fitting goals with scleral lenses is to minimize lens thickness and the fluid reservoir.[138,140] To our knowledge, this is the first study to evaluate corneal swelling in keratoconic subjects and to investigate how limbal clearance impacts hypoxia with scleral lens wear. Consistent with other reports of scleral lens wear, the increase in central and peripheral corneal thickness with more than two weeks of scleral lens wear for both low and high LC was approximately 4%.[139,195] This compares to physiological range of corneal swelling of 4.5% to 5.5% that occurs overnight with the eyelid limiting oxygen delivery.[196,197] The greatest quadrant changes in both 6mm and 8mm chord diameters occurred in the inferior and temporal quadrants. This may be

associated with the typical inferior-temporal decentration of scleral lenses, resulting in an uneven post-lens tear film thickness circumferentially that is greater in the inferior-temporal area. There was a small difference, but a trend of greater increase in peripheral corneal thickness with high LC. The association between peripheral corneal swelling and low LC suggests an alternate etiology contributing to the corneal change other than corneal hypoxia. Finally, no corneal striae were noted in this study, which has been reported to occur with mean 6.89% of corneal edema in normal eyes.[198]

Inadequate clearance between the scleral lens and cornea may result in mechanical insult to the corneal epithelium, as has been shown with flat-fitting corneal GP.[141-143] This is particularly important as the thickness of the post-lens tear film reduces as the lens settles.[159] Limbal bearing might be observed as localized corneal staining or fluorescein pooling in areas of corneal epithelial defects.[199] It is important to distinguish corneal staining caused by localized inappropriate contact between lens and ocular surface from that of diffuse corneal staining, which might be associated with inappropriate use of solution to insert lenses.[180] The number of cases with persistent corneal staining post-scleral lens wear is similar to that reported in the SCOPE study, a multi-center, cross-sectional study observing scleral lens practice and associated physiological outcomes.[200] Mechanical irritation may also present as epithelial bogging, which has been described as raised lesions in the corneal epithelium with a waterlogged appearance.[119] Walker et al hypothesized that this phenomenon occurs with limbal bearing, resulting in the breakdown of the epithelium, and accumulation of non-vital epithelial cells that have accumulated

inappropriately due to the compression by the scleral lens.[119] Some of these findings have a similar appearance to that of limbal stem cell deficiency (LSCD) and recurrent corneal erosion where there is altered structural integrity (adhesion to basement membrane), reduced cell proliferation, and delayed cell turnover.[201]

In this study, corneal staining, and epithelial bogging or indentation were differentiated and identified as positive and negative corneal/limbal staining, respectively. There were no statistical differences between the number of cases presenting with both positive and negative staining in low and high LC. The lack of statistical significance may be associated with a small sample size. Based on the findings, we hypothesize two possible mechanisms relating to the staining; one related to a hypoxic effect and the other a mechanical effect. Lin et al. describes an altered corneal epithelial barrier function due to mechanical insult with extended wear of soft contact lens wear, which is exacerbated by lens-induced hypoxia.[202] Similarly, Madigan et al.[203] reported a reduction of hemidesmosomes in corneal epithelium associated with soft contact lens with low oxygen transmissibility. These reports demonstrate the increased relative risk of corneal erosion in cases of long-term hypoxia. Similarly, while a lower LC may be associated with a greater negative pressure over the limbal area to create chronic mechanical pressure, a higher LC may create a more significant barrier to reduce oxygen permeability, the combined sources of stress imposed by scleral lens wear result in the irregular corneal surface integrity. On other hand, peri-limbal edema was observed in this study for both low and high LC lenses, which is consistent with results on localized limbal edema reported by Visser et al.[130] In their study, Visser et al. proposed that this was due to

mechanical stress – induced by lens adhesion or insufficient limbal clearance.[130] On the other hand, peripheral edema with high LC also appears to be consistent with the mathematical models of corneal edema published by Michaud et al.[138]

4.6 Conclusion

In summary, subjective responses improved for both low and high LC lenses likely due to the overriding effect of increased comfort with scleral lenses in general and that perhaps the 50µm difference in LC may not have been sufficient enough to elicit a statistical difference. Vision, comfort and dryness was improved over baseline with the high LC lenses. There was a slight decrease in limbal and bulbar hyperemia with low LC lenses compared to baseline but no difference between high and low LC lenses was found. Corneal swelling was noted in all corneal locations and especially at the 6mm zone, where there were significant differences for both low and high LC lenses compared to baseline. Quadrant specific analysis revealed greater degree of change in inferior-temporal quadrants, likely associated with lens decentration. Corneal response to scleral lens wears with either low or high LC appears to result in either peri-limbal staining likely a hypoxic response or negative staining possibly due to mechanical irritation or bogging.

Chapter 5

Comparative Analysis of Tear Protein in Keratoconic Scleral Lens Wearers with Varying Limbal Clearance

5.1 Overview

Purpose: The purpose of this study is to investigate changes in the level of inflammatory mediators in the tear film of keratoconic patients with scleral contact lenses wear with varying LC.

Methods: 11 keratoconic subjects, experienced in scleral lens wear, were recruited (all male, mean age: 38.5 ± 13.5 years, range 24-67; Stage 1 KC: 54.5%, Stage 2: 36.4%, Stage 3: 9.1%). Subjects attended two study visits on two separate days, and sample collection was attempted from both eyes (min. 0.2μl required). At each visit, immediately after lens removal, tears were collected with a microcapillary tube (10μL, 0.5mm in diameter), firstly from inferior tear meniscus using the flush tear method, and secondly from the pool of tears in the bowl of the inverted scleral lens. 50μm of non-preserved 0.9% sodium chloride solution was instilled into the inferior cul-de-sac via a micro-pipette. The subject was then asked to roll their eyes twice with the eyelid closed and the tear sample was collected between blinks for a sixty-second period. Tear cytokine and protease analysis was performed using a multiplex electro-chemi-luminescent array (Meso Scale Discovery, Rockville, MD) instrument. Levels of IL-1, -6, -8, TNF-α, MMP-1 and -9 were compared using a Student t-test statistical analysis.

Results: The median volume collected from the flush tear collection was 1.0 μL (Range 0.2 to 6.0 μL). The median volume collected from the post-lens tear film were 5.0 μL (Range 0.2 to 10.0 μL). A statistically significant difference was noted between

sample volumes from either collection method (p<0.05). Significant differences at the p<0.10 levels were found comparing low and high LC with IL-1β, TNF-α, MMP-1 and MMP-9 (all p<0.10) from the samples taken from the lens bowl. Scleral lens with high LC were associated with increased levels of IL-1β, TNF-α, and MMP-1 and decreased levels of MMP-9.

Discussion: For scleral lens studies with tear analysis it appears that collecting the sample from the bowl of the lens yields valuable results. Changes in the cytokine levels were found comparing low and high limbal clearance indicating that mid-peripheral lens fit is an important feature in regulating the inflammatory response of the keratoconic eye.

5.2 Introduction

KC is a corneal ectatic disorder whereby the cornea thins and changes biomechanically, ultimately causing irregular changes in optical and demonstrating characteristic keratoconic features.[1,4,9] As the condition progresses, the ectatic activity occurs primarily in the extracellular matrix, which is made up of 70% collagen, and is carried out by collagenases and gelatinases.[51,87] Kao et al[204] and Rehany et al[205] first reported higher than normal collagenolytic and gelatinolytic activity in a KC corneal culture in 1982.[204] In 1982, Kenney et al., similarly, reported increased gelatinolytic activity in keratoconic corneae.[206] These results paved the way for subsequent reports in identifying specific matrix metalloproteases (MMPs) involved in the pathogenesis of KC, such as MMP-1[90,92], MMP-2[206] and MMP-9.[51,87] MMPs are a family of zinc-dependent endopeptidases[87] synthesized by corneal epithelial cells, stromal keratocytes, and neutrophils normally necessary in normal corneal physiology and wound healing. The over-expression of MMPs ultimately results in the degradation of the basement membrane and stromal tissue in KC.[87]

In 1991, Fabre et al reported 4 fold increase in binding sites of IL-1 in cultured KC stromal sites.[61] Wilson et al.[58] has also proposed the presence of IL-1 as the cause of KC. Up-regulation of IL-1 is associated with cell apoptosis in the corneal stroma, which alters the biomechanical stability in keratoconic stromal tissue[58] and may occur as a response to mechanical trauma and/or oxidative stress secondary to vigorous eye rubbing. In addition, other pro-inflammatory cytokines, such as IL-6[52,69,70], IL-8[51,76], and TNF-α[51,65,70], have also been found to be up-regulated in this corneal ectatic disease. These cytokines are involved in the proteolysis within the stromal

tissue[57,207,208], corneal neovascularization[74,77,78], and other changes in corneal tissue. The involvement of cytokines in the pathogenesis of KC challenges the historic classification of KC as a non-inflammatory disease that presents with the absence of infiltrates.

The delicate balance and regulation of cytokines is important in corneal physiology. Any ocular stress, such as the introduction of a contact lens, can influence the levels of inflammatory mediators which have an impact on corneal physiology and may further play a role in exacerbating the progression of KC.[58,209] For instance, IL-6 levels have been reported to increase with contact lens wear.[68,210,211] Kallinikos et al. have also reported an increase in IL-8 with continuous wear of soft silicone hydrogel lenses.[210] Changes in IL-8 levels can also present with complications associated with contact lens wear, such as contact lens acute red eye (CLARE).[165] Different lens modalities will also have an impact on the varying degrees of change in IL-6 and IL-8 levels.[212] In the keratoconic population, an increase of IL-6, TNF-α, and MMP-9 have been found with soft contact lens and corneal RGP wear.[50,213] In addition, higher than normal levels of MMP-9 have been found subsequent to contact lens removal which can play a role in the integrity of the corneal epithelium.[214]

Understanding factors that influence tear film composition may aid in a better understanding of the impact of scleral lenses on the health of the cornea, specifically, the mechanism of complications commonly associated with scleral lens wear. In Chapter 5, we investigate changes in the level of various inflammatory mediators in the tear film of scleral lens wearers that may be associated with varying LC by 50μm.

Ultimately, results from this study aims to help clinicians determine the subclinical impact of varying scleral lens wear design has on keratoconic individuals.

5.3 Methods and Materials

5.3.1 Participants

This study was a continuation of the study presented in Chapters 3 and 4. As with Chapter 3, this study was approved by the Office of Research Ethics of University of Waterloo (ORE#21364) and performed under the Tenets of Helsinki. Informed consent was obtained from the same participants as in Chapter 3.

5.3.1.1 Inclusion and Exclusion Criteria

The same inclusion and exclusion criteria were followed in Chapter 3, Section 3.3.1.1.

5.3.2 Study Materials

5.3.2.1 Study Lenses

The study lenses were the same as described in Chapter 3. The final lens parameters for the pair of lenses with low and high LC are listed in Tables 3-2A and 3-2B and the lenses were worn for 2±1weeks.

5.3.3 Study Procedures

5.3.3.1 Study Visits

The same schedule for study visits was followed as in Chapter 3 and Chapter 4. As described in Section 4.3.3, each participant was fitted with two scleral lens designs bilaterally of high and low LC differing by 50µm. A wash-out period of a minimum of 72 hours was required between wearing the first and the second set of study lenses.

The order of which lenses were worn was randomized and double-masked to the participant and researcher.

At each delivery appointment visit, tears were collected from the ocular surface for baseline analysis. At each follow-up visit, tears were collected, after lens wear of minimum of 4-6 hours, from two sites; directly from the posterior surface of the scleral lens and from the ocular surface (lower conjunctival fornix). Figure 5-1 describes the study visits schedule.
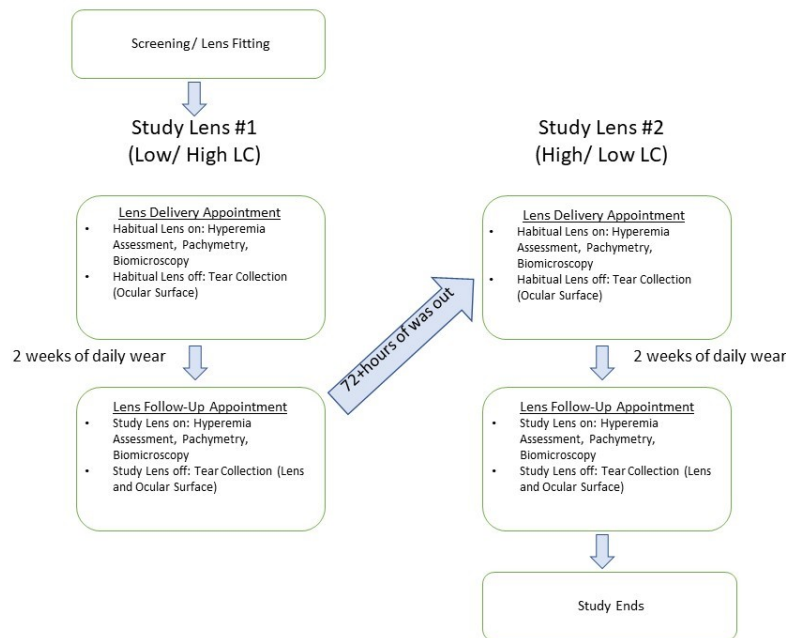
Figure 5-1: Flow chart of study visits.

5.3.3.2 Tear Collection from Scleral Lens

Participants were asked to remove their study lenses by holding the lens horizontally while positioning their chin and face downward. The post-lens tear film was, then, collected directly from the contact lens bowl using a microcapillary tube (10µL, 0.5mm in diameter), as illustrated in Figure 5-2. The maximum volume of tears that

would be collected was 10µl. The collected samples were subsequently transferred into a small vial for centrifugation at 4,000 rpm, for 20 minutes at 4°C. The supernatants of each sample were then stored in an -80° C freezer, awaiting analysis.
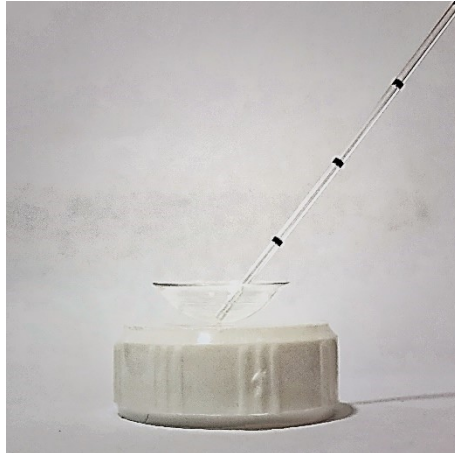
Figure 5-2: Microcapillary tube collecting tear sample from scleral lens bowl.

5.3.3.3 Tear Collection from Ocular Surface

Tears were collected using the flush tear technique before and after scleral lens wear, as depicted in Figure 5-2.[167] A volume of 60µL of sterile unit-dose saline was instilled into the inferior palpebral fold with a pipette. Tear collection was then performed utilizing a single use microcapillary tube. The tube was held at the outer canthus of the participant's eye. The tears were collected in between blinks for the duration of one minute. The collected samples were subsequently transferred into a small vial for centrifugation at 4,000 rpm, for 20 minutes at 4°C. The supernatants of each sample were then stored in a -80° C freezer, awaiting analysis.

Figure 5-3: Flush Tear Method. (Photo Credit: Shane Parker).

5.3.3.4 Tear Protein Analysis

Tear cytokine analysis was performed using the Meso Scale Discovery platform (MSD-ECL), as shown in Figure 5-4. This is an electrochemiluminescence detection system.[215,216] Preparation of the samples includes dilution and incubation with analyte-specific antibodies. These antibodies are tagged with ruthenium. Upon electrochemical stimulation, the ruthenium label will emit light as a result of a REDOX reaction.[215] The concentration of specific proteases and cytokines can be determined based on the amount of light emission. Preparation and analysis of samples are as per instructions supplied by manufacturers. In comparison to traditional methods of tear protein analysis, particularly enzyme-linked immunosorbent assay (ELISA), MSD-ECL allows for multiplex analysis and requires a significantly reduced test sample volume. The pro-inflammatory multiplex panel (human-1) was used to determine the levels of IL-1β, IL-6, IL-8, and TNF-α. This kit was validated according to published

material by Lee et al.[217] The calibration curves and subsequent concentrations using signal detection were derived using MSD DISCOVERY WORKBENCH® software by the manufacturers of the MSD Multi-Plex Assay System. They were done to show that each individual assay was independent of each other using individual detection antibodies and to determine the analyte concentrations (Figure 5-5).
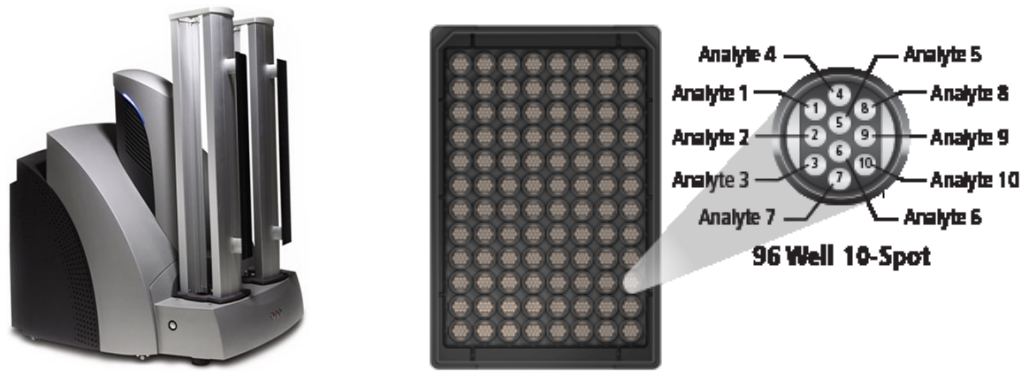
Figure 5-4: Meso-Scale Discovery Multiplex electro-chemiluminescent detection system and its set up for Pro-inflammatory 10-plex panel.
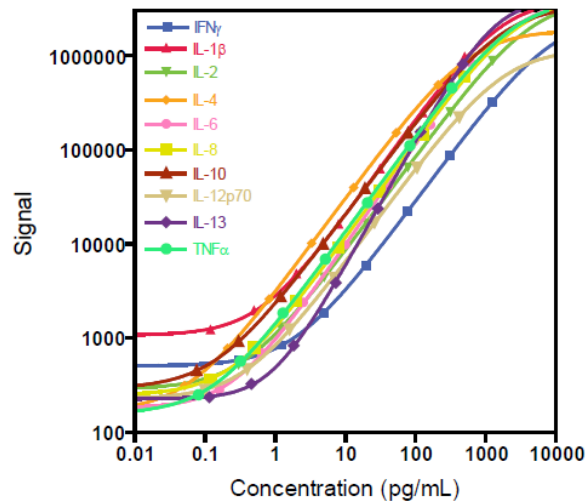
Figure 5-5: Calibration Curves for Human Pro-Inflammatory Panel 1 utilized by the MSD DISCOVERY WORKBENCH® software.

In addition, levels of MMP-1 and MMP-9 were measured using the human MMP 3-Plex Ultrasensitive kit, as shown in Figure 5-6. Standardized and validated calibration curves used by the MSD DISCOVERY WORKBENCH® are shown in Figure 5-7.
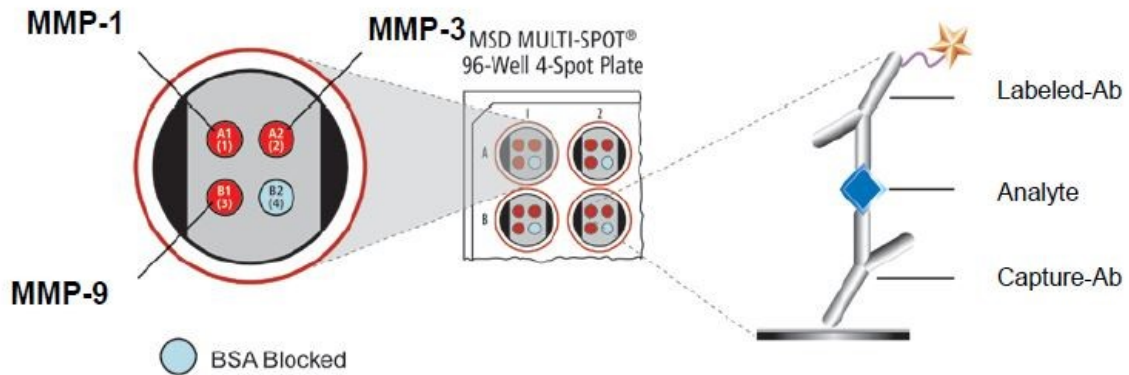
Figure 5-6: Meso-Scale Discovery Multiplex electro-chemiluminescent detection system and its MMP 3-Plex Ultrasensitive kit.
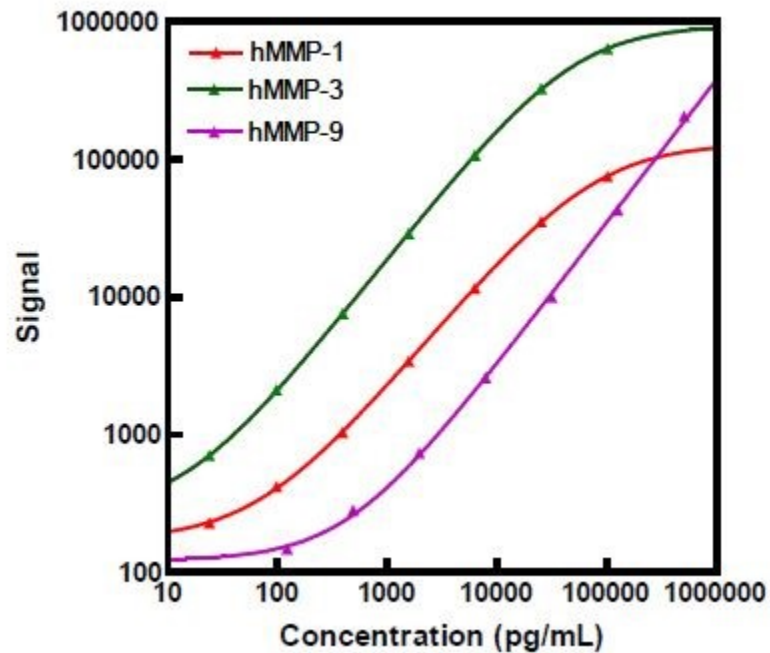
Figure 5-7: Calibration Curves for MMP 3-Plex Ultrasensitive Kit utilized by the MSD DISCOVERY WORKBENCH® software.

5.3.4 Statistical Analysis

Data were analyzed using Statistica 10 (Statsoft Inc., Tulsa, TX). Mean, standard deviations, medians and quartiles for each sample were determined and compiled for analysis. Outliers over 2 standard deviation were excluded. Student t-tests assuming unequal variance were used to determine statistical significance between samples and varying LC. P values of less than 0.05 were considered to be statistically different and in some cases (as with the cytokines) <0.10 due to large spread and variation in the measurements of these markers.

5.4 Results

5.4.1 Tear Sample Volume

A total of twenty-two sample pairs, from both the posterior surface of scleral lens bowl and from the ocular surface, were collected and compared. The median volume collected from the flush tear collection was 1.0µL (Range 0.2 to 6.0µL). The median volume collected from the post-lens tear film was 5.0µL (Range 0.2 to 10.0µL). A statistically significant difference was noted between sample volumes from either collection method ($p<0.05$).

5.4.2 Tear Cytokine Analysis

Levels of molecular markers of inflammation based on tear analysis collected from the ocular surface before and after wearing the study scleral lenses with low and high LC are listed in Table 5-1. No cytokine data was producible for eleven tear samples for reasons of inadequate tear volume necessary for analysis. For the samples that

were analyzed, no statistical significance was noted in levels of cytokine changes with varying LC.

Results from tear cytokine analysis, based on samples collected from the scleral lens bowl, are listed in Table 5-2, and depicted in Figures 5-8A-F. Significant differences at the p<0.10 levels were found comparing low and high LC with IL-1 (Figure 5-8A), TNF-α (Figure 5-8D), MMP-1(Figure 5-8E) and MMP-9 (Figure 5.8F) (all p<0.10).

Table 5-1: Median and range of cytokine levels in tear samples from ocular surface. Levels are represented for habitual optical correction (Baseline), and with study scleral lenses with low and high LC.

Cytokine	Baseline		Low LC		High LC	
	Median	*Range*	*Median*	*Range*	*Median*	*Range*
IL-1β (pg/mL)	5.24	1.89-57.28	6.02	2.02-145.72	12.64	1.26-259.54
IL-6 (pg/mL	16.94	4.10-78.52	8.18	2.39-66.66	22.00	3.54-114.28
IL-8 (ng/mL)	0.54	0.87-1.94	0.97	0.13-5.96	0.64	0.13-12.86
TNF-α (ng/mL)	0.20	0.08-0.53	0.53	0.38-0.68	1.86	0.34-2.30
MMP-1 (ng/mL)	n/a	n/a	n/a	n/a	n/a	n/a
MMP-9 (ng/mL)	n/a	n/a	n/a	n/a	n/a	n/a

Table 5-2: Median and range of cytokine levels in tear samples from scleral lens post-lens tear film. Levels are represented for study scleral lenses with low and high LC.

Cytokine	Low LC		High LC		
	Median	*Range*	*Median*	*Range*	
IL-1β (pg/mL)	7.35	0.09-77.23	8.39	0.24-177.33	p=0.117
IL-6 (pg/mL	12.97	0.02-622.58	10.02	0.43-589.75	p=0.734
IL-8 (ng/mL)	0.47	0.006-31.33	0.42	0.02-12.88	p=0.952
TNF-α (ng/mL)	1.15	0.090-1.35	1.11	0.07-8.09	**p=0.006**
MMP-1 (ng/mL)	1.00	0.19-5.09	2.69	0.12-4.07	**p=0.023**
MMP-9 (ng/mL)	32.17	4.74-357.90	26.37	6.31-177.85	**p=0.095**
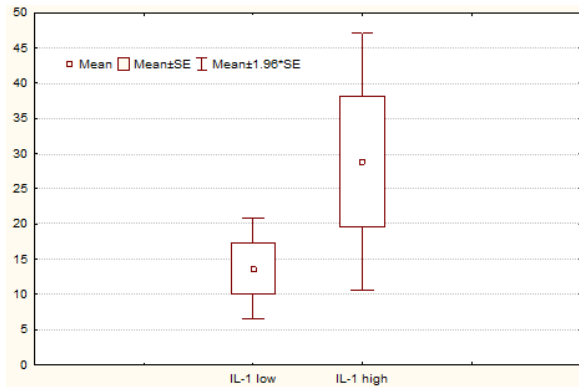
Figure 8A. IL-1β levels, post-scleral lens wear, for low and high LC, p=0.117

Figure 5-8D. TNF-α levels, post-scleral lens wear, for low and high LC, p=0.006.
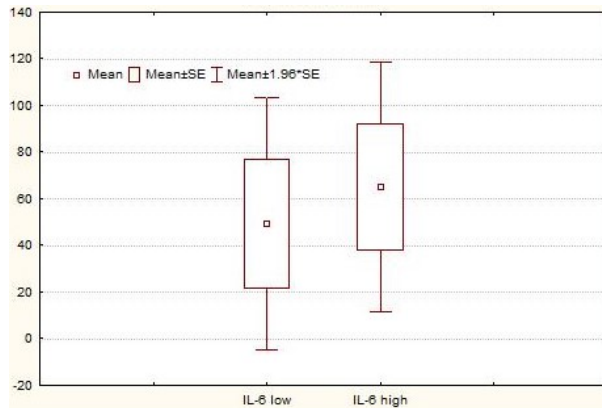
Figure 5-8B. IL-6 levels, post-scleral lens wear, for low and high LC, p=0.734.
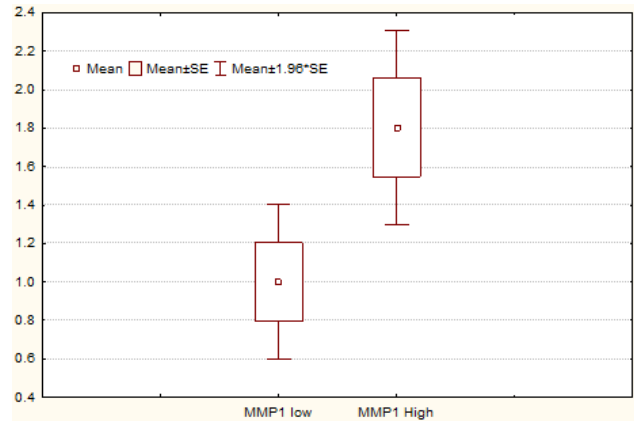
Figure 5-8E. MMP-1 levels, post-scleral lens wear, for low and high LC, p=0.023.
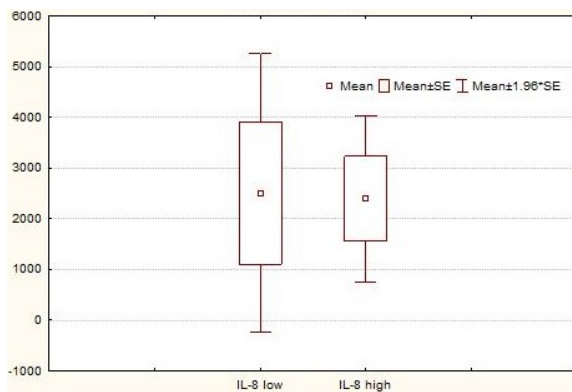
Figure 58C. IL-8 levels, post-scleral lens wear, for low and high LC, p=0.952.
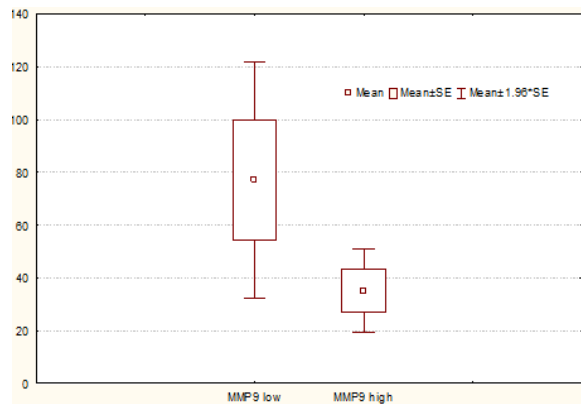
Figure 5-8F. MMP-9 levels, post-scleral lens wear, for low and high LC, p=0.095.

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5.5 Discussion

The composition and concentration of proteins present in the tear film will vary in response to biological, pathogenic, or environmental factors. Tear protein analysis has helped us understand the pathophysiology and effect on the corneal integrity caused by KC, diabetes, and dry eye.[48] Corneal RGP and short term scleral lens wear have been shown to have an effect on the tear film chemistry in individuals with normal corneae and KC.[50,108,218] However, the influence of the fitting relationship and interaction between the scleral lens and the corneal, limbal and conjunctival surfaces on these markers is not well understood. This is particularly important for the limbal area as limbal stem cell deficiencies has been reported in contact lens wearers.[219,220] To better understand how scleral lens wear might influence the limbal area, where the corneal epithelial stem cells are located, this thesis chapter examines and compares specific tear composition before and after standardized fitting of scleral lenses with varying LC.

An effective means of collecting the tear sample is essential for tear composition analysis. Tear collection can be done in various ways, including Schirmer strips[221,222], microcapillary tube collection from fornices[43], and impression cytology using a cellulose acetate filter.[223] The main challenges are to ensure there is adequate volume for analysis and an ability to truly represent the status of the tear film.[221,224] Mechanical irritation of the ocular surface during tear collection can stimulate reflex tearing, and alter the tear protein composition from the pure basal state, obscuring the results.[225] Markoulli et al. first reported on a flush tear method as a means for a faster, more comfortable and therefore easier collection.[167]

In this study, tear collection was taken from the ocular surface using the flush tear method. While this method was able to avoid volumetric, and therefore concentration, errors due to reflex tearing, the primary challenge that was faced was to maintain and generate an adequate volume for analysis. In several cases, the minimum volume collected over the one-minute tear collection period was as little as 0.2μL from the ocular surface. This is less than the 0.5μL volume necessary for analysis through the MSD system. This is significantly less than volumes collected with the flush tear method in normal corneae reported in a pilot project in preparation for this thesis study.[226] Difficulties in recovering adequate sample sizes have been reported to be associated with lower tear volume[107], higher tear osmolarity[227,228] and surface tension.[229] These features can often be found in those with ocular surface diseases[230] and can present as a co-morbidity in keratoconic eyes.[107]

On the other hand, collection of tear samples from the posterior scleral lens bowl was able to recover a greater tear volume up to 10μL. These samples originated from the post-lens tear film between the scleral lens and the anterior corneal plane during lens wear, and are theoretically continuous with the tear film from the ocular surface. The 0.2μL to 10.0μL range in tear volume was then compared to a mathematical model to calculate the volume of the post-lens tear film (Figure 5-9). Assuming the scleral lens bowl and the anterior chamber of tear fluid are spherical caps, the volumes can be calculated from the lens parameters (Chapter 3, Section 3.4.2.) and corneal data (Chapter 3, Section 3.4.1). The formula in Figure 5-9 calculates the volume using the sagittal depth and sagittal height compared to the back-surface optic zone radius of the lens and anterior corneal keratometric values. Across the

scleral lens fits in this study, the mean theoretical post-lens tear film, with a mean central corneal clearance of approximately 200μm, was averaged to be 155.37μL. While the post-lens tear film cannot be recovered in its entirety during the removal of scleral lens, the sample volume collected from posterior bowl of the scleral lens still far exceeds reports from flush tear methods collected from ocular surface of 27.2±11.7μL[167]. One shortcoming of this mathematical model is that the calculated volumes, based on the assumption that the scleral lenses are spherical caps, are exaggerated because the asphericity (positive eccentricity) of the back surface of the scleral lens and cornea will reduce the volumes relative to a curvature with zero eccentricity (that is, a sphere).

Theoretical Lens Volume = 404.85mm^3
$$V = \frac{\pi h^2}{3}(3r\text{-}h)$$
- Mean Sag depth @ 16.0mm = 4.544mm
- Mean Back Optic Zone Radius = 7.759mm

Theoretical Anterior Chamber Volume = 249.48mm^3
- Mean Sag height @ 15.0mm = 3.714mm
- Mean Simulated K's = 6.998mm

Theoretical Post-Lens Tear Film
 = Lens Volume – Anterior Chamber Volume
 =155.37mm^3
 = 155.37μL

Figure 5-9: Volume and calculation for theoretical volumes of post-lens tear film assuming scleral lens and anterior cornea are spherical caps.

In this study, the limited tear volume collected from the ocular surface presented as a limitation. While the MSD facilitated tear analysis with a small sample volume, each multiplex requires a minimum of 0.5μL of sample. In more than one instance of tear collection from the ocular surface, only 0.2μL were collected. As such, not all tear film cytokines of interest in the study could be analyzed, namely the MMPs analysis. On the other hand, the post-lens tear film can act as an easier source of adequate tear volume and facilitates in tear protein analysis. One shortcoming of this method is that this method does not provide any baseline data on tear proteins.

Further analysis of tear cytokines focused on tear samples from the post-lens tear film, which is assumed to be continuous with the tear film on the ocular surface. Despite greater recovery of tear sample volume by means of collection from the post-lens tear film, the various cytokines examined in this study exhibited large ranges, which can present as a challenge in the statistical analysis. This is likely due to the influence of a large age range in the participants that present with various stages of the KC. As such, the basal levels of inflammatory mediator may be inconsistent depending on the stage of their of corneal ectasis.[70] It is for this same reason that tear samples from the right and left eyes cannot be pooled together as the severity of ectactic disease is asymmetrical.[3,6-9] While unable to control the age range, the study had only recruited males to minimize the impact hormones, such as estrogen, has on the cytokine levels and MMP activities.[231,232] As opposed to absolute concentrations, the relative cytokine level is still valuable in providing an insight into how varying the LC may affect the local inflammatory response.[233-235]

In this study, IL-1β levels were noted to increase with the increase of LC in the scleral lens design which was not statistically significant likely due to a small sample size. IL-1β is a potent inducer for other cytokines, such as IL-6 and -8 and TNF-α.[55,56] Therefore, changes in levels of this cytokine may implicate changes in the latter tear proteins. Martín-Montañez et al.[236] showed a higher level of IL-1β associated with omafilcon A (Dk/t at -3.00D = 44, lower Dk) relative to that associated with comfilcon A (Dk/t at -3.00D = 160), which has a higher oxygen permeability. On the other hand, Yüksel Elgin et al.[212] reported elevated IL-1β associated with contact lens wear using silicone hydrogels, compared to corneal GPs. Corneal GPs permit greater tear exchange and can reduce the hypoxic response. Furthermore, IL-1β has been cited to be involved in the regulation of keratocyte apoptosis and corneal tissue organization.[58] An increases in IL-1β, which was found to be associated with scleral lens wear with high LC in this study, can have an implication to promote disease progression in KC.

A general trend of increase in levels of IL-6 in this study was also seen with increasing the LC. Elevated IL-6 has been linked to soft contact lens wear.[68,218,237] While all the studies differ in methodologies, Schultz and Kunert[68] reported increased levels of IL-6 with soft lens wear of 43.8±5.3pg/5μL (8.76ng/mL); Thakur and Wilcox[218] reported elevated levels of IL-6 of 2.505±0.951ng/mL with soft contact lens wear especially when worn overnight, while Poyraz et al.[237] reported IL-6 levels of 33.1±15.0pg/mL with 6 months of soft lens wear and 34.4±17.5pg/mL with the same duration of corneal RGP wear. Furthermore, an IL-6 increase with corneal injury as a result of

ocular desiccation in cases of ocular surface disease[63-66], and eye rubbing in cases of KC has been reported.[71]

In this study, varying the LC resulted in no change in IL-8. IL-8 is a pro-inflammatory chemokine that plays a role in neutrophil chemotaxis.[56,237] It has been associated with angiogenic activities by attracting neutrophils along the vascular wall.[56,75,238] A lack of change in the proangiogenic cytokine indicates there is no difference in neovascularization activities with varying the LC, which were not observable within the two weeks of study lens wear. IL-8 is up-regulated in response to the mechanical trauma on the corneal epithelium normally from eye rubbing.[71,210] IL-8 levels have also been reported to increase with corneal RGP wear, likely due to some mechanical stimulation over a two hour period.[76] In association with scleral lens wear with low and high LC, the presence of some limbal clearance should result in no mechanical insult on the ocular surface at the corneal and limbal area.

TNF-α is a pro-inflammatory cytokine with pro-lymphangiogenic and pro-angiogenic effects. This mediator is produced by corneal epithelial cells to promote vasodilation, edema, and leukocyte recruitment.[77] In the keratoconic population, TNF-α has been reported to approximately increase five-fold with contact lens wear.[50] In this study, scleral lens wear with high LC resulted in statistically significant higher levels of TNF-α. TNF-α is known to contribute to IL-6 production in cytokines[239] and may increase with IL-6 levels in cases with high LC.

MMP-1 and MMP-9 is part of a family of collagen-degrading enzymes that play an important role in normal corneal physiology. MMP-1 was significantly increased with increased LC. Mckay et al.[240] has reported corneal tissue response of increased MMP-

1 in acute hypoxic conditions. It is important to note that healthy corneal tissue should have no MMP-1.[92] Conversely, there was a general trend (at p=0.1) of a decrease in the level of MMP-9 with higher LC. MMP-9 is critical in the proteolysis of collagen type VII and other components of the extracellular matrix of the corneal epithelial basement membrane.[84-86] Elevated MMP-9 levels in the tear film is indicative of an inflammatory response to stress or injury to the corneal epithelium[94] and has been linked to recurrent corneal erosions[86], dry eye[54,241-244], KC,[87] and scleral lens wear in keratoconic and normal corneae.[108,226] Up-regulation of MMP-9 is regulated and promoted by IL-1 and TNF-α.[49,80], and is inhibited by the tissue inhibitor of MMP-1 (TIMP-1)[84-86]. In this study, the inversely-related changes in IL-1 and TNF-α may be related to the down-regulation of MMP-9 by TIMP-1.

In scleral lens wear, a thicker post-lens tear film placed over the limbal area can pose a barrier to oxygen transmissibility, resulting in a pro-inflammatory environment and corneal hypoxia.[138] On the other hand, inadequate limbal clearance can induce mechanical insult to the ocular limbal surface. With scleral lens wear, increases in IL-1 and MMP-1 levels are indicative on how higher LC can result in hypoxia in the limbal zone. Conversely, an increased level of MMP-9 with decreased LC is suggestive of the presence of mechanical insult on the limbal tissue as a result of low LC. The limbal zone must be carefully considered to minimize its impact on the ocular health as it is an important area of the cornea which houses the corneal stem cells necessary for the re-population of mainly epithelial cells within the cornea.[146]. Higher LC is associated with greater increases in various cytokine levels over a two-week period,

creating a more pro-inflammatory condition. This change may be sufficient to cause a slow progression of corneal ectasia.[50,245]

5.6 Conclusion

Tear collection directly from the post-scleral lens tear film after lens removal allows for a better recovery of cytokines compared to the flush tear method. It may be a preferred method for tear film analysis for scleral lens research, particularly with subjects with low tear volume. The objective of this study was to identify changes in tear cytokines with scleral lenses that varied in LC by 50μm. Higher LC was associated with higher levels IL-1β, IL-6, TNF-α and MMP-1 levels and lower levels of MMP-9. There was no change in IL-8 levels with varying LC. The limbal zone of the scleral lens can have an impact on the corneal physiology of the limbus. As such, the limbal zone has a significant impact on the clinical performance of a scleral lens.

Chapter 6

Tear Cytokine Changes and Relationship to Clinical Performance of Scleral Lenses with Varying Limbal Clearance and Discussion

6.1 Introduction

This thesis study was a prospective cross-over, dispensing study that fitted twenty-two keratoconic eyes with two pairs of scleral lenses with a low and a high limbal clearance. Following a two-week, full-time, lens wear duration, clinical performance and tear cytokines analysis was performed to better understand how varying LC impacts the ocular health. All data regarding fitting characteristics, clinical performance, and associated tear cytokines changes are summarized in Table 6-1.

Table 6-1: Summary of limbal zone fitting characteristic clinical performance, tear cytokine associated with scleral lenses with low and high LC.

	Low LC		High LC	
	Nasal	Temporal	Nasal	Temporal
Initial LC	120.89 ± 37.87	198.98 ± 70.21	145.67 ± 51.12	252.43 ± 104.71
Final LC	106.40 ± 35.09	141.08 ± 83.01	148.83 ± 50.59	185.78 ± 69.75
Subjective Assessment				
Comfort	80 (55-95)		**90 (70-100)**	
Dryness	**82.5 (50-100)**		**90 (60-100)**	
Burning	85 (65-100)		95 (70-100)	
Vision	90 (60-100)		**92.5 (70-100)**	
Hyperemia	Nasal	Temporal	Nasal	Temporal
Limbal	0.92±0.62	**0.77±0.34**	1.03±0.83	0.95±0.52
Bulbar	1.60±0.52	**1.30±0.34**	1.63±0.57	1.40±0.46
Pachymetry				
Centre cornea	469.7±37.7µm		**485.6±32.7µm**	
Periphery at 6mm	**590.3±42.7µm**		**578.9±51.3µm**	
Periphery at 8mm	650.7±56.7µm		651.9±54.7µm	
Cytokines				
IL-1 β (pg/mL)	7.35 (0.09-77.23)		8.39 (0.24-177.33)	
IL-6 (pg/mL	12.97 (0.02-622.58)		10.02 (0.43-589.75)	
IL-8 (ng/mL)	0.47 (0.006-31.33)		0.42 (0.02-12.88)	
TNF-α (ng/mL)	1.15 (0.090-1.35)		1.11 (0.07-8.09)	
MMP-1 (ng/mL)	1.00 (0.19-5.09)		2.69 (0.12-4.07)	
MMP-9 (ng/mL)	32.17 (4.74-357.90)		26.37 (6.31-177.85)	

6.2 Correlations Among Results

The limbal zone of a scleral lens plays an important role in the overall fitting success. Various ocular sequelae were reported in a case series of scleral lens fittings that had an appropriate central ocular surface-to-lens fitting relationship. This study wanted to identify possible relationships between low and high LC with potential mechanical or hypoxic side effects. Comparing ocular health findings, subjective comfort, and tear cytokine analysis can help clinicians and researchers better understand how varying LC can impact the ocular health, clinically and sub-clinically.

6.2.1 Statistical Analysis

Statistics were analyzed using Statistica 10 (Statsoft Inc., Tulsa, TX). To quantify correlations between cytokine levels and clinical parameters, the Pearson correlation coefficient (r) was determined along with its statistical significance. P values of less than 0.10 for tear data were considered to be statistically different.

6.2.2 Correlation Results

Only statistically significant correlations are reported.

6.2.2.1 Correlation between Limbal Clearance and Clinical Parameters

<u>Low LC in nasal quadrant</u>

- Negatively correlated with peripheral corneal pachymetry in the inferior quadrant at 8mm. (r=-0.6603, p=0.038)

<u>High LC in temporal quadrant</u>

- Correlated with peripheral corneal pachymetry in the inferior quadrant at 6mm. (r=0.6656, p=0.036)

- Correlated with peripheral corneal pachymetry in the inferior quadrant at 8mm. (r=0.6322, p=0.050)

6.2.2.2 Correlation between Tear Cytokines and Clinical Parameters

<u>IL-1</u>

- Overall subjective comfort was correlated with IL-1 levels with low LC (r=0.6341, p=0.049)

- Bulbar hyperemia in nasal quadrant with high LC is negatively correlated with IL-6 levels with low LC (r=-0.6596, p=0.038)

<u>IL-6</u>

- Bulbar hyperemia in nasal quadrant with high LC is negatively correlated with IL-6 levels with low LC (r=-0.6596, p=0.038)

- Peripheral pachymetry in Superior quadrant at 6mm with Low LC is negatively correlated with IL-6 levels with high LC (r=-0.7882, p=0.007)

- Peripheral pachymetry in superior quadrant at 6mm with Low LC is negatively correlated with IL-8 levels with high LC (r=-0.8517, p=0.002)

- Peripheral pachymetry in Inferior quadrant at 8mm with High LC is correlated with IL-6 with high LC (r=0.6606, p=0.038)

<u>TNF-α</u>

- Peripheral pachymetry with low and high LC inferior quadrant at 6mm is correlated with TNF-α levels with low LC (r=0.6747, p=0.032 and r=-0.6537, p=0.040)

- Peripheral pachymetry in temporal quadrant at 6mm with high LC is negatively correlated with TNF-α levels with low LC (r=-0.5449, p=0.10)

- Peripheral pachymetry at temporal quadrant at 6mm and 8mm with high LC are correlated with TNF-α levels with high LC (r=0.6886, p=0.028 and r=0.6908, p=0.027, respectively)

- Peripheral pachymetry with low LC nasal and temporal quadrants at 6mm are correlated with TNF-α levels with high LC (r=0.6979, p=0.025 and r=0.7703, p=0.009, respectively)

MMP-1

- Limbal hyperemia in nasal quadrant with high LC is negatively correlated with MMP-1 levels with low LC (r=-0.7410, p=0.014)

- Bulbar hyperemia in nasal hyperemia with high LC is negatively correlated with MMP-1 levels with low LC (r=-0.7410, p=0.014)

- Peripheral pachymetry with High LC in Superior quadrant at 6mm is negatively correlated with MMP-1 levels with low LC (r=-0.6459, p=0.044)

MMP-9

- High rating regarding no burning sensation was correlated to MMP-9 levels with high LC (r=0.7760, p-0.008)

- Limbal hyperemia in temporal quadrant with low LC is negatively correlated with MMP-9 levels with high LC (r=-0.6766, p=0.032)

- Bulbar hyperemia in temporal quadrant with low LC is negatively correlated with MMP-9 levels with high LC (r=-0.6766, p=0.032)

- Peripheral pachymetry with Low LC in Temporal quadrant at 6mm is negatively correlated with MMP-9 levels with high LC (r=-0.7991, p=0.006)

- Peripheral pachymetry with Low LC in Temporal quadrant at 8mm is negatively associated with MMP-9 with high LC (r=-0.8425, p=0.002)

6.2.2.3 Correlation between Tear Cytokines and Limbal Clearance

A strong correlation between IL-6 levels from high LC and IL-8 levels from high LC was noted (r=0.9291, p=0.000). A similarly strong correlation was found between MMP-9 levels with low LC and IL-8 levels with low LC (r=0.9938, p=0.000). MMP-9 with low LC was negatively correlated with TNF-α levels with low LC (r=-0.6072, p=0.063). IL-6 with high LC was negatively correlated with TNF-α levels with low LC (r=-0.9928, p=0.0001).

6.3 Discussion

Introduction of any foreign object, such as a scleral lens, is a sustained environmental change which can result in a chronic state of altered ocular homeostasis.[45,47] If the immune system is not able to rectify the change, the ocular inflammatory response can result in tissue damage.[45] In early stages of ocular inflammation, only biochemical changes occur, which is commonly asymptomatic. Cytokines are markers for subclinical inflammatory changes.[43,48] Through the vasculature system, the impact of cytokines are amplified through a cascade to impact adjacent tissues.[144] Ultimately, these changes become observable through biomicroscopy as classical signs inflammation of rubor which presents as limbal and bulbar injection, tumor as corneal edema, and dolor as reports of pain or discomfort. The main purpose of this study is to examine the various subclinical changes and clinical ocular findings

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associated with scleral lens wear with varying limbal clearance in keratoconic patients.

6.3.1 Hyperemic Complications

Injection in the limbal and bulbar conjunctival areas are persistently two of the most common complications associated with scleral lens wear.[119,126] Injection is indicative of the presence of distress to the ocular surface.[155] In the case of scleral lens wear, inflammatory response originate in the limbal vasculature.[144] Hyperemia is a result of vasodilation of limbal and bulbar conjunctival vasculature can be detected by biomicroscopy, as well as by Oculus Keratograph 5® R Scan (Section 4.3.2.3).

Vasodilation in the limbus and bulbar conjunctiva occur as a result of increased level TNF-α produced by corneal epithelial cells.[77] In this study, while no difference in hyperemia in the limbal and bulbar regions was observed subsequent to scleral lens wear comparing low and high LC, high LC was associated with higher levels of TNF-α in the post-lens tear film.

Papas[246] has reported an association between limbal redness and contact lens-induced hypoxia. Similarly, Maldonado-Codina et al.[247] have noted greater contact lens-induced limbal and bulbar redness associated with low oxygen transmissible hydrogel lens wear compared to high oxygen transmissible silicon hydrogel lens wear.

6.3.2 Hypoxic Complications

Despite the use of lens materials with high oxygen transmissibility, corneal hypoxia has been reported in scleral lens wear and has been theoretically associated with a

thicker post-lens tear film.[139,156,157] Hypoxia causes the cornea to shift into anaerobic metabolism[248], which results in corneal swelling[194]. Hypoxic changes in the corneal tissue may be observed through biomicroscopy as striae and loss of transparency when corneal thickness has increased by 6.89%[198] and greater than 15%[249], respectively. Sub-clinically, corneal pachymetry using the Scheimpflug imaging (as described in Section 3.3.1.1) can provide a method for close monitoring of corneal hypoxic changes.[250] On the other hand, tear analysis may allow a closer examination of the impact of hypoxia which was found to be associated with greater clearance at the limbal zone of the scleral lens in this study. Up-regulation of IL-1β has been reported to be associated with contact lens wear of lower oxygen permeability.[212,236]

A scleral lens wear system must provide peripheral oxygen transmissibility of no less than 32.6 x 10^{-9} $cm^2.mlO_2/s.ml.mmHg$ to avoid corneal swelling.[251] According to a theoretical study used to analyze CCC by Fatt[252] and Michaud et al.[138] (Figure 6-1), the limbal clearance must be no greater than 5.40μm in order to avoid inducing corneal hypoxia in the peripheral cornea, assuming oxygen transmission through the lens and post-lens film thickness is the only source of oxygen. This is certainly consistent with the concept that thinner clearance allows for better oxygen delivery[138–140] In practice, oxygen to the limbal tissue might be supplied by limbal vessels.[126] For this reason, it can be argued that LC is not the sole cause of limbal edema. Furthermore, Bergmanson et al. argues that perhaps the minute degree of tear exchange that occurs in scleral lens systems might be an alternate source of oxygen delivery to the corneal and limbal epithelium.[253]

$$\frac{Dk}{t_{Scl}} = \frac{1}{\left(\frac{t_1}{Dk_1}\right)+\left(\frac{t_2}{Dk_2}\right)}$$

t_1 = Limbal Clearance
Dk_1 = Oxygen Permeability of Tear = 80
t_2 = Peripheral Lens Thickness ≈ 300µm
Dk_2 = Oxygen Permeability of Lens Material (Boston XO) = 100

Figure 6-1: Fatt Formula to calculate theoretical oxygen delivery in scleral lens system.[138,252]

In this study, pachymetry analysis was focused on the peripheral cornea to investigate the influence varying LC has on corneal hypoxia. A central corneal edema of approximately 4% was noted in the central corneal thickness for all scleral lens fits of both low and high LC. A significant increase in peripheral corneal thickness with all scleral lens wear since all LC exceeded 5.40µm in this study. The change was smaller with the low LC lenses compared to high LC (Figures 4-7A and 4-7B). This is consistent with the tear cytokines analysis which showed a relative lower level of IL-1β with low LC, which provides higher oxygen transmissibility.

6.3.3 Mechanical Complications

Dolor is observable as symptoms of pain or discomfort which may result from mechanical insult to the ocular surface. In this study, no pain was reported in association with scleral lens wear. In addition, both low and high LC lenses were able

to provide the same level of comfort to the keratoconic subjects. Previous reports have found little association between change in tear cytokines to ocular comfort associated with lens wear.[254] This is with the exception of IL-1β, which have shown positive correlation with severity of dry eye syndrome.[66]

Limbal bearing may be observed in instances of inadequate LC, where the lens is inappropriately resting on the ocular surface.[199] This may cause the breakdown of the limbal epithelium and can be seen as localized corneal staining. When examining the corneal surface, similar incidences of positive corneal staining were found with both low and high LC lenses. On a biochemical level, levels of MMP-9 were inversely related to magnitude of LC. In addition, MMP-9 levels demonstrated a statistically significant negatively correlation with clearance in the limbal zone. Elevated MMP-9 levels in the tear film are indicative of an inflammatory response to stress or injury and have been linked with the presence of inflammation in a disrupted corneal epithelium, such as recurrent corneal erosion[86], dry eye[54,241-244], KC,[87] and complications secondary to contact lens wear.[50,214,255] It can be deduced that a higher LC may be protective against mechanical insult on the limbal tissue.

6.3.4 Limbal Complications

The limbus is an important area of the cornea as it houses the limbal vasculature which is supplies oxygen to the limbal stem cells and contributes to the avascular cornea and,.[144-146,256] Damage to the limbal epithelium, the preferential site for corneal epithelial stem cells can result in defective tissue remodeling and corneal epithelization, resulting in the conjunctivalization of the limbus.[257-261] This condition is referred to as Limbal Stem Cell Deficiency (LSCD). Iatrogenic causes for acquired

LSCD include poor-fitting contact lens wear which compromises the health of the limbal stem cells.[220] Clinical finding associated with LSCD include chronic stromal inflammation, corneal neovascularization, conjunctival epithelial ingrowth.[261] In LSCD, upregulation of IL-6 and IL-8, in addition to other cytokines in its signaling pathway including IL-1β and TNF-α, has been reported to result in angiogenesis and corneal neovascularization.[75] In addition, excessive extra cellular matrix breakdown in cases of LSCD is associated with upregulation of both MMPs-1 and -9.[259]

In this study, increasing the LC in scleral lens design trended towards a positive correlation with levels of in IL-1β, IL-6, TNF-α, MMP-1 and a negative correlation with MMP-9. There was inadequate data in tear cytokine analysis from the ocular surface, by means of the flush tear method (Section 5.3.3.2) to demonstrate how scleral lens wear with varying LC can change levels of the inflammatory markers from baseline.

A clinical finding observed with scleral lenses with both low and high LC is negative corneal/limbal staining. Walker et al. has described it as epithelial bogging.[119] The presence of epithelial disruption has a similar presentation to the negative corneal staining finding in cases of limbal stem cell deficiency. Hypoxia can retard the limbal stem cell growth and differentiation.[262] In scleral lens wear, it is hypothesized that the combined hypoxic and mechanical effect posed by the scleral lens and the post-lens tear film, regardless of high and low LC, may be disruptive to the limbal stem cells' health. Lin et al. describes altered corneal epithelial barrier function due to mechanical insult with extended wear of soft contact lens wear, which is exacerbated by lens-induced hypoxia.[202] Chronic hypoxia can result in a reduction of hemidesmosomes in the corneal epithelium, which ultimately increases the relative

risk of erosion.[203] Similarly, Visser et al[130] has also associated peri-limbal edema with mechanical stress imposed by insufficient limbal clearance.[130] This study also demonstrated changes on the ocular surface associated with hypoxia and mechanical irritation with both low and high LC.

Chapter 7
Study Summary

7.1 Study Summary

The study aimed to answer some questions about the overall safety profile of scleral lenses. Anecdotally, there has been reports of ocular sequelae associated with scleral lens wear despite achieving an ideal central lens-corneal fitting relationship and use of high oxygen permeable material.[162] This suggests that the limbal zone of the scleral lens also plays a key role that can impact the ocular health, both clinically and sub-clinically. This is particularly important in keratoconic cornea, where even a small change in the regulation of cytokines can have a profound impact on the pathophysiology of corneal ectasia.[58,209]

Excessive or inadequate post-lens tear film over the limbal stem cell tissue can result in a disturbance to the ocular health. Despite oxygen supply through the limbal vasculature and tear exchange,[126,253] oxygen delivery is challenged by a post-lens tear film.[138-140] According to a theoretical model, a limbal clearance of 5.40µm or less can reduce hypoxic concern, but inadequate limbal clearance can result in mechanical insult of the ocular surface. The disruption to the corneal epithelium can result in superficial punctate keratopathy, identifiable as positive corneal staining.[141-143] The mean settling in the limbal zone in this study, or the difference between initial and final limbal clearance, was 16.27µm. This means that the appropriate LC could be a minimum of 25 µm to prevent corneal epithelial damage. This suggests that the ideal LC may be different depending on the individual scleral lens wearers. For instance,

in individuals who have an endothelial deficiency, LC should be kept at the minimum suggested.

Overall, this study was able to demonstrate the high clinical performance in comfort and vision that scleral lenses can bring to keratoconic subjects. At the same time, it highlights some physiological changes that present with an ideal central lens-cornea fitting relationship and low and high LC, differing by approximately 50µm. Tear cytokine analysis confirms that scleral lens wear can have an impact on the inflammatory response from the ocular surface, and will change in accordance to varying LC.

7.2 Study Limitations and Future Research

There were several limitations in this study. The primary short-coming was a small sample size of eleven participants. This created a subsequent challenge faced during subject recruitment which was a lack of control over the age of the participants. The wide range of age and stage of KC may have had an influence on the levels of inflammatory mediator found.[71] Further study with an expanded sample size would better illustrate scleral lens changes in different age groups and at different stages of KC. Another limitation of this study included the challenge to remain double-masked. The researcher running the protocol was likely able to observe the LC during the image scan and may have been biased during subsequent testing in the study protocol, such as biomicroscopy. Improvement to the study protocol includes including more researchers to delegate different component of the study protocol. In addition, the study duration (2±1 weeks) may not have been reflective of long-term impact of scleral lens wear. Despite a full-time wear schedule of daily wear exceeding

the normal corneal epi cell lifespan of 7 to 10 days[264], cytokine levels induced by varying LC may not demonstrate changes until beyond one month of lens wear.[237] The long term impact on lens performance and tear cytokine levels beyond this period of time needs to be studied further. Finally, the small tear volumes that were collected from the ocular surface and lens bowl, due to low tear volume[107], high tear osmolarity[227,228], and surface tension[229], posed a challenge for examining a larger array of inflammatory mediators. To overcome the challenges posed by inadequate recovery of tear volume, an alternative tear collection methods that allow for closer proximity to the ocular surface might be a superior method, such as impression cytology with the use of a cellulose acetate filter.[223]

Comments:	
Signed	Date

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Appendix A
Subjective Ratings of Ocular Symptoms

Date _____ Study __Limbal Clearance Study Investigator _____ ID _____ Lens 1 and 2

The following questions relate to a number of symptoms which you may or may not be experiencing with the contact lenses you are wearing in the study. Please select a value **between 0 and 100** which most adequately describes how you feel about your **study lenses** and **enter this in the box next to each question's scale**, R=right eye; L=left eye

1. How would you rate your <u>comfort</u> with your study lenses?

    0   100

    Very poor comfort   **excellent comfort**

    R ☐
    L ☐

2. How would you rate your <u>dryness</u> with your study lenses?

    0   100

    Very dry   **not dry at all**

    R ☐
    L ☐

3. How would you rate <u>burning</u> with your study lenses?

    0   100

    Severe burning   **no burning**

    R ☐
    L ☐

4. How would you rate your <u>clarity of vision</u> with respect to cloudy/filminess (blinking to clear) with your study lenses?

    0   100

    Very poor   **excellent**
    (constantly having to blink to clear)   **(never having to blink to clear)**

    R ☐
    L ☐