Sensitivity Across the Ocular Surface—Fundamental Findings and Clinical Applications

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

Current understanding of sensitivity and sensation experienced across the ocular surface remains limited. This project explored the regional variation of corneal sensitivity and transducer function, interaction of sensory and autonomic nerves in the lacrimal functional unit, and the ocular surface sensitivity in Dry Eye and with silicone hydrogel (SH) lens wear.

Experiments were undertaken, using Belmonte esthesiometer to deliver pneumatic mechanical, chemical and thermal stimuli and Cochet-Bonnet esthesiometer for tactile stimuli, to the cornea and conjunctiva. Psychophysical methods were used to determine the thresholds of stimulus detection, and the magnitude of sensations to suprathreshold stimulation was estimated assuming Steven's power law. Additionally, tear secretion in response to corneal sensory input was determined by tear meniscus height measured using Optical Coherence Tomography.

Sensitivity to pneumatic cool and mechanical stimuli varied slightly across the cornea while chemical sensitivity was not different between regions. The transducer function was also similar between central and peripheral cornea but different between stimulus modalities. In comparison, the reflex tearing response to suprathreshold stimuli was greater with central corneal stimulation. Also, corneal and conjunctival hypersensitivity was found in the dry eye symptomatic group, and it appeared to be associated with symptom severity, tear film stability and corneal epitheliopathy. Refitting with SH lenses after an initial no-lens interval led to increased conjunctival pneumatic mechanical sensitivity, while corneal tactile sensitivity showed a decrease. In addition, corneal staining induced by certain lens-solution combination appeared to be accompanied by increased corneal and conjunctival sensitivity.

In conclusion, the position-invariant corneal sensitivity to pneumatic mechanical, chemical and thermal stimuli suggests that the distribution of human corneal sensory fibres may be more homogeneous than previously hypothesised. The mechanisms mediating the sensory aspect of corneal nociception may be similar across the cornea, while, perhaps due to the importance of the visual axis, the tear reflex response to central and peripheral cornea seems to be driven by different neural circuitry, perhaps at the higher levels of the sensory processing pathway. It appears that alteration in sensory processing of the ocular surface occurs in Dry Eye and accompanies SH lens-solution-induced corneal staining. This altered sensitivity seems to be more prominent in the conjunctiva than in the cornea.

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Dedication

To God Most High, Creator of heaven and earth.

With love and gratitude for their unwavering support, I dedicate this work to my parents, Ruhai Situ and Wanfen Mo.

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List of Symbols and Abbreviations

^	raised to power of
↑	increase
↓	decrease
°	celsius degree
ACh	acetylcholine
ANOVA	analysis of variance
ARVO	The Association for Research in Vision and Ophthalmology
ATD	Aqueous Tear Deficient
BUT	tear film break-up time
C	central cornea
C-B	Cochet-Bonnet esthesiometer
CGRP	calcitonin gene-related peptide
CL	contact lens
CO_2	carbon dioxide
Conj	conjunctiva
CRCERT	The Cooperative Research Centre for Eye Research and Technology
DED	Dry Eye disease
DK	oxygen permeability
Dk/t	oxygen transmissibility
EW	extended wear
FL	fluorescein
HEMA	Polymacon
I	Inferior cornea

IL	Interleukin
K	cornea
KCS	keratoconjunctivitis sicca
LASIK	laser-assisted in situ keratomileusis
LG	lissamine green
LN	natural log transformed
MGD	meibomian gland dysfunction
min	minute
ml	millilitre
mm	millimetre
MPS	multipurpose solution
MUC	mucin
N	nasal cornea
NCA	non-contact esthesiometer
NCCA	non-contact corneal aesthesiometer
NIBUT	non-invasive tear film break-up time
NSDE	non-Sjögren Syndrome Dry Eye
OCT	Optical Coherence Tomographer
OFX	Opti-Free Express multipurpose solution
os	ocular surface
OSDI	Ocular Surface Disease Index
PCV	proportional directional control valve
PHMB	polyhexamethylene-biguanide
PMMA	polymethyl methacrylate

PO	thalamus posterior nucleus
PRT	phenol red thread
PV	PureVision lens
r^2	the square of correlation coefficient
RB	rose bengal
ReNu	ReNu multipurpose solution
RGP	rigid gas-permeable
S	superior cornea
SCL	soft contact lens
SD	standard deviation
SEM	standard error of the mean
SH	silicone hydrogel
SI	primary somatosensory cortex
SII	secondary somatosensory cortex
SS	Sjögren Syndrome
SSN	superior salivatory-facial nucleus
T	temporal cornea
TMH	tear meniscus height
V	trigeminal nerve
V1	the first branch of the trigeminal nerve
V2	the second branch of the trigeminal nerve
VAS	visual analogue scale
Vc/C1	subnucleus caudalis-upper cervical spinal cord junction region
Vi/Vc	trigeminal nucleus interpolaris-caudalis transition region

VIP	vasoactive intestinal peptide
Vsp	spinal trigeminal nucleus
WC	water content
ZI	zona incerta

Chapter 1
General introduction

1.1 Background and research questions

Understanding the neural mechanisms contributing to corneal and conjunctival sensation is essential for gaining insight into more complex issues such as ocular discomfort reported in Dry Eye disease (DED), contact lens wear and post refractive surgery.

The cornea and conjunctiva, which constitute the anterior ocular surface of the eye and are broadly subject to environmental challenges, are densely innervated.[1-4] This sensory innervation serves important reflex functions to protect the eye[5-7] and plays important roles in regulating the secretion of the tear film thus maintaining a homeostatic environment of the ocular surface.[8-11] In addition, sensory nerves contribute to local inflammation that (among other things) follows ocular irritation through the release of peptides and neurotransmitters from their peripheral endings.[12, 13] Furthermore, together with the sympathetic innervation, they exert various "trophic" or nutritive effects on their target cells such as playing roles in epithelial metabolic activity, maintaining the cellular integrity of the epithelium, and promoting wound healing of the injured tissues.[5-7, 14-17]

The innervation of the cornea and conjunctiva is by the peripheral axons of primary neurons from the somatosensory trigeminal system.[6] Sensory nerves enter the cornea and conjunctiva via branches of the ophthalmic division of the trigeminal nerve (V) and subsequently branch extensively.[5-7, 18] The nerves terminate mostly as free endings within the superficial layers of the epithelium,[2, 3, 6, 7] although special nerve endings with morphological structure have been reported in the limbal area.[19] The terminals or receptors of the sensory nerves detect and encode the sensory signals. Different functional types of receptors, such as mechano-nociceptors, polymodal nociceptors and cold receptors, have been identified.[12, 20] These functional types of receptors are preferentially activated by a specific form of stimulus energy and contribute distinctly to various ocular somatosensory sensations.[12]

The measurement of ocular surface sensitivity is one way to assess the functioning of the sensory nerves.[21, 22] Cochet-Bonnet type of esthesiometers[23, 24] has been the most common means for sensitivity measurement. However, this type of esthesiometer suffers from several shortcomings in its design[21, 25-27] and is restricted to touch sensitivity. The recent development of pneumatic

esthesiometers enabling delivery of controlled air pulses at various temperature and mixture of air with CO_2 (to the ocular surface) allows measurements of ocular surface sensitivity over a wider range of thermal, mechanical and chemical stimuli.[28-30] Alterations in the sensory nerve function as reflected by the changes in corneal sensitivity have been reported in various conditions including DED, contact lens wear, corneal disease and post corneal and refractive surgery.[31-38] Corneal sensitivity has been proposed as a useful clinical indicator of corneal health and during the healing process following corneal surgery and disease.[21, 22]

Besides being an indicator of the function of sensory nerves, sensitivity across the cornea has been suggested, perhaps, to represent its neural density.[3] Using Cochet-Bonnet esthesiometry, a marked decline of sensitivity towards the periphery has been reported.[24, 25, 39] This variation in touch sensitivity has been suggested to match the distribution of neural density observed in cats and rabbits almost perfectly− greater neural density towards the center than in the periphery.[3, 40] However, previous data are limited due to the stimuli used. With the advantages of recently developed pneumatic esthesiometers,[28-30] sensitivity can be studied using thermal, mechanical and chemical stimuli. It is unknown whether the variation of corneal sensitivity using different stimulus modality would be similar to that demonstrated with tactile stimuli. Additionally, recent investigations on human corneal nerves in fresh cornea[41] and in living eyes observed using confocal microscopy[42] have revealed a novel arrangement of the subbasal plexus which is dissimilar to that seen in rabbit corneas, suggesting a rather homogeneous distribution of corneal nerves endings in human compared to rabbit.[2, 42] However, the distribution of different functional type of receptors has not been elucidated.

Although the study of corneal and conjunctival sensitivity provides valuable information about the function of sensory nerves of the ocular surface, it could not give a complete picture of the sensory system, because sensitivity measurement reveals only one point on a sensory scale and does not describe the relationship between the value of stimulus magnitude and the resultant sensation.[43] To better understand the transducer mechanism of a sensory system, psychophysical magnitude functions, the relationship between the magnitude of a sensory attribute and corresponding physical values of the stimulus,[44] have been used to explore the relationship between various intensities of stimulus and the elicited sensation of the ocular surface (cornea and conjunctiva).[28, 45, 46] A few investigators have examined the sensation magnitude of the stimulation to central cornea and conjunctiva,[28, 45, 46] but the sensations evoked by mechanical, chemical and thermal stimulation at various locations of the cornea have not been quantified. As mentioned earlier, the uniqueness of

morphological structure of nerve endings in the limbal area has been observed but how this structure relates to the sensation of the cornea remains largely unknown.[19] It is also not known whether the transducer mechanisms for sensation evoked by different modalities of stimuli at various locations, e.g. central vs. peripheral corneal stimulation, would be similar.

The concept of the lacrimal functional unit has been proposed recently, in which the ocular surface including the cornea, conjunctiva, and the meibomian and lacrimal glands (main and accessory glands) acts as a complex integrated functional unit that is interconnected by sensory and autonomic nerves.[11, 47] The functional unit controls the secretion of the three major components of the tear film in a regulated fashion[9, 10] and responds to environmental, endocrine and central neural influences.[8, 11, 47, 48] The overall function of the unit is to maintain the integrity of the tear film and the clarity of the cornea to ensure the quality of the principle optical component imaging objects onto the retina.[8] Dysfunction or disease in any part of the functional unit results in alteration of the tear film and thus compromises the normal functioning of the ocular surface, with one possible outcome being the development of DED.[8, 11, 47-49]

One important aspect of the functional unit is the part played by the sensory nerves of the ocular surface. The sensory impulses arising from the ocular surface have been considered to be the major driving force for tear secretion.[50-52] However, it is not fully understood how sensory input relates to the graded outflow of the efferent autonomic nervous system in the functional unit. In addition, activation of the sensory nerves at the ocular surface may contribute to the symptoms of dry eye. Although both decreased and increased corneal sensitivity have been reported in certain groups of dry eye patients,[32, 33, 53-55] the relationship between ocular surface sensitivity and clinical signs and symptoms of dry eye has not been well established. Moreover, the role of conjunctival sensitivity played in DED has not been studied.

Contact lenses interact with several parts of the lacrimal functional unit, such as the cornea, conjunctiva and the eyelids. Reduced functioning of corneal sensory nerves as measured by corneal sensitivity has been reported in both rigid and soft contact lens wearers,[38, 56-61] although the study outcomes reported in the literature varied due to the differences in lens materials, wearing conditions and measuring methods. The possible mechanisms contributing to this reduction in corneal sensitivity include metabolic alteration related to lens-induced hypoxia and mechanical adaptation.[21, 38, 62-64] However, most of the previous results have been based on wearing earlier generation low oxygen permeability (DK) hydrogel lens materials and the measurements have been largely limited to

tactile sensitivity. The recent developments of highly oxygen permeable silicone hydrogel (SH) lens materials offer certain advantages over traditional hydrogel lenses for contact lens wearers, by eliminating lens-induced hypoxia and producing less detrimental effects on corneal homeostasis.[65] It remains largely unknown whether SH lens wear affects ocular surface sensitivity differently, in particular when lens-induced hypoxia has been reduced or eliminated. Also, it is unclear how tactile, mechanical and chemical sensitivity changes with SH lens wear over time. Moreover, perhaps in relation to the unique nature of SH lens materials, accumulating evidence has shown that the interaction between certain SH lenses and preserved multipurpose solutions (MPSs) affects the ocular surface, manifested as asymptomatic corneal fluorescein staining.[66] Whether the interaction between the lens material and care regimens would have an impact on ocular surface sensitivity remains unclear, although one study has suggested that this may be the case.[67]

To summarize, as described briefly in this section and more detailed in the following chapter, current understanding of sensitivity and sensation across the ocular surface and how they relate to DED and contact lens wear remains limited.

1.2 The objectives and hypotheses of the thesis

This thesis will address the fundamentals of sensitivity across the ocular surface and the relevance to the clinical aspects, in terms of regional variation of corneal sensitivity, transducer function of the central and peripheral cornea, interaction of sensory and autonomic nerves in the lacrimal functional unit, and ocular surface sensitivity in DED and with silicone hydrogel lens wear.

Objectives of the thesis

- to measure corneal sensitivity at multiple corneal positions using pneumatic stimuli, at room temperature and at ocular surface temperature (with and without CO_2 added) delivered by a computer controlled Belmonte esthesiometer;
- to characterize the suprathreshold behaviour with mechanical and chemical stimulation of central and peripheral cornea, and to compare the psychophysical magnitude function for each modality and location;
- to investigate the relationship between mechanical and chemical stimulation at various corneal locations and the efferent output as determined by tear secretion in the lacrimal

functional unit, and to define the psychophysical function of stimulated lacrimation and its relationship to threshold to detect stimulus;

- to investigate conjunctival and corneal sensitivity in subjects with and without symptoms of ocular dryness, stratified by age and gender, and to establish the relationships of dry eye symptoms and corneal and conjunctival sensitivity to pneumatic stimulation, tear film stability and clinical ocular surface characteristics in these subjects;
- to investigate the effects of SH lens wear and lens-solution interactions on ocular surface sensitivity.

Thesis hypotheses

- The corneal sensitivity measured using pneumatic stimuli, at room temperature and at ocular surface temperature (with and without CO_2 added) delivered by a computerized Belmonte esthesiometer decreases with eccentricity;
- The relationship between sensation magnitude and the intensity of stimulus of central and peripheral cornea is a power function. The size of the power exponent of the psychophysical magnitude function is different between the central and peripheral cornea and between stimulus modalities;
- Tear secretion induced by mechanical and chemical stimulation is monotonically related to the intensity of the stimulus applied. The tearing induced by suprathreshold stimuli is different between stimulus modality and positions. The stimulus intensity required to induce reflex tearing is higher than the intensity to elicit a sensation;
- Corneal and conjuncitval sensitivity is different between subjects with and without symptoms of ocular dryness, age groups and gender. There is a relationship between dry eye symptoms and corneal and conjunctival sensitivity to pneumatic stimulation, tear film stability, and clinical ocular surface characteristics;
- Corneal and conjuncitval sensitivity is not constant during SH lens wear and is affected by the interaction between SH lens and MPS.

Chapter 2
Review of literature

2.1 Sensory innervation of the ocular surface

2.1.1 Sensory innervation of the cornea

The sensory innervation of the ocular surface originates as the peripheral axons of the primary sensory neurons located in the ipsilateral trigeminal ganglion.[1] The innervation is particularly rich in the cornea, which makes it the most densely innervated epithelial surface in the body.[2]

Corneal sensory nerves are predominately derived from the ophthalmic branch of the trigeminal nerve.[3, 4] Nerve bundles penetrate the cornea at about the mid-stromal level of the periphery in a radial fashion.[5, 6] Some smaller branches that run in the subconjunctival and episclera tissues enter the peripheral cornea superficially.[7]

When they enter corneal stroma, the nerve bundles lose their perineurium and myelin sheath within approximately 1 mm of their entrance from the limbus perhaps to facilitate corneal transparency.[5-7] The stroma nerve trunks divide repeatedly into smaller branches as they course anteriorly and form the sub-epithelial plexus that lies at the interface between Bowman's layer and the anterior stroma.[6]

Nerve fibres in the sub-epithelial plexus penetrate Bowman's layer and run parallel to the corneal surface as single or multiple leashes, between Bowman's layer and the basal epithelial cell layer.[6] The leashes consisting of mixture of straight and beaded nerve fibres form the sub-basal plexus, and the beaded fibres then form branches that enter the more superficial corneal epithelium where they terminate eventually as free nerve endings.[4] In the peripheral cornea, sensory nerves enter epithelium directly from the conjunctival plexus near the limbus.[7]

Recent studies have revealed a novel distribution of the sub-basal nerve plexus in human cornea. The sub-basal nerve plexus appears to converge to a whorl-like pattern in inferior-central cornea, and outside this area the nerve fibre bundles in the remainder of the cornea were arranged in a parallel pattern.[8, 9] The density of the sub-basal nerve bundles therefore seems to be equal over a large central to peripheral area,[6, 8, 9] which is different to the gradually decreased neural density from the center toward the limbus found in rabbit corneas with a radiating density pattern.[2] Thus, evidence suggests that in humans there is a more homogeneous distribution of nerve endings over the central and central-peripheral cornea.[4]

2.1.2 Sensory innervation of the conjunctiva and eyelids

Similar to the cornea, the conjunctiva receives sensory nerves from a plexus of sensory fibres originated mainly from the divisions of ophthalmic nerve, with a small portion from the maxillary nerves.[10, 11] The bulbar conjunctiva is supplied by the long ciliary nerves,[12] the lacrimal and frontal (supraorbital division) branches of the ophthalmic nerve, and the infraorbital division of the maxilliary nerve.[13] Sensory nerves traverse under the epithelium and in the stroma (substantia propria). Most conjunctival sensory fibres terminate as naked nerve endings as in the cornea, but some with encapsulated endings. This special type of ending is particularly numerous in the limbus (the palisade zone).[14]

2.2 The physiology of the ocular surface sensory innervation

The primary sensory neurons that innervate the cornea and conjunctiva can be classified as finely myelinated (Aδ type) or unmyelinated (C type) fibres, based on their size, presence of a myelin sheath around the axon and conduction velocity.[15] In human cornea, the majority of the fibres are of C type.[6] The peripheral axons of the sensory neurons (primary afferents) terminate as naked free nerve endings and are called nociceptors.[16]

The areas of the ocular surface covered by individual afferent fibres sensitive to stimulation are known as the receptive fields and stimulation within the receptive field results in depolarization of receptors and impulse firing of the afferent fibre. Receptive fields of individual corneal neurons vary in size ranging from 1 to 100 mm^2.[5, 7, 17, 18] A typical receptive field in cat or rabbit cornea identified using mechanical stimulation (mechanoreceptive field) extends over 5-20% (10-20 mm^2) of the corneal surface.[5, 17] In most cases (70% of the neurons in cat cornea), receptive fields extend to the adjacent limbus and bulbar conjunctiva, while about 30% with small receptive fields are restricted to the cornea.[5] The adjacent receptive fields overlap extensively.[5, 19]

2.2.1 Functional type of primary ocular sensory neurons

Electrophysiological recordings of single sensory neurons innervating the eye have been carried out in cat and rabbit.[3, 5, 17, 20-25] According to their responsiveness to different stimulus modalities (i.e. mechanical, chemical and thermal), subclasses of functional type of neurons that are preferentially activated by a specific type of energy have been identified. Generally, the responses of corneal sensory neurons are either unimodal or bimodal/polymodal. Three types of unimodal corneal neurons, including cold sensitive, mechanosensitive and chemosensitive units that are sensitive to

single modality of stimulation, have been described in rabbits,[24, 25] although "pure" chemosensitive units have not been found in cats and their existence in the rabbit cornea has been questioned.[26] Other neurons that respond to both high-threshold mechanical and high-temperature stimuli (bimodal units) and to all three types of stimulation (polymodal units) have been identified in rabbit and cat,.[21, 24, 25, 27]

About 20% of corneal fibres, all finely myelinated (Aδ type), are classified as mechno-nociceptors.[5, 24] They are activated only by mechanical forces of the magnitude that could damage the cornea.[5, 17, 20, 24, 25, 28] The mechano-nociceptors are rapidly adapting or phasic sensory receptors as they respond to brief or sustained stimuli with one or several short-lasting impulse discharges.[3, 17, 20, 24, 25, 28] They may be responsible for the acute, sharp pain produced by a mechanical contact with the corneal surface.[3]

Cold-sensitive receptors are another subclass of corneal sensory fibres that represent 10-15% of the total population, and belong to Aδ and C type fibers.[5, 24, 25] They discharge spontaneously at rest and increase firing rate in response to the subtle reduction in normal temperature of the cornea, while they become transiently silent when the temperature increases.[17, 22] The cold-sensitive receptors are able to detect and encode the intensity of the stimulus, responsible for the non-noxious cooling sensation resulting from reduction of corneal temperature.[29, 30]

The majority of corneal sensory fibres (about 70%) are classified as polymodal nociceptors.[3] They are equally responsive to near-noxious mechanical energy, heat or noxious cold and chemical irritants, and are also activated by a large variety of endogenous chemical mediators released by damaged tissue, inflammatory cells, or extravasated plasma.[27, 31] Polymodal nociceptors are found on both Aδ and C corneal fibres but most of them are of C type.[3] In response to their natural stimuli, polymodal nociceptors give a continuous, irregular impulse discharge that persists with the full length of the stimulus duration and the firing frequency of the discharge is roughly proportional to the intensity of the applied stimulation.[3] Compared to mechano-nociceptors, polymodal nociceptors have a slightly lower mechanical threshold.[20, 29] The polymodal nociceptors may not only signal the presence of a noxious stimulus but also encode the intensity and duration of the stimulus.[3] Polymodal nociceptors are considered to be the principal source of nerve impulse activity induced by chemical irritant, heat and noxious cold, in addition to their contribution to the sensation evoked by mechanical force.[3, 5] They probably also account for the after-sensation of pain elicited by noxious stimuli.[3]

It has been suggested that mechanically insensitive 'silent' nociceptors may exist in the cornea.[24] This type of receptor is activated only in the presence of local inflammation, responding to mechanical and thermal stimuli as well as a variety of endogenous chemicals.[3]

Although most electrophysiological studies identifying different types of sensory receptors have been carried out on the cornea, the same functional classes of sensory receptors have been also reported in the bulbar conjunctiva[20] and other ocular structures.[5] In addition, a small number of low threshold mechanosensory receptors has been found in the bulbar conjunctiva in cat, immediately adjacent to the cornea. These receptors have very small receptive field and are restricted to the limbal border responding to sustained mechanical stimulation but not to heat or cold.[5]

2.2.2 Higher-order ocular neurons

Information from stimuli encoded by sensory receptors is carried centripetally by trigeminal ganglion neurons to the higher levels in the central nervous system. The spinal trigeminal nucleus (Vsp) in the lower brain stem is the first site for synaptic integration of peripheral signals from the craniofacial regions in which second-order neurons are activated.[32] The ocular surface is represented mainly in two spatially distinct regions of the Vsp: The trigeminal nucleus interpolaris-caudalis (Vi/Vc) transition and subnucleus caudalis-upper cervical spinal cord (Vc/C1) junction regions.[33-35] The second order neurons responsive to noxious mechanical, chemical or thermal stimuli have been found in both Vi/Vc and Vc/C1 regions.[36, 37] The unique dual presentation of trigeminal organization has lead to a suggestion that neurons in Vi/Vc and Vc/C1 regions process corneal input differently; Vi/Vc corneal neurons may play a role in specialized ocular functions such as blink and tear reflexes and represent an endogenous antinociceptive control pathway while Vc/C1 neurons may mediate sensory-discriminative aspects of pain sensation.[38] The dense connection fibres between the two regions may serve to recruit and organize output from neurons located at Vi/Vc and Vc/C1.[38] Recently, a specific set of neurons that may be involved in reflex tearing and fluid homeostasis of the ocular surface have been identified at the Vi/Vc region.[39]

The second-order neurons in the Vsp, carry nociceptive information from the ocular surface to the contralateral thalamus.[40] Neurons that receive corneal projection have been identified in the thalamus posterior nucleus-zona incerta (PO/ZI) area and the superior salivatory-facial nucleus region (SSN) of the brainstem.[41] The distribution of these neurons varies and is associated with the input from the sensory modality of the peripheral nociceptor.[41] For example, CO_2-responsive rostral unit (in the Vi/Vc region) and caudal units (in the Vc/C1 region) project within PO/ZI or to SSN, whereas

among the units *not* responded to CO_2 (i.e. mechanical only), rostral units project only to SSN and caudal units only project to PO/ZI.[41]

From the thalamus, sensory information arising from the trigeminal system reaches several areas of the cortex. Ocular sensory presentation in the cortex has been described in primary (SI) and secondary (SII) somatosensory area, as well as in the area 3b and 1 in the lateral sulcus, mainly on the contralateral side.[13, 15] The great complexity and diversity of the central projections of the trigeminal neuclei, will not be discussed further here.

2.3 The lacrimal functional unit and the roles of sensory afferent nerves

2.3.1 The lacrimal functional unit

The concept of the lacrimal functional unit was proposed in 1998 to describe the relationship between the ocular surface and lacrimal glands and provide a framework for understanding how the integrated system malfunctions in dry eye patients.[42] The lacrimal functional unit comprises the ocular surface (cornea, conjunctiva, and meibomian glands), the lacrimal glands (both the main and accessory), and the sensory afferent and efferent sympathetic and parasympathetic nerves that connect them (Figure 2-1). Acting through the central nervous system, the components of the functional unit are linked into a homeostatic loop (i.e. a relatively stable state of equilibrium between the different components in the functional unit) by a complex sensory, sympathetic and parasympathetic neural network.[43-45] The function of this integrated unit is to regulate tear film secretion and respond to environmental, endocrine and central neural influence, for the purpose of protecting and maintaining the health of the ocular surface.[43-45] Dysfunction of any part of the integrated unit may lead to the alteration of the other components, compromising the ability of the functional unit to respond to environmental and physiological changes.[42, 43, 45]

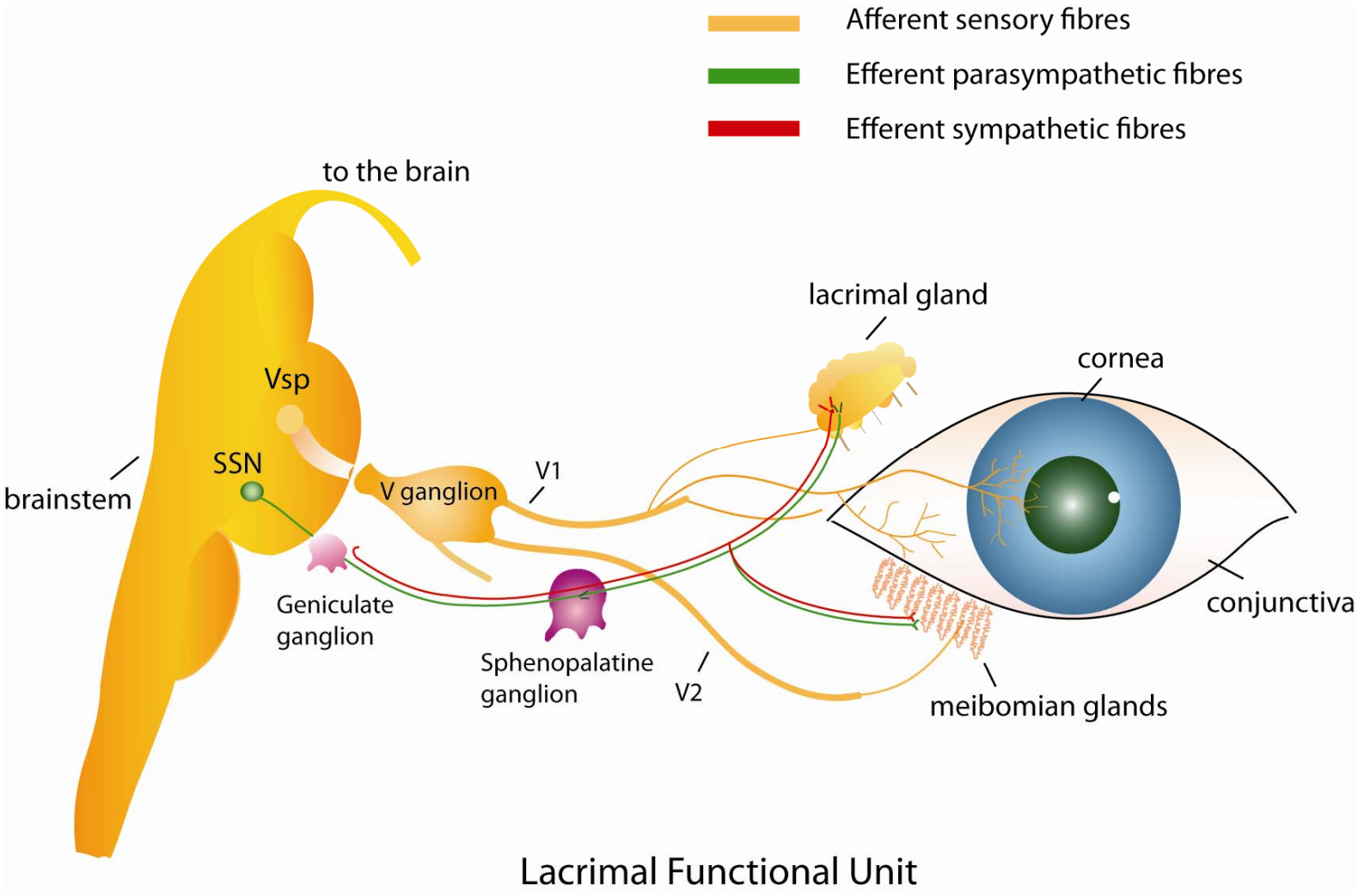

Figure 2-1 Lacrimal functional unit

2.3.2 Secretion of the tear film and its neural regulation

Traditionally, the pre-corneal tear film has been described as a structure with three distinct layers: the mucin layer coating the epithelial surface, the aqueous layer making up the majority of the tear film, and the thin anterior lipid layer slowing evaporation.[46, 47] Recent studies have suggested that the components of mucus, protein, and aqueous in the pre-corneal tear film mix to form a hydrated gel decreasing in density toward the superficial lipid layer.[48, 49]

The mucins in the tear film are hypothesised to play integral roles in ocular surface lubrication, anchoring of the aqueous components of the tears and stabilizing the lipid components.[50] They also function to prevent adherence of inflammatory cells, foreign bodies and pathogens to the ocular surface.[51] Ocular mucins can be classified as membrane-associated and secreted and have been numbered in the order of their molecular characterization, for example MUC1, MUC2 etc. The membrane-associated (or transmembrane) mucins include MUC1, MUC4 and MUC16 have been reported to be expressed by ocular surface epithelia and the lacrimal gland.[52] They are concentrated on the tips of the microplicae, facilitating the formation of glycocalyx at the corneal and conjunctival epithelia and tear film interface, and may also be involved in the epithelial cell cycle and apoptotic activity.[50] The secreted mucins are further sub-classified as gel-forming and soluble on the basis of their ability to form polymers. The gel-forming mucins such as MUC5AC are secreted by the conjunctival goblet cells while the small soluble mucin MUC7 is produced by acinar cells of the lacrimal glands and conjunctiva.[53] Although the expression of ocular mucins is not fully understood, evidence has suggested that activation of nerves induces goblet cell secretion.[54] It has been hypothesised that activation of sensory nerves of the ocular surface may in turn, activate the parasympathetic and sympathetic nerves surrounding the goblet cell and the subsequently released neurotransmitters stimulate secretion of the goblet cell.[54, 55]

The aqueous component of the tear film contains not only water, but also numerous electrolytes, proteins (including peptide growth factors), vitamins, anti-microbial, cytokines, immunoglobulins, and hormones, all of which serve to maintain a trophic and protective environment for the ocular surface.[51] The main and accessory lacrimal glands are the major contributor to the aqueous phase of the tear film, and the secretion of the main lacrimal gland is controlled by parasympathetic and sympathetic nerves.[56] Acting through the central nervous system, the afferent sensory nerves in the functional unit signal the input from the ocular surface, and this in turn, activates the efferent parasympathetic and sympathetic nerves in the lacrimal gland resulting in the release of

neurotransmitters.[57, 58] The major neurotransmitters that lead to the secretion are acetylcholine (ACh) and vasoactive intestinal peptide (VIP) released by parasympathetic nerves, as well as the sympathetic neurotransmitter norepinephrine.[56] The ACh and norepinephrine interact with specific receptors present on the surface of lacrimal gland cells to activate distinct but overlapping signal pathways leading to lacrimal secretion.[57] Although the mechanisms that underlie the secretion of water, electrolytes and protein of the lacrimal gland are different, they are under tight neural control through the lacrimal functional unit.[56] Besides the lacrimal glands, both cornea and conjunctiva are additional sources of electrolytes and water.[51] The conjunctiva also transports fluid into and out of the tear film and modifies the composition by absorbing or secreting water, electrolytes and proteins including mucins.[54] The sympathetic nerves stimulate the conjunctival stratified squamous cells to secrete electrolytes and proteins.[54]

The most superficial layer of the tear film is the lipid layer that minimises tear evaporation, spreads over the surface of the tear film, and interacts with the aqueous layer and other tear film components to provide a smooth optical surface for the cornea.[59] The lipid layer also prevents the skin-surface fatty acids such as sebaceous lipids from entering the tear film and the spilling of tears onto the skin.[51] The major tear lipid is meibomian lipid that is the secretion of meibomian gland, with additional amounts produced by the glands of Moll and possibly the lacrimal glands and glands of Zeiss.[59] It has been proposed that the structure of the lipid layer has two phases; the thicker outer layer containing nonpolar lipids such as wax, esters, sterol esters, hydrocarbons, and triglycerides, and the thin inner polar meibomian lipids that are mainly phospholipids.[60] The nonpolar lipid phase determines the function of retarding evaporation but its functional integrity is dependent on the stability of the underlying polar lipids.[60] The polar lipids serve as a surfactant that mixes with the non-polar lipids and interacts with the aqueous phase of the tear film.[51] The innervation of the meibomian glands is not completely understood but abundant parasympathetic and sympathetic nerves have been found contacting the meibomian glands.[61, 62] Although sensory innervation is suggested by substance P- and calcitonin gene-related peptide (CGRP)-positive axons, the role of sensory input in controlling lipid secretion is unclear.[63] Despite the principal control of the meibomian glands appearing to be hormonal, neural regulation may also be involved in stimulating lipid secretion onto the ocular surface.[43]

2.3.3 The roles of sensory input and the two states of the functional unit

As discussed in the preceding section, the sensory innervation of the ocular surface, together with parasympathetic and sympathetic nerves, regulates the secretion activities of the lacrimal and meibomian glands and the goblet cells. Thus, the secretion of major tear film components is controlled by the lacrimal functional unit in a coordinated fashion.

The part that sensory signals arising from the ocular surface plays is an important aspect of the functional unit.[44] It is hypothesised that the neural pathway to control tear production is a reflex arc, involving the activation of the afferent sensory nerves and the efferent autonomic nerves.[56, 64-66] Sensory signals arising from the ocular surface are carried centripetally by trigeminal ganglion neurons. The afferent signals then pass to the superior salivatory nucleus (SSN) located in the brainstem, and efferent fibres extend, via the facial nerve, to the pterygopalatine ganglion where postganglionic fibres arise that terminate in the lacrimal glands and other ocular surface tissue.[42, 44] To protect the ocular surface from the spectrum of challenging situations, it has been suggested that the lacrimal functional unit may operate differently depending on environmental conditions and pathology.[43] Under normal condition (without stressful stimulation), sensory nerves on the ocular surface provide sub-threshold sensory signals to the functional unit regulating resting tear flow, whereas noxious stimuli activate sensory afferents in the functional unit triggering a series coordinated reflexes including reflex lacrimation to protect the eye from potential damage.[43] Very few studies have investigated the relationship between sensory stimulation of the ocular surface and tear secretion.[67]

2.4 Psychophysical measures of sensory threshold and magnitude

2.4.1 Classical methods of threshold measurement

The relationship between a physical stimulus and the internal sensory and perceptual response can be determined by psychophysical methods.[68] The sensory threshold is an important theoretical concept used in psychophysics that refers to a limit separating stimulus values that elicit a sensation from those do not elicit a sensation. The absolute detection threshold is defined as the minimum amount of stimulus energy required to produce the first detectable sensation under ideal conditions.[69] Due to the random fluctuation in the stimulus and in the activity levels of the neuron, and subject's attention and psychological bias, threshold values show some variability from trial to trial within a measurement session and across several sessions. As a result, a carefully measured threshold

becomes the probability of detecting various levels of the stimulus in order to estimate a statistical value that best describes threshold under a particular set of conditions. Typically, in a "yes-no" trial, threshold has been defined as the stimulus value where the subject detects the stimulus 50% of the time.[69] Recognizing the statistical nature of the thresholds and the necessary methodological consequences, G.T. Fechner[68] developed three methods of threshold measurement: The methods of constant stimuli, limits, and adjustment.

The method of constant stimuli is the procedure using a fixed set of stimulus values (usually between five and nine different values in the set), covering a range such that the lowest values are expected to be slightly lower than the threshold and would almost never be detected, while the highest values are expected to be slightly higher than the threshold and should almost always be detected.[69] Each stimulus is presented many times and in a random order and the subject is asked to report whether the stimulus is detected after each stimulus is presented. If the probability of detection (often the percent "yes" responses) is plotted against the stimulus intensity (called a psychometric function or probability of detection curve), a threshold from this typically S shape or *ogival* curve can be determined.[69] The method of constant stimuli is considered to provide a highly accurate estimation of the absolute thresholds when it is used properly.[69] However, it is subject to the effects of psychological bias such as guessing and in addition, because this method requires stimuli to be presented many times, it is quite time consuming and rarely used in clinical measurements.[69]

In the method of limits, the stimulus value is first presented well above or below threshold; on each successive presentation, the stimulus value is systematically changed step by step until the transition stimulus value where the response changes from "yes" to "no" or vice versa, is reached.[69] The stimuli could be presented as either an ascending or descending series. If the series is ascending, the stimulus value is started below the expected threshold and increased step by step until the subject eventually reports the presence of the stimulus, whereas if the series is descending, the stimulus value is decreased in successive steps until the subject do not detect the stimulus. The series terminates at this transition point and the ascending and descending procedures are repeated several times. The average of the transition points obtained from all the series is the threshold. Although the method of limits is perhaps less accurate than the method of constant stimuli, it is an efficient and frequently used technique for estimation of sensory thresholds.[69] It is also used to estimate the location of the threshold when choosing the stimulus values before applying the method of constant stimuli.[69]

The method of limits usually gives satisfactory results if the two potential errors with the method are properly controlled.[69] The first is an error of expectation in which the subject may falsely anticipate the arrival of the stimulus at threshold level and prematurely report the change of sensation that has occurred before it actually happens.[69] This will affect the result by giving a deceptively low threshold on ascending trials and a falsely high threshold on descending trials. The second error is habituation which is opposite to the first type of error inasmuch as it affects the result by falsely increasing thresholds on ascending trials and decreasing thresholds on descending trials.[69] To some extent, the tendency of expectation and habituation can be corrected by varying the starting point for successive series.[69] In an attempt to avoid the limitation of the method of limits, a few variations such as staircase methods have been developed.[70]

The method of adjustment is generally easily used when the stimulus values can be changed continuously.[69] Similar to the method of limits, the starting stimulus value in the method of adjustment is set either far below or above the threshold. The subject is asked either to increase the stimulus level until it is just detectable, or to decrease the stimulus level until the sensation disappears. The trials will be repeated many times and the mean of these trials is taken as the threshold. The method of adjustment is a simple and direct method for estimating threshold and gives the subject active participation in the measurement. As with the method of limits, the potential errors of expectation and habituation can be corrected by randomising the starting point when using this method.[69]

However, all three classical psychophysical methods have a drawback of lacking controls on observer response criteria.[69, 71] The threshold estimated using these methods may not be truly free of the effects of response bias, the tendency that subjects favour one response over another, based on the factors other than the stimulus itself.[69, 71] Forced-choice procedures[70] and signal detection theory methods[72] have been developed to overcome the weaknesses of the classical psychophysical techniques of determining sensitivity.

2.4.2 Measuring the magnitude of sensations

Although the study of sensitivity by measuring thresholds as described in the previous section provides valuable information about the senses, it measures only one point on a sensory scale in units of stimulus energy but not sensation units.[69, 73] It does not reveal the relationship between the value of stimulus magnitude and the resultant sensation and thus alone, could not give a complete picture of a sensory system. Because sensation changes are not usually linear with the changes in the stimulus,

the relationship between sensations and stimuli has to be determined experimentally. The plot of the magnitude of a sensory attribute against corresponding physical values of the stimulus is called psychophysical magnitude function (or transducer function).[69]

Psychophysical magnitude functions can be obtained only when it is possible to measure both the stimulus and its resultant sensation response. Many techniques for scaling of sensory magnitude have been developed to measure the relationship between the magnitude of sensation and stimulus strength,[74] generally classified into three groups: (a) those that require the subjects to make simple ordinal discrimination judgments between stimuli; (b) those that require subjects to adjust stimuli to partition the sensory continuum into equal sensory intervals; (c) those that require subjects to assign numbers to stimuli that presumably represent sensation magnitude or to adjust stimuli to match numbers presented by the experimenter.[74]

One of the most frequently used scaling methods in psychophysics is magnitude estimation,[74] in which the subject is required to make direct numerical estimations of the sensory magnitudes produced by various stimuli. There are mainly two ways of applying the magnitude estimation technique to construct a psychological scale. In the first one, the subject is presented with a reference stimulus and told that the sensation it produces has a certain value (modulus), for example 100. Subsequently, a series of test stimuli are presented and the subject is asked to assign a number to these stimuli to indicate their perceived magnitudes relative to value of the modulus.[69] In the other variation of the method, no defined modulus is given to the subject,[69] and a series of test stimuli that vary in intensity or other dimensions are randomly presented, and the subject assigns a number to the elicited sensation.

The measurement of sensation magnitude is a fundamental problem in psychophysics. In 1860, Fechner[68] founded psychophysics and developed the procedures to measure sensation. He also suggested that sensation magnitude increased with the logarithmic increase of the stimulus intensity.[68] Fechner's logarithmic law was challenged by S. S. Stevens in 1950s, primarily based on magnitude-estimation data.[75] Stevens[75] proposed that sensory magnitude was proportional to the stimulus intensity raised to an exponent and this later became known as Stevens' power law. This law is expressed as

$$\psi = \kappa \varphi^{\alpha},$$

where ψ (psi) is the sensory magnitude, κ (kappa) is a constant determined by the absolute size of assigned numbers, φ (phi) is stimulus intensity and α (alpha) is an exponent that is characteristic of the stimulus used. Several studies have investigated the suprathreshold exponent values of stimuli delivered to the central cornea and conjunctiva,[76-79] mainly using visual analogue scaling,[80] a method in which the subject places a mark on a line with two anchors, one at each extreme, e.g. extreme pain to no pain.

2.5 Ocular surface sensitivity

Measuring sensitivity is one way to assess the functioning of the sensory nerves. In the experiments described in this dissertation, the function of the ocular surface is reflected by the responses to controlled corneal and conjunctival stimulation. Several types of esthesiometers have been developed to measure ocular surface sensitivity[76, 81-88] and a number of studies have investigated corneal and conjunctival detection thresholds with various stimuli, different types of esthesiometers and psychophysical methods.[76, 81-84, 86, 89-93] However, the body of work examining conjunctival sensitivity is much less comprehensive compared to the cornea. Corneal sensitivity has been proposed as a useful clinical indicator of corneal health in diseases, contact lens wear, and during the healing process following various corneal injuries and refractive surgery.[91, 94-103]

2.5.1 Measurement of ocular surface sensitivity

2.5.1.1 Cochet-Bonnet type of esthesiometers

The first esthesiometer used to quantify corneal sensitivity was built by von Frey[83] in 1894, using calibrated horse hairs of different lengths attached to glass rods. Based on von Frey's concept that the force produced by a long hair axially on the corneal surface is proportional to the diameter and the length of the hair, Boberg-Ans[81] invented a device using a single nylon thread of constant diameter but of varying length to measure sensitivity; this was further improved by Cochet and Bonnet.[82]

Cochet-Bonnet esthesiometers have used hair or nylon filaments of variable diameter and length to deliver tactile stimuli to the ocular surface. There are two models of Cochet-Bonnet esthesiometers. The first model using a 60 mm nylon monofilament of 0.08 mm in diameter produces pressures ranging between 2 and 90 mg per 0.005 mm^2 on the surface. However, this model has not been used commonly due to difficulty in obtaining the 0.08 mm nylon filament and is no longer manufactured. The second model uses a nylon monofilament, 60 mm in length and 0.12 mm in diameter, and this

produces pressures ranging from 11 to 200 mg per 0.0113 mm^2 (Figure 2-2). Most of the sensitivity results reported in the literature have been obtained with this model, and Cochet-Bonnet esthesiometers are the most commonly used esthesiometers in clinical practice because of convenience and relative ease of use.[98]

However, the Cochet-Bonnet esthesiometers have certain limitations[85, 88, 104] including (i) a truncated range of stimulus intensities; (ii) poor stimulus control. It is difficult to align the nylon thread with the ocular surface (particularly the 0.8 mm diameter filament) and control the amount of the force applied (i.e. making sure that the 5° of bend in nylon thread for which the instrument is calibrated is occurring). Factors such as gravity, previous use, and the ambient humidity also affect the bend of the thread; (iii) potential disruption of the epithelial surface by the stimulus; (iv) patient apprehension resulting from perceived invasiveness. Each of these disadvantages of the Cochet-Bonnet esthesiometer could affect threshold accuracy and reproducibility.

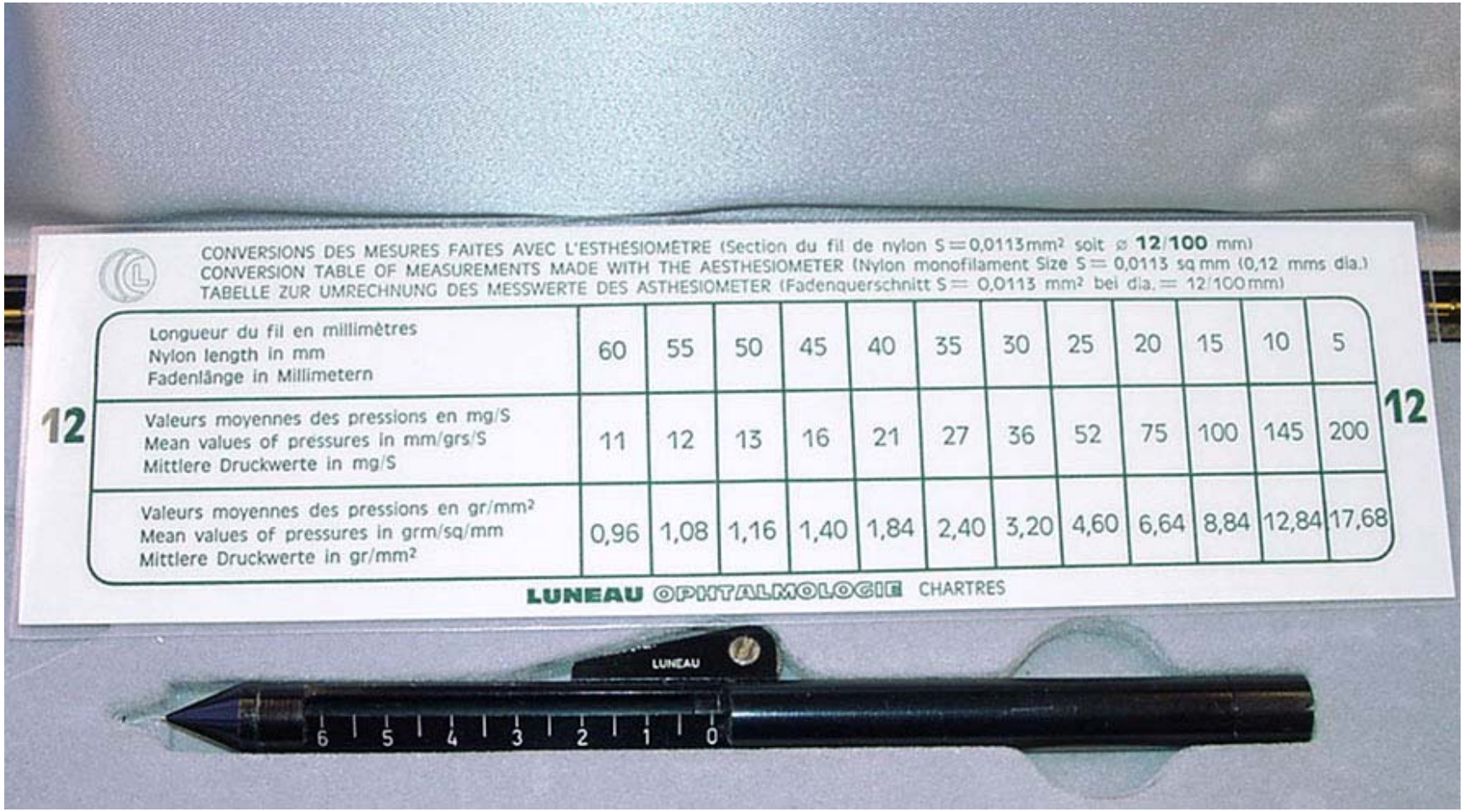

Figure 2-2 Cochet-Bonnet esthesiometer

2.5.1.2 Pneumatic esthesiometers

To overcome the weaknesses of the Cochet-Bonnet esthesiometers, more sophisticated devices have been developed, which presumably provide greater precision.[83, 85-88, 105] These devices have used various types of probes directly placed on the cornea surface or an air jet to determine the threshold for a localized mechanical force. However, these devices are limited to measuring mechanical sensitivity. Although a temperature-controlled saline jet esthesiometer and a CO_2 laser esthesiometer have been developed to measure thermal sensitivity, they have not been widely applied.[106, 107]

Recently, the Belmonte esthesiometer was developed. This delivers air pulses of controlled flow rate, temperature and air-CO_2 mixtures to the ocular surface, allowing measurement of sensitivity over a range of mechanical, thermal and chemical stimuli.[76] The Belmonte esthesiometer consists of two gas cylinders, one containing medical grade compressed air and one of 98.5% CO_2, connected through two pressure regulators and two unidirectional regulators to an electronic proportional directional control valve (PCV) (Figure 2-3). The PCV adjusts the flow of air and CO_2 separately, producing gas mixtures with a controlled proportion of CO_2 and air. The final flow of the gas mixture is adjusted with a flowmeter and supplied to a probe with internal diameter 0.8 mm on a mount with fine position control. The probe contains a temperature controlling device comprising a thermode, a servo-regulator, a Peltier cell that warms the gas and a solenoid valve to control the output of gas. A CO_2 meter monitors the CO_2 concentration of the gas mixture. During stimulation, the gas is transiently directed to the tip of the probe by changing the direction of flow from the PCV. A pulse with a defined CO_2 concentration, temperature and flow rate from the tip of the probe flows towards the ocular surface for specific intervals (ranging typically from 1 to 10 seconds).

A modified Belmonte esthesiometer was manufactured at CRCERT at the University of New South Wales.[108, 109] The design (Figure 2-4) is similar to the original device but with different electronic flowmeters and temperature controllers, a heating coil at the tip of the probe to keep the stimulus delivered approximately at corneal temperature and a smaller inner diameter (0.5 mm) of the probe. The temperature sensor provides feedback to maintain a steady temperature independent of air flow and ambient temperature. The modified Belmonte esthesiometer is mounted in an American Optical non-contact tonometer housing with an optical range finder to allow a “precise” stimulus distance control from the corneal apex.[108, 109]

Using the CRCERT- Belmonte esthesiometer as a platform, a computer controlled Belmonte esthesiometer has been developed at the University of Waterloo, with computer controlled mixing of

air and CO_2 flowrate and temperature.[110-112] Additionally, a video camera continuously monitors the distance between, and orthogonal alignment of the tip of the esthesiometer and the ocular surface. Custom software has been developed for the selection of stimulus attributes and psychophysical method used. Subject responses are recorded using a button box (Figure 2-5).

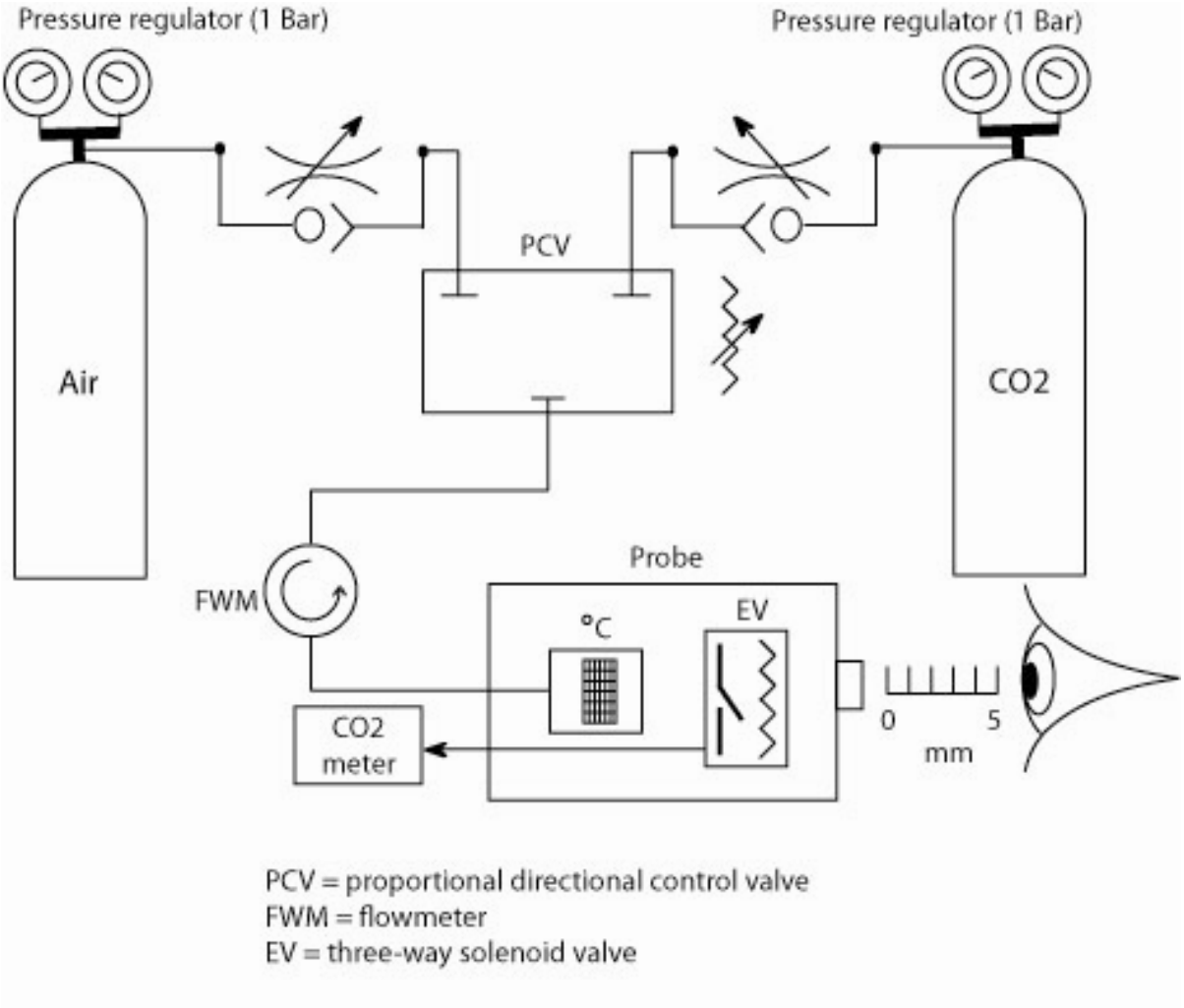

Figure 2-3 The design of Belmonte esthesiometer (adapted from Belmonte et al 1999[76])

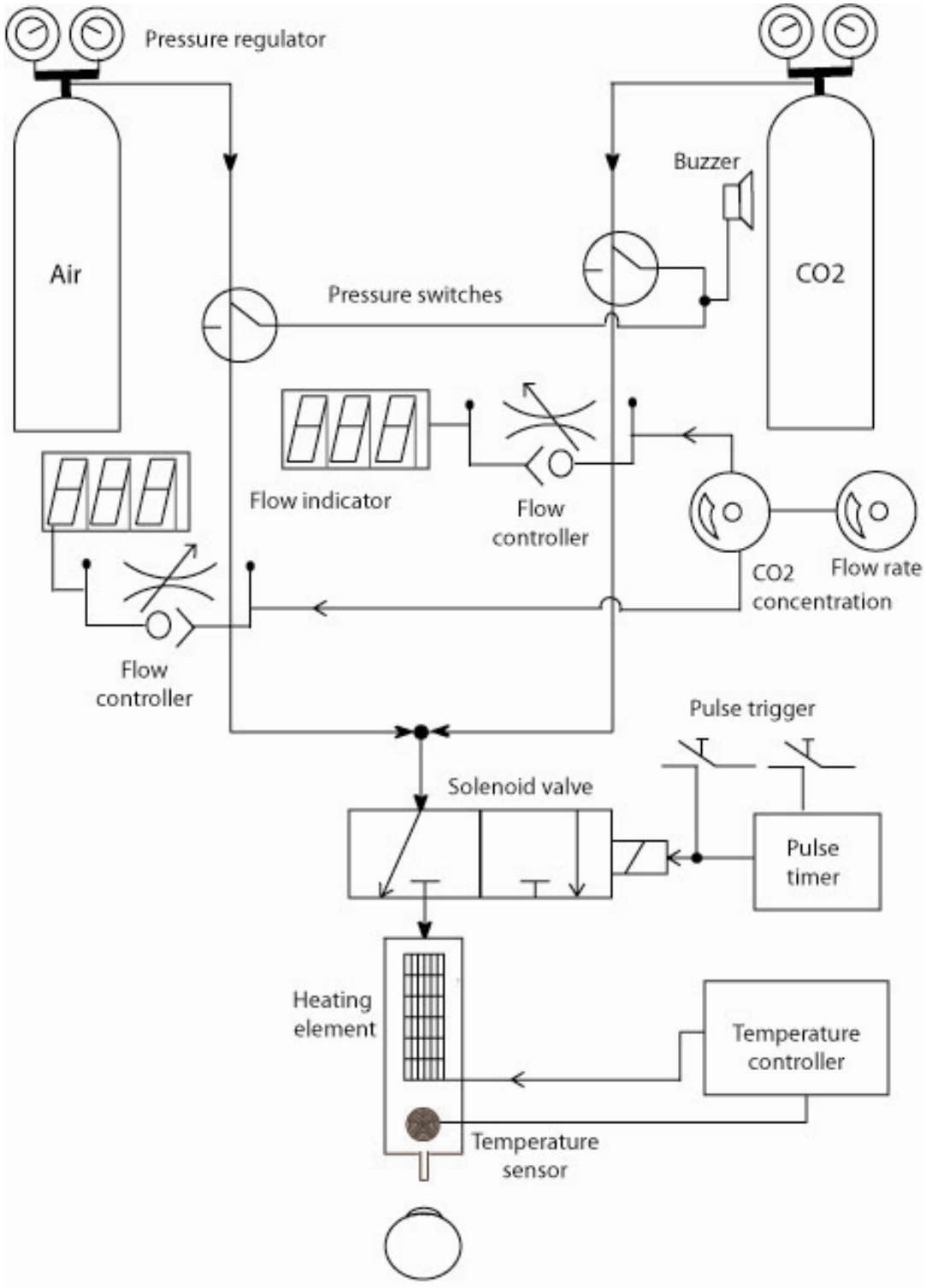

Figure 2-4 The modified Belmonte esthesiometer (adapted from Stapleton et al 2004[107])

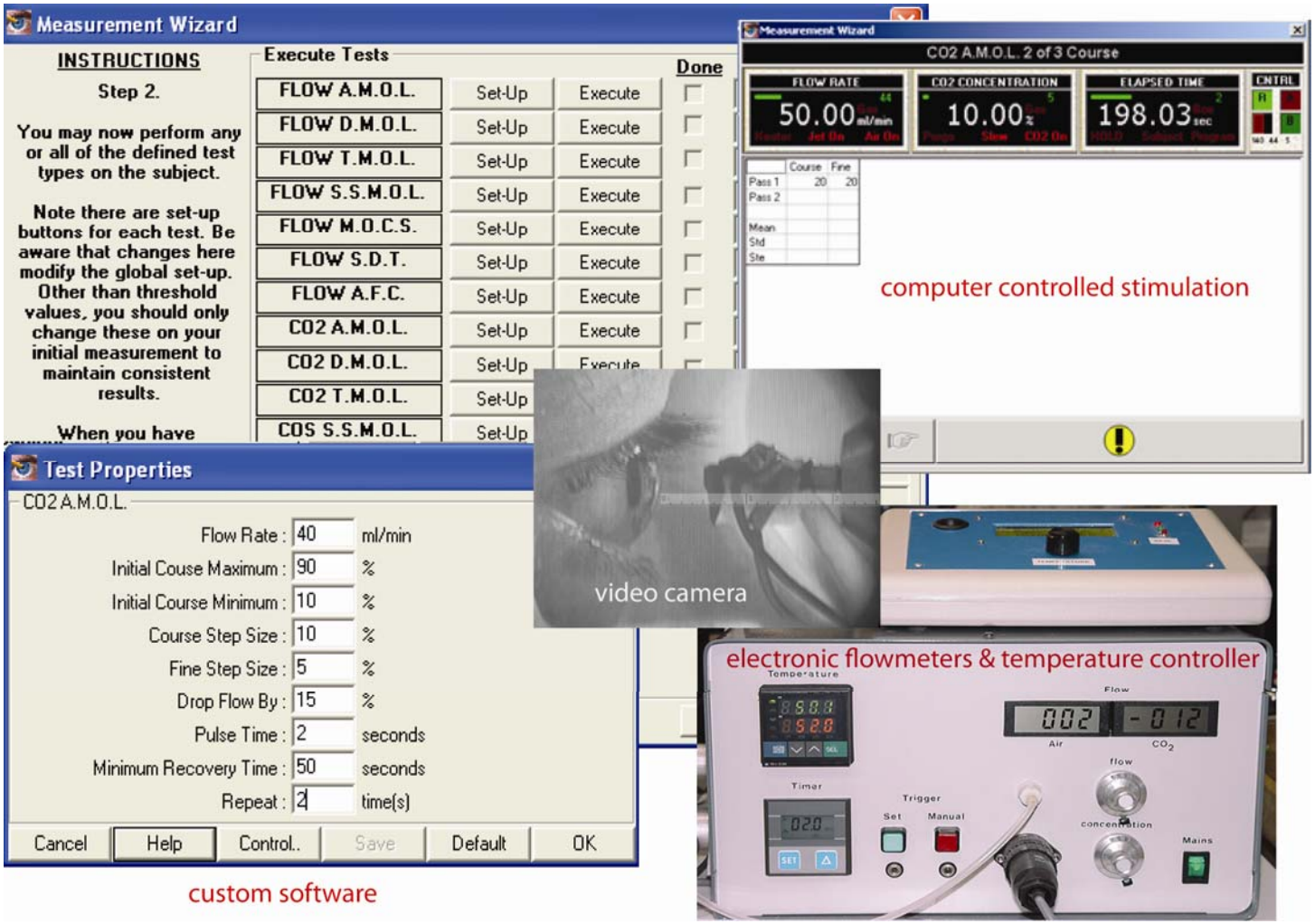

Figure 2-5 Computer controlled Belmonte esthesiometer

2.5.2 Physiological variation of ocular surface sensitivity

2.5.2.1 Regional variation

A few studies have examined sensitivity across the cornea and adjoining tissues. Due to the predominant use of the Cochet-Bonnet esthesiometer, most published reports are of touch sensitivity, showing that it is higher in the center and declines significantly towards the corneal periphery.[81, 82, 92, 113] The distribution of the corneal sensitivity across the periphery is not uniform with the lowest being the superior region that is usually covered by the upper eyelids,[81-83, 113] (although a few investigators have reported slightly different patterns).[83, 114] The regional variation of corneal sensitivity described in these studies seems to match the density of corneal innervation obtained in rabbits and cats.[2, 115] Using this type of device, conjunctiva has been shown at least eight times less sensitive than the cornea.[83] Interestingly, the variation of corneal sensitivity across the ocular surface, measured using pneumatic esthesiometers has been found to be less compared to that measured using Cochet-Bonnet esthesiometers.[90, 108, 116] Little or no difference between corneal and conjunctival sensitivity has been reported, especially when a cooling stimulus is applied.[86, 89, 90, 93, 108]

Although there are fewer studies on conjunctival sensitivity, some regional variation of conjunctival touch sensitivity has been reported. Sensitivity of the lid margin is higher than in the bulbar and tarsal conjunctiva areas[92] and temporal limbal conjunctiva has been found to be more sensitive than inferior limbal conjunctiva.[117]

2.5.2.2 Other physiological variation

Besides the regional variations, a few studies have reported a decrease of corneal and conjunctival sensitivity with age,[92, 103, 118, 119] although other investigators using pneumatic stimulus have not found an association between age and corneal sensitivity.[95, 120] Investigators using the Cochet-Bonnet esthesiometer have reported a significant reduction in corneal sensitivity after the fifth decade of life,[114, 119] while Murphy et al[121] showed a decline in sensitivity to an air-jet stimulus as early as after the second decade of life. It has been speculated that this loss of corneal sensitivity with the passing years may be due to the reduction in innervation density of the cornea and associated with the development of arcus senilis.[113, 122]

Gender has been considered to be another factor attributing to the variation of corneal and conjuncitval sensitivity. Although hormonal changes occurring as part of the menstrual cycle or

pregnancy had been reported to induce fluctuations of corneal sensitivity in females,[123, 124] reports of gender differences in corneal and conjunctival sensitivity have varied between studies.[113, 120] In a recent study, no overall gender differences in pneumatic corneal mechanical and chemical sensitivity was reported, but mechanical sensitivity in the sub-group of premenopausal women (<55 years) was higher than males of similar ages.[118]

Corneal sensitivity has been reported to vary with time of day, being the lowest in the morning upon eye opening and the highest in the evening.[108, 125, 126] Within the first four hours following eye opening, sensitivity has been shown to return to the baseline measurement.[125, 126] Although the basis of this overnight reduction in corneal sensitivity is not fully understood, hypoxic change of the cornea induced by eyelid closure has been hypothesised to be the primary cause.[127]

Eye color is a physiological factor that has been reported to affect corneal sensitivity. Some investigators have found association between corneal sensitivity and iris color but not others.[110, 118, 128, 129] The basis of the differences is not clear. For example, Millodot who initially reported the eye color effect, examined inter-ocular differences in sensitivity in subjects with heterochromia-iridis and found none.[130]

2.6 Ocular sensitivity in Dry Eye disease

2.6.1 Corneal sensitivity in Dry Eye disease

Corneal sensitivity has been measured using Cochet-Bonnet and pneumatic esthesiometers in dry eye patients.[95, 102, 122, 131-136] Both corneal hypersensitivity and hyposensitivity in dry eye patients have been reported in the literature.

Using a Cochet-Bonnet esthesiometer, Xu et al[102] first reported *decreased* corneal sensitivity in both Sjögren Syndrome (SS) and non-SS Dry Eye (NSDE) groups. They found that corneal sensitivity was positively correlated to tear function index but inversely correlated to ocular surface conditions determined by Rose Bengal (RB) and fluorescein (FL) scores. Later, Pflugfelder et al[134] showed a *decrease* in corneal touch sensitivity in SS Aqueous Tear Deficient (ATD) and meibomian gland dysfunction subgroups but not in NSDE group. The *reduced* corneal sensitivity has been reported to be similar between primary and secondary SS patients.[136] In a study investigating the correlation between dry eye symptoms and objective clinical signs in SS patients, Adatia et al[131] found that corneal sensitivity was inversely correlated with both FL and lissamine green (LG) staining of the ocular surface but the overall symptom severity was inversely correlated to lissamine

green staining of the cornea. However, no significant correlation between sensitivity and dry eye symptoms was found.

Using a Belmonte esthesiometer, reports on sensitivity change in dry eye patients have been less clear. de Paiva and Pflugfelder have compared corneal sensitivity to mechanical and chemical stimuli (air jet without and with CO2 added, respectively) in normal, dry eye patients, and post LASIK patients with and without DED.[95] They found that patients with DED and post-LASIK with DED had an *increase* in sensitivity to the pneumatic mechanical stimuli. A significant inverse correlation was reported between mechanical thresholds and the severity of corneal FL staining. Conversely, Bourcier et al[133] have shown *decreased* corneal mechanical and chemical sensitivity in dry eye patients compared to controls. A positive significant correlation between thresholds and corneal staining scores (FL and LG) (without providing data on the strength of the correlation) was reported but no correlations were found between thresholds and tear film break-up time (BUT) and Schirmer score. A significant correlation between thresholds and the intensity of symptoms of burning and stinging was reported but the strength of the correlation was not reported in this paper. They also found that the differences in threshold between SS and NSDE were not significant. When comparing corneal innervation and sensitivity in primary SS and in NSDE, Benitez-del-Castillo et al[122] reported *decreased* corneal mechanical, chemical and thermal sensitivity in dry eye patients and a correlation between the density of subbasal nerves and sensitivity. Sensitivity was correlated with Schirmer test scores, RB staining and symptoms. In a recent study, Tuisku et al[135] provided evidence that primary SS patients had *increased* corneal mechanical sensitivity, alterations in corneal nerve morphology (nerve sprouting and thickened stromal nerves) and an increased number of antigen-presenting cells. However, the corneal nerve density in these patients was found to be similar to that in controls. They also found that corneal mechanical sensitivity appeared to be correlated with corneal staining scores and subjective symptoms evaluated using Ocular Surface Disease Index (OSDI) and visual analogue scales, but not with corneal nerve density.[135]

The contradictory results in corneal sensitivity measured using pneumatic esthesiometer do not seem to be easy to reconcile. The reasons for these discrepancies remain unknown and more studies are needed to better understand the part played by ocular surface sensitivity in the natural history of different forms of DED, the role of sampling and the many (non-standardised) technical differences in pneumatic esthesiometry that are used in these experiments.

Table 2-1 is a summary of reported corneal sensitivity in Dry Eye.

Table 2-1 Summary of reports of corneal sensitivity in Dry Eye

Author(s)	sample	Instrument	Corneal sensitivity	note (correlations)
Xu et al (1996)	59 DE (15 SS and 44 non-SS); 26 control	C-B	↓ in both DE groups vs. control	↓ sensitivity - ↑ staining scores (RB and FL)
Pflugfelder et al (1998)	40 DE (20 ATD: 11 SS & 9 non-SS; 20 MGD); 10 control	C-B	↓ in S-ATD and both MGD DE groups vs. control non-S-ATD vs. control: no difference	N/A
Adatia et al (2003)	18 SS	C-B	No value reported	↓ sensitivity - ↑ staining scores (FL and LG); ↓ LG staining - ↑ overall symptom severity
Benitez del Castillo et al (2004)	21 DE (11 SS, 10 non-SS); 21 control (11 <60 y, 10>60 y)	C-B	Appeared ↓ DE vs. control no statistical difference between groups	No r or plots presented (reported correlations between sensitivity vs. Schirmer's, RB staining, and # sub-basal nerves)
Villani et al (2007)	35 SS (15 SSI and 20 SSII); 20 age/gender matched control	C-B	↓ in SS group vs. control Similar between SSI vs. SSII	No r or plots presented (correlation between sensitivity vs. fiber tortuosity)
De Paiva & Pflugfelder (2004)	DE n=20; Post-LASIK:20 non-DE, 6 DE; 20 control	Modified Belmonte esthesiometer	Mechanical:↑ in DE vs. control & ↑ in post-LASIK DE vs. non-DE Chemical: no difference	↑ mechanical sensitivity - ↑ FL staining severity
Bourcier et al (2005)	44 DE; 42 control	Belmonte esthesiometer	Mechanical, chemical & thermal: ↓ in DE group vs. control	SS vs. non-SS: no difference ↓ sensitivity - ↑ staining scores
Benitez del Castillo et al (2007)	21 DE (11 SS, 10 non-SS); 20 control (10 <60 y, 10>60 y)	Belmonte esthesiometer	Mechanical, chemical & thermal: ↓ in DE groups vs. controls	↓ sensitivity - ↓ Schirmer's test; ↓ sensitivity - ↓ # sub-basal nerves; ↓ sensitivity - ↑ symptoms

Tuisku et al (2008)	20 DE (primary SS); 10 age/gender matched control	Modified Belmonte esthesiometer	Mechanical: ↑ in SS vs. control	↑ mechanical sensitivity - ↑ FL staining, VAS and OSDI scores no correlation sensitivity vs. nerve density; alterations in corneal nerve morphology (nerve sprouting & thickened stromal nerves) ↑ antigen-presenting cells

2.6.2 Conjunctival sensitivity in Dry Eye disease

Even though the conjunctiva is not only supplied by free nerve endings, as in the cornea, but is also involved in the secretion of major components of the tear film, there are no reports of conjunctival sensitivity in DED in the literature.

2.7 Contact lens wear and ocular surface sensitivity

2.7.1 Contact lens wear and corneal sensitivity

Studies have shown that contact lens wear reduces corneal sensitivity.[101, 113, 137, 138] The degree of reduction in sensitivity may depend on lens material, the length of lens wear during the day and over the long term, and lens wearing modality (i.e. daily vs. extended wear).

Early reports were of marked reduction in corneal sensitivity after both short (a few hours) and longer-term wear of polymethyl methacrylate (PMMA) lenses.[83, 100, 139] The magnitude of sensitivity loss with longer wear of PMMA lenses may relate to chronic lens use and has been found to be more pronounced in the corneal center than in the periphery.[139, 140] Similar to PMMA lens wear but to a lesser extent, the wearing of rigid gas-permeable (RGP) and soft (HEMA) contact lenses has been reported to induce decreased touch sensitivity of the cornea.[83, 99, 101, 141-143] The effect of RGP contact lens wear on corneal sensitivity may depend on the DK of the materials[113, 141, 143] inasmuch as it has been reported that RGP lenses with greater permeability produced a smaller reduction in touch sensitivity than occurs with PMMA lens wear.[113] With soft lens wear, the extent of the sensitivity reduction may vary depending on the water content of the lenses[99, 137] and the central cornea has been reported to be less affected than the periphery.[137] On the other hand, soft lens wear not affecting sensitivity has also been reported.[144]

The effects of contact lens wear on corneal sensitivity measured using pneumatic stimuli have varied. Murphy et al[101] have reported reduction in corneal sensitivity to a cooling stimulus in long term soft and RGP contact lens wearers compared to the non-lens wear group, whereas du Toit et al[120] have found no change after non-contact lens wearers wore hydrogel lenses for six months. Stapleton et al[108] recently investigated the effect of short-term hydrogel and SH lens wear on corneal and conjunctival sensitivity of un-adapted lens wearers. They found no changes in corneal sensitivity after 6 hours of hydrogel or SH lens wear compared to no lens wear,[108] and they previously showed no change of the central cornea but an increase in sensitivity of the inferior cornea in long term soft

contact lens wearers.[145] On the other hand, Feng[112] found increased corneal sensitivity (using pneumatic stimuli at ocular surface temperature) in un-adapted lens wearers after wearing toric hydrogel lenses for 1, 3, and 6 hours and the sensitivity returned to baseline measurement by 2 weeks. In addition, no association between the duration of lens wear and the magnitude of sensitivity reduction has been reported.[101] The topographical variation in sensitivity reduction has been shown to be similar between long term soft and RGP lens wear.[101]

2.7.2 Recovery of reduction in corneal sensitivity after cessation of contact lens wear

The decreased corneal sensitivity associated with contact lens wear has been shown to be reversible and it has been reported that sensitivity returned to normal levels after lens wear was ended.[113] The time course for recovery of corneal sensitivity varied depending on the duration of wear and the type of lens worn. Millodot[146] found that following 8 hours of PMMA lens wear, a significant recovery of touch sensitivity occurred within 1 hour after lenses were removed, while the recovery following long term PMMA lens wear took longer (i.e. months) and was related to the number of years of lens wear.[100] In another investigation, a partial or complete recovery of sensitivity occurred one week after refitting long-term PMMA wearers with RGP lenses.[141] Using a saline jet stimulus, it has found that the recovery of thermal sensitivity after 8 hours of PMMA wear took 24 hours.[147] Similarly, a rapid recovery of touch sensitivity following soft lens wear has also been reported; sensitivity showed full recovery within four hours after lens removal.[99, 137]

2.7.3 Interaction of contact lens and care solution and corneal sensitivity

A recent study described a clinical investigation of two contact lens care products and their relationship to corneal sensitivity, comfort and corneal staining and suggested that reduction in touch sensitivity was associated with the use of certain lens care product and decreased comfort with lens wear.[148]

2.7.4 Contact lens wear and conjunctival sensitivity

Investigations on the effect of contact lens wear on conjunctival sensitivity are scarce. Using the Cochet-Bonnet instrument, decreased lid margin and tarsal conjunctival sensitivity as a result of PMMA, RGP and hydrogel contact lens wear have been reported.[149, 150] In contrast, increased inferior bulbar conjunctival sensitivity to pneumatic stimuli has been noted in un-adapted lens wearers after 1, 3 and 6 hours of toric hydrogel lenses wear[112] and in short-term wearers of high oxygen

transmissibility (Dk/t) lenses.[108] Similar findings have been reported in lens wearers who had worn low Dk/t lens for longer and who were symptomatic of ocular discomfort.[145, 151]

Table 2-2 is a summary of reported corneal and conjunctival sensitivity with contact lens wear.

Table 2-2 Summary of reported corneal sensitivity with contact lens wear

Author(s)	Lens type & wearing mode	sample	Instrument and stimulus	Sensitivity	note
Boberg-Ans (1955)	PMMA	9	Boberg-Ans (tactile)	↓ after 1-2 hrs of wear	No details presented
Knoll & Williams (1970)	Soft & rigid lenses	11 rigid and 19 soft lens wearers 20 control	C-B	↓ rigid lens group vs. SCL and control	SCL group vs. control: no difference
Millodot (1974)	SCL	Adapted wearers (1 day - 3 month)	C-B	↑ immediately after 8 hrs of wear; ↓1 hr after removal	N/A
Millodot (1976)	PMMA	Adapted wearers	C-B	↓ after 8 hrs of wear	N/A
Millodot (1978)	PMMA	1-22 yrs of hard lens wear (n=91)	C-B	↓ related to the years of lens wear	N/A
Larke & Hirji (1979)	SCL EW	57 in 3 groups: solution; no solution Controls (specs)	C-B	↓ in lens wear vs. control	N/A
Bergenske & Polse (1987)	RGP	10 adapted PMMA wearers	C-B	↑ after refitting with RGP	N/A
Velasco et al (1994)	SCL 8 hrs of wear	Adapted wearer 44 (38% WC) 23 (55% WC)	C-B	↓ (38%WC > 55%WC; 8 hrs > 4 hrs; N/S/I >C/T)	Full recovery 4 hrs after lens removal
Sanaty et al (1998)	PMMA	20 adapted wearer; 20 age-gender matched controls	C-B	↓ vs. controls (central > peripheral)	Related to the years of lens wear

Tan et al (1997)	12 hrs of wear	12 symptomatic & 12 asymptomatic	NCA	cool stimulus: ↑ to in symptomatic (K & Conj)	(room & os temperature) os temperature no different
Stapleton et al (1999)	Daily & EW	14 lens wearers 14 age-gender matched controls	Modified Belmonte esthesiometer	↑ in CL wear vs. control (inferior K & Conj)	(os temperature) Central K: lens wearers vs. control no difference daily vs. EW: no difference
Murphy et al (2001)	RGP & Soft	40 each lens group (adapted wearers) 40 controls	NCCA	↓ in CL wear vs. control	(room temperature) No relationship between sensitivity and duration of wear
du Toit et al (2001)	SCL 6-month DW	100 neophyte	Non-contact esthesiometer	No change after 6 months of wear	(room temperature)
Feng (2003)	SCL (toric)	48 neophytes	Modified Belmonte esthesiometer	↑ after 1, 3 and 6 hours of wear and returned to baseline by 2 weeks	(os temperature) Both K and conj sensitivity
Stapleton et al (2004)	SCL & SH (6 hrs of wear)	10 neophytes	Modified Belmonte esthesiometer	↑ conj with SH wear vs. no lens and low DK wear	(os temperature) No difference in K sensitivity
Epstein (2006)	SCL + 2 MPSs	8 wearers	C-B	↓ ReNu vs. OFX use	N/A

2.7.5 Possible mechanisms related to altered sensitivity with contact lens wear

The mechanisms of corneal sensitivity change in contact lens wearers are not fully understood. Possible factors that may partly account for the loss of corneal tactile sensitivity in contact lens wear have been proposed, including sensory adaptation to mechanical stimulation, metabolic impairment of the cornea due to hypoxia and corneal acidosis suppressing nerve function. Additionally, it has been speculated that the stromal acidosis and hypoxia caused by contact lens wear[152, 153] may produce structural or morphological changes in corneal nerves which may also contribute to the reduction in corneal sensitivity.[91]

The first experiment to explore the underlying basis of the reduction in corneal sensitivity with contact lens wear was carried out by Polse.[154] He found that corneal edema induced by wearing 100% nitrogen gas goggles for 2 hours was not accompanied by altered corneal sensitivity. There was however decreased sensitivity without corneal swelling when PMMA lenses were worn.[154] He therefore suggested that sensory adaptation to mechanical stimuli but not hypoxia might contribute to the reduction of sensitivity in contact lens wearers.[154] An early study also showed that the lenses producing less mechanical stimulation gave rise to a smaller decrease in corneal sensitivity.[150]

Because the recovery of sensitivity in contact lens wear has been reported to be longer when compared to adaptation in the other sensory systems (i.e. hours vs. minutes), Tanelian and Beuerman[147] suggested that the reduction in corneal sensitivity could not be attributable to simple adaptation, reflecting a decrease in peripheral neural activity as a result of continuous stimulation. They postulated that the reduction in corneal sensitivity in contact lens wear may involve functional changes in the corneal sensory receptors resulting from altered metabolism in the receptor or epithelium of the cornea, or due to central habituation (which they believed was unlikely).[147]

On the other hand, studies have also shown corneal sensitivity decreasing under overnight closed eye conditions[125, 127] or exposure to reduced partial pressure of atmospheric oxygen.[155] Millodot[143] was the first to hypothesise that a metabolic disturbance due to hypoxia of the cornea accounted for the loss of sensitivity with contact lens wear. He compared the effect of three rigid lenses with different oxygen permeability and concluded that epithelial oxygen availability was related to the changes in corneal sensitivity.[156] Moreover, Millodot and O'Leary[155] investigated the latency of reduction in sensitivity caused by hypoxia and suggested that the period of anoxia in Polse's experiment (2 hours) may have been too short to induce a reduction in sensitivity. Additional

evidence, such as improved corneal sensitivity after switching from PMMA to RGP lens wear[141] and the effect of lens water content on the magnitude of sensitivity reduction,[137] support the hypothesis that oxygen availability is related to the decline of sensitivity with contact lens wear.

How decreased oxygen to the cornea directly affects the function of corneal nerves is not clear. It has been proposed that hypoxia may disturb the synthesis of acetylcholine (or acetyltransferase, the enzyme used to synthesize acetylcholine) and this may affect the activity of the corneal nerves.[113]

In addition to the mechanical and metabolic theories, alteration in the pH of the cornea has been suggested to be a cause of sensitivity reduction.[91] Corneal stromal acidosis resulting from accumulation of CO_2 and lactate under the contact lens could lead to a depression of nerve function.[152, 153]

Recently, using in vivo confocal microscope, studies have demonstrated no differences in the morphology and distribution of corneal nerves and keratocyte density between contact lens wearers and controls.[157, 158] The investigators suggested that the decreased corneal sensitivity in contact lens wearers appeared not to be accompanied by decreased nerve density in the cornea.[157, 158]

The current understanding of the effects of contact lens wear on the sensory aspect of the ocular surface and the clinical implications of these changes is still limited. The effect of SH lenses is largely unknown and how these new materials and/or lens care regimens affect the function of ocular surface nerves still have to be investigated.

Chapter 3

Variation of corneal sensitivity across the cornea

Chapter 3 was published as follows:

Eccentric variation of corneal sensitivity to pneumatic stimulation at different temperatures and with CO_2

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	Situ	Simpson	Fonn
Experimental design	Y	Y	-
Data collection	Y	-	-
Data analysis	Y	Y	-
Write-up publication	Y	Y	Y

3.1 Overview

The purpose was to measure corneal sensitivity at multiple corneal positions using pneumatic stimuli, at room temperature and at ocular surface temperature (with and without CO_2 added), in fifteen healthy participants. Sensitivity of central, mid-peripheral, and peripheral cornea was measured using a computer-controlled modified Belmonte esthesiometer to deliver pneumatic cool (air at 20°C), mechanical (air at 50°C), and chemical stimuli (air at 50°C with CO_2 added). The ascending method of limits and method of constant stimuli were adopted to determine the threshold to these stimuli at each location. Sensitivity across the cornea using pneumatic stimuli at different temperatures and chemical stimuli varied only slightly. These patterns of variation are different to what has been previously reported using Cochet-Bonnet esthesiometry.

3.2 Introduction

The cornea is richly supplied by sensory nerves and is one of the most sensitive tissues of the body.[1, 2] Corneal nerves have protective functions and also play important roles in maintaining a healthy ocular surface and corneal wound healing.[3, 4] Alteration of corneal nerve function has been reported among contact lens wearers and those who have undergone refractive surgery.[5-10] Advances in dry eye research have also suggested a possible link between corneal neural function and the integrity of the ocular surface.[11-14]

Corneal sensory nerves are primarily from the ophthalmic division of the ispilateral trigeminal nerve, via the anterior ciliary nerves, and the nerve plexus of the surrounding conjunctiva.[15] After entering the cornea, the nerve bundles branch extensively and terminate within the superficial layer of the cornea as free nerve endings with large overlapping receptive fields.[16-18] On the basis of electrophysiological findings in cat and rabbit cornea, different modality-specific corneal sensory fibres have been identified,[16, 19-23] for example, mechano-nociceptors, polymodal nociceptors and cold receptors. Mechano-nociceptors, which belong to the group of Aδ fibres, respond to mechanical forces and are most likely responsible for producing acute, sharp pain by a mechanical contact with the corneal surface. Polymodal nociceptors, on both Aδ and C fibres, are responsive to noxious mechanical energy and to chemical and temperature changes.[19-22, 24, 25] The cold receptors on C fibres (and perhaps Aδ), signal downward temperature variation in the non-noxious range.[26, 27]

One function of corneal nerves can be evaluated by measuring corneal sensitivity. This is an important clinical indicator of corneal health and the healing process following various corneal

injuries and refractive surgery.[28, 29] Until recently, corneal sensitivity has been measured with tactile stimuli, usually delivered by a Cochet-Bonnet esthesiometer. Using this device, corneal sensitivity varies as a function of corneal eccentricity; the sensitivity is greater in the centre than in the periphery.[30-32] The variation of corneal sensitivity described in these studies is limited to touch only, because of the stimulus. The recent development of pneumatic esthesiometers[33-35] has allowed measurements of corneal sensitivity over a range of stimuli comprising mechanical, chemical and thermal. In addition, using this type of device, a number of studies have reported the sensory experiences evoked by different modalities of stimulation of the human cornea.[24, 36, 37] However, there are no reports of corneal sensitivity to pneumatic mechanical and cool, and chemical stimuli at various locations of the cornea. It is unknown whether the variation of corneal sensitivity demonstrated with tactile stimuli would be similar if different stimulus modalities were used.

In the present study, we therefore used a Belmonte esthesiometer [33] to deliver pneumatic mechanical and cool, and chemical stimuli to various positions on the cornea and examined the variation of corneal sensitivity to these different types of stimuli. These variations were also compared to those previously reported using the tactile stimuli of a Cochet-Bonnet esthesiometer.

3.3 Methods and Materials

3.3.1 Subjects

15 healthy participants, consisting of 6 females and 9 males, aged from 21 to 46 (mean ± SD, 30.2 ± 8.3) were studied. They had no history of eye or systemic diseases or surgery. Two of them had previously used daily wear contact lenses but had not been wearing them for at least one month prior to their participation. This study followed the tenets of the Declaration of Helsinki for research involving human subjects and received the clearance from the University of Waterloo, Office of Research Ethics (Waterloo, Ontario, Canada).

3.3.2 The Belmonte Esthesiometer

The design principle of Belmonte Esthesiometer has been previously described in detail.[33] A computer-controlled modified Belmonte Esthesiometer was used to deliver the pneumatic mechanical and cool, and chemical stimuli. In brief, this instrument contains computerized flow controllers mixing air and CO_2 as well as controlling stimulus temperature. A video camera continuously monitored the distance between, and orthogonal alignment of the tip of the esthesiometer and the

ocular surface. Custom software was used to select stimulus duration and record subject responses from a button box.

3.3.3 Procedures

Measurements were performed on the right eye only, at the central (C), nasal and temporal mid-peripheral (N2 and T2, pupil edges under room illumination, approximately 2-2.5 mm from the apex) and peripheral cornea (N1 and T1, approximately 1 mm to the visible margin between transparent cornea and limbus) along the horizontal meridian (Figure 3-1) for the pneumatic mechanical and cool sensitivity experiments, and at three locations (N1, C and T1) for the chemical experiment. Subjects were instructed to view fixation targets at 3 meters and the tip of the esthesiometer was rotated to ensure the stimulus was delivered perpendicular to the corneal surface regardless of eccentricity. The tip of the esthesiometer was 5 mm from the surface of the cornea and this was continuously verified using the video camera (set using the optical focusing mechanism in the esthesiometer mount eyepiece).

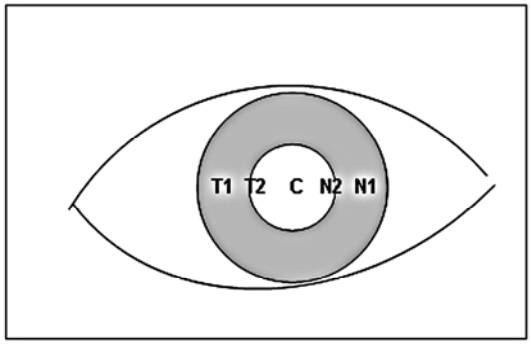

Figure 3-1 The stimuli location for T1, T2, C, N2 and N1

Corneal thresholds with different stimuli were measured in three separate sessions at approximately the same time of the day. All measurements were conducted at least four hours after subjects awoke.

The pneumatic mechanical and cool stimuli consisted of a series of air pulses with flow rate varying from 0 to 200 ml/min. Chemical stimulation was induced by increasing the concentration of CO_2 in the air. The stimulus temperature was set at 20°C (room temperature) for pneumatic cool stimuli (a mechanical stimulus with cooling effect),[34, 35, 37-41] and at 50°C which was approximately 33°C at the ocular surface [24, 33, 36] to eliminate a thermal effect for the pneumatic mechanical and

chemical stimuli. The stimulus duration was one second with an interval between stimuli after the subject's responses of 10 seconds for the pneumatic cool and mechanical stimulation and 45 seconds for the chemical stimulation (to allow for purging of previous gas mixture). Two seconds prior to each stimulus, a short tone was given by the computer to instruct the subject to blink and look at the fixation target. The subject was informed that they were to blink freely and also to close their eyes or look down between stimuli. Subjects could interrupt the trials if necessary.

Using the ascending method of limits,[42] a gross threshold for the pneumatic mechanical or cool stimulus was first estimated at each location. On the basis of this, the stimulus intensities for a method of constant stimuli[42] were selected to determine pneumatic mechanical and cool thresholds. Six levels of stimuli (ranging around ± 25 ml/min of the initial gross threshold) were randomly presented at each location and each presented 4 times. The midpoint (50%) and the slope of the psychometric function were estimated using nonlinear regression fits of the logistic function: Likelihood of detection = 1 – (1 / (1 + (Stimulus intensity / Threshold) ^ Slope)). In the chemical sensitivity experiment, the air flow was set at half of the initially estimated mechanical threshold and then CO_2 was added in increments of 3% CO_2. The ascending method of limits as described previously was used to determine the chemical threshold (to minimise subject fatigue resulting from the long two-step procedure in the pneumatic mechanical and cool sensitivity experiment). The chemical threshold was the average of three first reports of stimulus detection.

3.3.4 Data analyses

Statistical analyses were performed using Statistica 7.0 (StatSoft Inc. Tulsa, OK USA). Data were expressed as mean ± SEM. Repeated Measures ANOVA and post hoc Tukey tests were used to compare the differences in thresholds and slopes from the psychometric functions at each location. Significance was set at $p \leq 0.05$.

3.4 Results

Chemical thresholds did not differ with location ($F_{(2, 28)} = 1.38$, $p > 0.05$). For pneumatic mechanical and cool experiments, ANOVA showed significant differences in thresholds between locations ($F_{(4, 56)} = 5.98$, $p < 0.01$). The differences in threshold between locations were not affected by gas temperature ($F_{(4, 56)} = 0.60$, $p > 0.05$, Figure 3-2). Tukey HSD tests showed that threshold to pneumatic cool stimuli at T1 was different to other locations except for location C (all $p < 0.05$), and there was a

difference between C and N2 (p=0.05). For mechanical threshold, only the difference between T1 and N2 was significant (Tukey HSD test, p=0.05).

For pneumatic cool and mechanical stimulation, there was no difference in the slope of psychometric function between locations and no interaction between location and temperature ($F_{(4, 56)}$= 0.85 and 1.76 for location and interaction between location and temperature, respectively, all p>0.05).

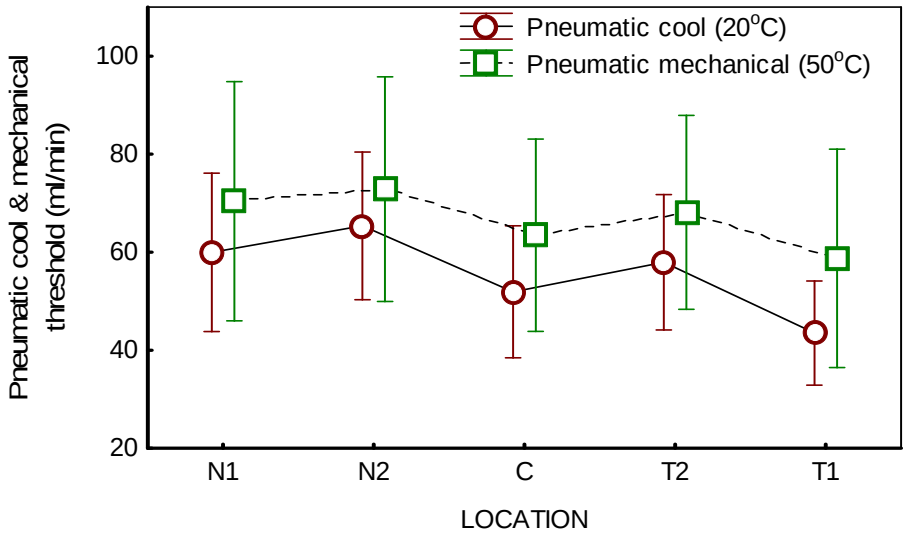

Figure 3-2 Plots of threshold variation for pneumatic cool and mechanical stimulation

Note: Vertical bar denote 95% confidence intervals

3.5 Discussion

This study examined the variation of corneal sensitivity with pneumatic mechanical and cool, chemical stimulation at different corneal positions. The pneumatic mechanical and chemical thresholds of the central cornea in our study are similar to those published previously measured using Belmonte esthesiometers.[12, 25, 33, 36, 41, 43] The thresholds to pneumatic cool stimuli were generally lower than the pneumatic mechanical threshold at each corresponding location consistent with previous results.[37, 41]

Corneal sensitivity partly reflects the function of sensory nerve fibres of the cornea and perhaps represents their density.[2] Aδ and C fibres are the smallest diameter and most abundant axons of the

peripheral nerve bundles.[44] They terminate as free nerve endings in the superficial layers of the cornea and function as nociceptors and cold detectors.[2, 16, 20, 22, 45, 46] For many years, our understanding of the anatomy of the sensory system in human cornea was largely based on the data from species other than human. It had been shown that neural density was greater toward the center than the periphery in cats and rabbits.[2, 47] Rózsa and Beuerman (1982) studied density and organization of free nerve endings in rabbit corneal epithelium using light microscopy, and reported that the neural density was greatest at the apex and suggested that this distribution was matched to mechanical sensitivity almost perfectly.

In the present study, corneal sensitivity to mechanical and pneumatic cool stimuli varied only slightly across the horizontal meridian, but the pattern was unlike the previously reported neural density variation.[30-32] This small variation with position is similar to the corneal cooling threshold pattern reported by Murphy et al.[39] Recent studies, describing the architecture of human corneal nerves in fresh cornea [46, 48] and in living eye observed using a confocal microscopy,[49] have revealed a novel arrangement of the subbasal plexus when compared to that seen in rabbit corneas. Nerve bundles in the subbasal plexus form a regular dense meshwork with approximately equal density over a large central and peripheral area, suggesting a homogeneous distribution of nerve endings extending out to the peripheral cornea in human.[46] If, as Rózsa and Beuerman have suggested, sensitivity maps onto neural density, then results from the current study are in accord with this finding about the distribution of human corneal nerves.

Functional heterogeneity of corneal sensory fibres has been demonstrated in electrophysiological investigations in animals.[16, 17, 19, 21, 26] Studies have shown that different functional types of receptors are activated by the specific modality of stimuli, for example mechanical stimuli activate both mechano-nociceptors and polymodal nociceptors, while chemical and thermal stimuli activate polymodal nociceptors or chemoreceptors; the cold-sensitive receptors respond to decrease in temperature.[16, 19-22, 24, 25, 50] However, how these specific types of receptors are distributed among the two types of nerve fibres is unknown and the relationship between the structural and functional specialization of the corneal nerve fibres has not been established in humans. In the current study, the patterns of variation in mechanical and chemical thresholds are comparable, suggesting that the distribution of the functional types of neurons is probably similar across the cornea. Thresholds to pneumatic cool stimuli appeared to be lower than the mechanical threshold at each corresponding location although the difference was not statistically significant in this study. Similar results were

found in a study in which sensitivity at central and inferior cornea were compared.[41] The differences between thresholds with air pulses at 20°C (room temperature) and 50°C (ocular surface temperature) most likely reflects the contribution of the population of corneal "cold" receptors.[51]

In the present work, higher sensitivity to pneumatic cool stimuli was exhibited at the temporal periphery of the cornea. The actual reason for this variation is unclear. Corneal cooling could result from convection and evaporation that would additionally activate "cold" sensitive receptors.[27, 51] The convection and evaporation rate may not be equal on the ocular surface. It has been shown that the nasal portion of the eye is warmer than the temporal side when measured using a thermography.[52] This may contribute to the asymmetric pattern in pneumatic cool thresholds between temporal and nasal periphery.

There are generally two types of instruments used to measure corneal sensitivity: Contact devices such as the Cochet-Bonnet esthesiometer and pneumatic devices. It has been reported that the "mechanical" threshold measured using these two types of esthesiometries was not correlated.[35, 53] Cochet-Bonnet esthesiometry uses a small diameter nylon thread to deliver a direct mechanical stimulus to the corneal surface. In contrast, pneumatic esthesiometry uses an air column to induce a mechanical effect on the cornea.[34] Our experiment points to an additional difference between Cochet-Bonnet and pneumatic esthesiometers. Sensitivity measured by Cochet-Bonnet esthesiometry has typically been reported to decline markedly across the cornea,[30-32, 54] whereas in our study there were remarkably similar thresholds in the centre and periphery of the cornea, regardless of stimulus type. It has been hypothesized that tactile and pneumatic stimuli may somehow recruit different functional classes of sensory receptors.[51] Presumably, the mechano-nociceptors, a specific subgroup of Aδ fibres, are responsible for the touch sensation induced by this type of measurement, while the stimuli from pneumatic esthesiometer are considered to activate both Aδ and C fibres, involving both mechanosensory and polymodal receptors. In addition, several shortcomings of Cochet-Bonnet esthesiometry[29, 53] such as poor stimulus control and the perceived invasiveness resulting in patient apprehension, may influence the results of corneal sensitivity measurement. How this would differ across the cornea, though, is unclear. Perhaps those stimuli closer to the visual axes are more visible and therefore induce more apprehension and this criterion change appears as greater sensitivity.

Another possible experimental reason for an inability to find a difference between the central and peripheral thresholds, as has been found using the Cochet-Bonnet esthesiometer, may be due to lack of statistical power. This observation is not strictly correct however, because we did show that

temporal pneumatic cool threshold was lower than in other positions. In addition, we believe that a more pertinent observation with these stimuli was the very slight variation in thresholds across the cornea, particularly when compared to that reported using Cochet-Bonnet.[30, 31] This relative constancy occurred for each of the three stimulus types, and is in accord with the findings of other studies using pneumatic stimuli that there is much less difference in threshold between cornea and conjunctiva[35, 36, 43] than when measured using a Cochet-Bonnet esthesiometer.

In summary, corneal sensitivity to pneumatic mechanical and cool stimuli varies slightly across the cornea and chemical sensitivity is approximately constant. This pattern, without a marked increased sensitivity in the central cornea, is different to the variation previously described with tactile stimuli. The distribution of corneal sensory fibres in human may be more homogeneous than previously believed.

Understanding the regional distribution of corneal sensitivity to pneumatic mechanical, chemical and cool stimuli may be helpful clinically in understanding the health of the cornea, particularly when regional differences might be anticipated, such as in refractive surgery planning and post-operative follow up.

Chapter 4

Transducer function of central and peripheral cornea

4.1 Overview

Purpose: To investigate the psychophysical transducer functions of central and peripheral cornea to mechanical and chemical stimulation.

Methods: Nine healthy subjects participated in the study. Mechanical and chemical stimuli, at and above thresholds, were delivered to the central and temporal peripheral cornea (about 2mm from limbus) using the Belmonte pneumatic esthesiometer. The magnitude of the evoked sensation was estimated with a modulus free technique. The relationship between the physical intensity of various stimuli and the normalised sensory magnitudes was estimated and compared between the two locations in the cornea.

Results: The sensations evoked by mechanical and chemical stimuli in central and peripheral cornea were nociceptive in nature. The size of the power exponents at the central and peripheral cornea were similar (paired t-test, $p>0.05$) while the exponents for mechanical and chemical stimulation were different (paired t-test, $p<0.05$).

Conclusion: This study demonstrated that Stevens' Power Law also applies to the processing of pneumatic mechanical and chemical stimuli at the central and peripheral cornea. It appeared that transducer functions characterizing suprathreshold scaling were similar regardless of position, but there may be different suprathreshold functioning in response to mechanical and chemical stimulation.

4.2 Introduction

The cornea is subject to broad environmental challenges and is densely innervated[1, 2] by sensory nerves that predominantly originate from neurons located in the ipsilateral trigeminal ganglion.[3] These neurons (or primary afferents) can be classified as finely myelinated (Aδ type) and unmyelinated (C type) fibers based on their size, presence of a myelin sheath in the axon and conduction velocity.[4] The peripheral axons of the neurons, terminate throughout the superficial layers of the corneal epithelium as "free nerve endings",[5] although specialized neural terminals have been described in the limbus and perilimbal bulbar conjunctiva.[6] Different modality-specific corneal sensory neurons have been identified in cats and rabbits,[4, 7-13] these being primarily,

polymodal nociceptors, cold receptors and mechanonociceptors. Polymodal nociceptors are responsive to noxious mechanical, chemical and temperature changes and the cold receptors signal downward temperature variation in the non-noxious range, while mechano-nociceptors respond to mechanical forces and are most likely responsible for producing acute, sharp pain in response to mechanical contact with the corneal surface.[12-14] In addition, a small number of low-threshold mechanical receptors have been found in the limbus.[13] Separate populations of sensory neurons detect submodalities of the stimuli and the encoded information is subsequently processed at various levels of the central nervous systems and interpreted as the perceived sensations.[15] In humans, sensations evoked by stimulation to the cornea are largely unpleasant or painful in nature.[16-20] Because of these unique features, the sensory innervation of the cornea provides a useful model to study pain mechanisms.

The measurement of sensation magnitude is a complex problem in psychophysics because the relationship between sensory magnitude and stimulus intensity is usually not linear.[21] The plot of the magnitude of a sensory attribute against the corresponding physical values of the stimulus is called psychophysical magnitude (or transducer) function, and is essential in understanding the operation of each sensory system.[21] In humans, magnitude estimation is a frequently used scaling method to quantify sensory attributes in relationship to physical stimuli;[22 21] the participant is required to provide numbers that represent the sensation magnitude produced by the physical stimuli.[22] Stevens[23] proposed that this relationship between sensation magnitude and stimulus intensity was a power function and the size of power exponent could be used to predict the magnitudes estimated from other stimulus intensities.[23]

Very few studies have investigated the exponent of corneal power function [17, 19, 24, 25] and there are no reports of the suprathreshold sensation intensities evoked by mechanical, chemical and thermal stimulation at various corneal locations. Previous studies have shown little variation between central and peripheral corneal detection thresholds when measured using pneumatic esthesiometers,[26-28] but it is unknown whether the mechanisms responsible for corneal sensations evoked by suprathreshold pneumatic stimulation would be similar between locations.

Therefore this study was conducted to characterize the suprathreshold behaviour with mechanical and chemical stimulation of the central and peripheral cornea, and to compare the psychophysical transducer function for each modality and location.

4.3 Methods and materials

4.3.1 Subjects

Nine healthy subjects (5 females and 4 males; aged from 21 to 44, mean ± SD, 28.9 ±7.4), who were non-contact lens wearers and had no history of ocular or systemic diseases or surgery, participated in the study that was conducted in accordance with the guidelines of the Declaration of Helsinki. Ethic clearance was received from the University of Waterloo, Office of Research Ethics (Waterloo, Ontario, Canada). Informed consent was obtained from each subject.

4.3.2 The Belmonte esthesiometer

A computer-controlled Belmonte esthesiometer that has been described in detail elsewhere[17, 20, 28] was used to deliver the mechanical and chemical stimuli to the central and temporal peripheral cornea (about 2mm from limbus) of the right eye.

The mechanical stimuli consisted of a series of air pulses with flow rate varying from 0 to 200 ml/min. Chemical stimulation was induced by increasing the concentration of CO_2 (from 0 to 90%) in the air with the flow rate at 70% of the mechanical threshold. The temperature of the stimulus was set at 50 ºC, which (based on our calibration) dropped to approximately 33 ºC at the ocular surface.

4.3.3 Procedures

At the initial assessment, subjects received a training session using the central cornea of the left eye. In order to define the stimulus range, detection thresholds to mechanical and chemical stimuli at both test locations were estimated using the ascending method of limits, (averaging of three first reports of stimulus presence). Mechanical and chemical stimuli were then set at nine levels with equal separation, ranging from threshold to a maximum of five times (5x) the mechanical threshold and three times (3x) the chemical threshold. Because the physical limits of the esthesiometer were 200 ml/min flow rate and 100% CO_2, the stimuli maxima had to be modified occasionally. In those cases, rather than measurement being 5x or 3x the threshold, 200 ml/min flow rate or 100% CO_2 were used. In addition, in two subjects with low chemical thresholds, stimuli ranges were extended to 5x. The duration of each stimulus was two seconds.

Measurements were made during four separate sessions at approximately the same time of the day. All measurements were conducted at least four hours after subjects awoke to minimize the influence of diurnal effects.[29] The order of the test location and the stimulus modality was randomly assigned

and in each session, the intensity of the stimulus was presented in random order, with a minimum inter-stimulus interval of two minutes. Each stimulus was presented three times.

Sensory magnitude was scaled using magnitude estimation with a modulus free technique (no numerical value assigned to a standard stimulus).[22] Following each stimulus, subjects were asked to assign a number to the magnitude to the stimulus; they were instructed to choose whatever range of numbers felt most comfortable, to place no upper limit on the numbers and to assign the numbers that directly reflected their subjective impression of the sensation they perceived.

4.3.4 Data analysis

For each modality and location, all sensation magnitudes (the numbers assigned) by a single subject were standardized after the experiment by dividing individual responses to each stimulus intensity by the overall mean response of the subject to all the stimuli; this normalized the numerical ranges chosen by each subject.[30] In addition, because the stimulus intensities were scaled based on subject's detection thresholds, "times threshold" was used to standardize stimulus intensity.

These standardized sensation magnitudes and stimulus intensities were used in the data analysis, performed using Statistica 8.0 (StatSoft Inc. Tulsa, OK, USA), and $p \leq 0.05$ was considered to be statistically significant. Non-linear regression analysis was used to derive Stevens' power functions,[23] estimating the exponent n and constant a for each subject's data fit to $y=ax^n$ (where y is the sensation magnitude, a is a scaling constant, x is the stimulus intensity, and n is an exponent characteristic of the stimulus used). Paired t-tests were used to compare the differences in the size of the power exponent and the constant between modalities and locations. In addition, the group average of sensory responses corresponding to the stimulus intensity was fit with non-linear (logistic) function for each modality and location.

4.4 Results

The sensations evoked by mechanical and chemical stimuli to central and peripheral cornea were generally described to be unpleasant or painful, although for mechanical stimulus at threshold it sometimes described as "blowing air" or "windy". Detection thresholds to mechanical and chemical stimulation for central and peripheral cornea are shown in Figure 4-1. There was no significant difference in threshold between the two locations (paired t-test, $p=0.08$ and $=0.74$ for mechanical and chemical stimuli, respectively).

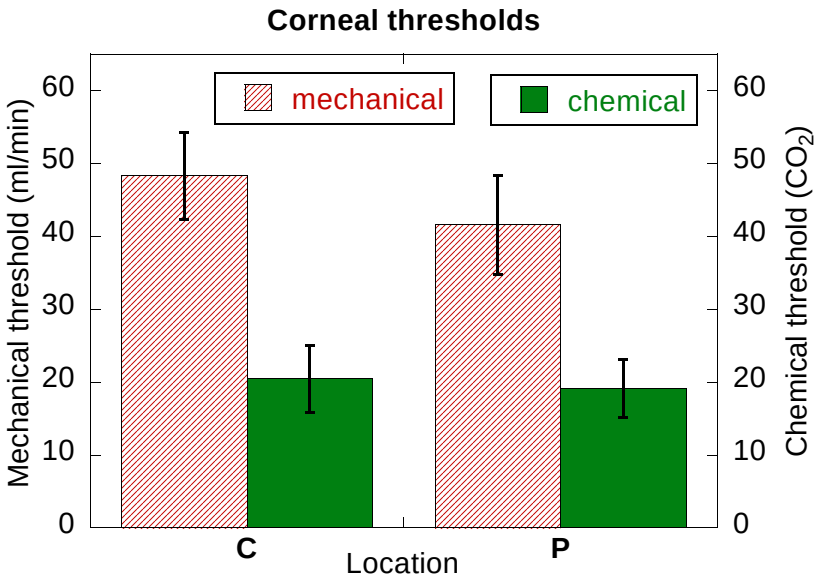

Figure 4-1 Mechanical and chemical thresholds at different locations

Note: Values were mean ± SEM.

The power function exponents and constants (mean ±SEM) are presented in Tables 4-1 and 4-2, respectively. Significant differences in exponents were found between stimulus modalities (paired t-test, p=0.03 and 0.04 for central and peripheral stimulation, respectively), with greater exponents for mechanical stimulation than chemical ones. Exponents were similar for the stimulation of central and peripheral cornea, although the location difference for mechanical stimulation approached significance (paired t-test, p=0.06 and 0.93 for mechanical and chemical stimulation, respectively). There was also a significant difference in scaling constants between modalities (paired t-test, $p<0.01$ and p=0.02 for central and peripheral stimulation, respectively) but not between locations (paired t-test, p=0.29 and 0.28 for mechanical and chemical stimulation, respectively).

Table 4-1 Power exponent (*n*)

	Central	Peripheral	Paired t-test p
Mechanical	1.38 ± 0.19	1.19 ± 0.14	0.06
Chemical	0.97 ± 0.25	0.96 ± 0.13	0.93
Paired t-test p	***0.03***	***0.04***	----

Table 4-2 Constant (*a*)

	Central	Peripheral	Paired t-test p
Mechanical	0.32 ± 0.04	0.34 ± 0.03	0.29
Chemical	0.54 ± 0.04	0.49 ± 0.05	0.28
Paired t-test p	***<0.001***	***0.02***	----

Transducer functions are not only well fit by power functions,[16, 31] the "goodness" of fit of the curve to the data for the logistic models were also high (all $r^2 \geq 0.97$). To illustrate this, figures 4-2 to 4-5 show the grouped data fit using logistic functions (the inset demonstrates the grouped data of power function fit for comparison, all $r^2 \geq 0.94$ for the fits of power function) : These too are able to differentiate the modality and location effects that are manifest as the power function exponent and scaling constant differences. The forms of the curve were different between mechanical and chemical stimuli but very similar between locations.

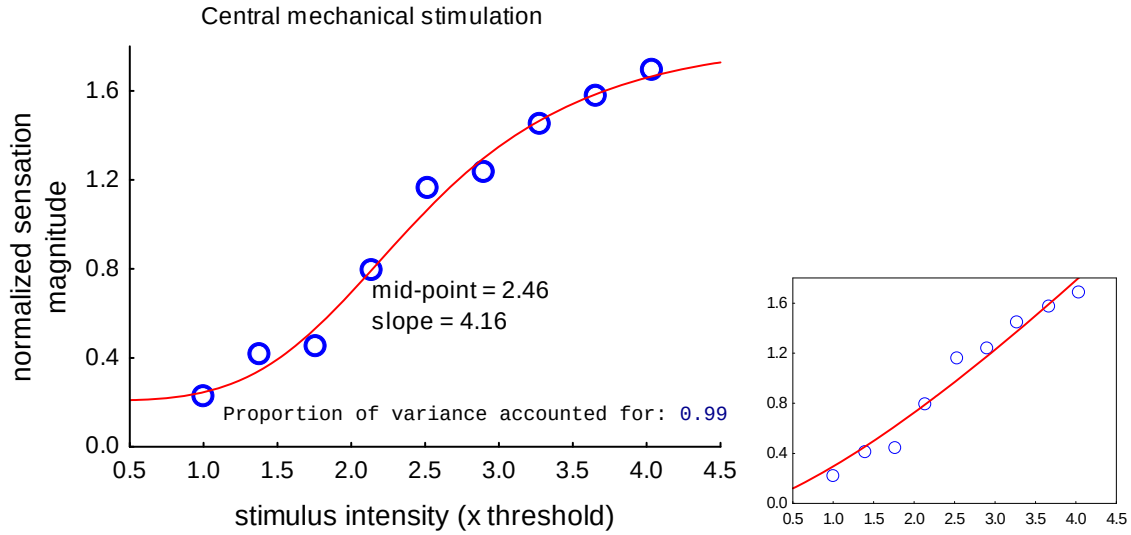

Figure 4-2 Plots of central mechanical stimulation

Note: Group data fit with logistic function (left), (Inset) fit with power function (right)

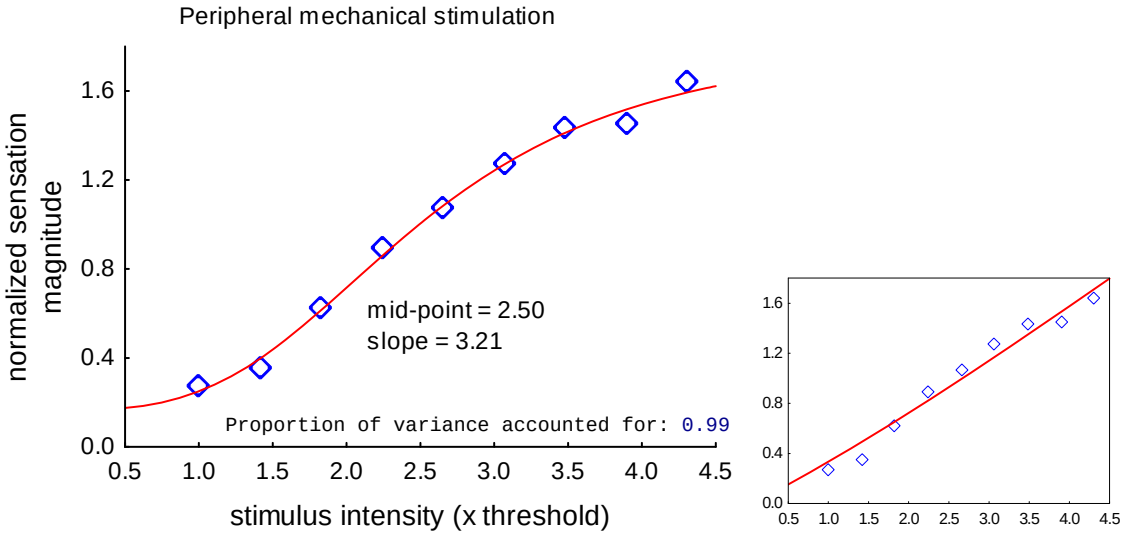

Figure 4-3 Plots of peripheral mechanical stimulation

Note: Group data fit with logistic function (left), (Inset) fit with power function (right)

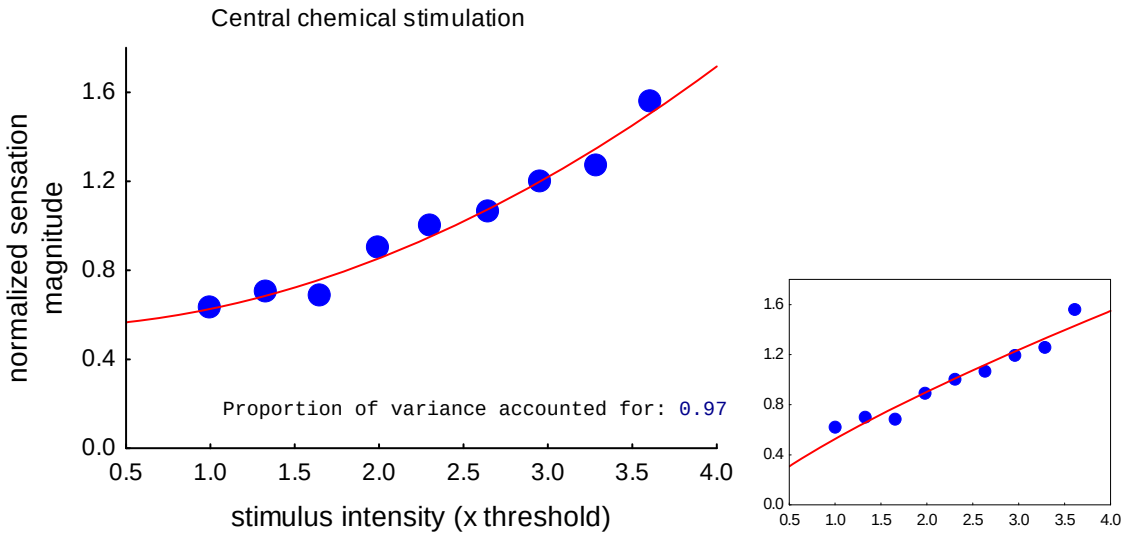

Figure 4-4 Plots of central chemical stimulation

Note: Group data fit with logistic function (left), (Inset) fit with power function (right)

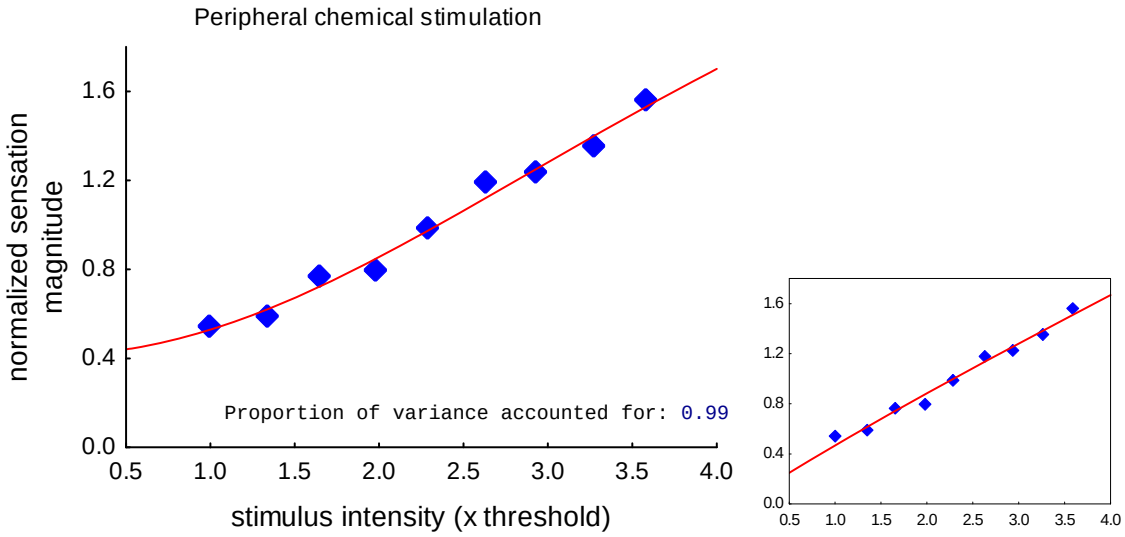

Figure 4-5 Plots of peripheral chemical stimulation

Note: Group data fit with logistic function (left), (Inset) fit with power function (right)

4.5 Discussion

The present study compared the psychophysical transducer or magnitude functions for mechanical and chemical stimulation of central and peripheral cornea. Similar to previous findings, sensation elicited by noxious mechanical and chemical stimulation of human cornea has been predominantly along the pain continuum.[16, 18-20] The magnitudes of the sensations were power functions of stimulus intensity and seemed to be stimulus modality dependent. The averaged exponents of mechanical and chemical transducer functions at the central cornea in the study were similar to those previously reported.[17, 19, 24, 25]

The psychophysical magnitude function for a particular sense modality or test condition represents the relationship between the magnitude of a sensory attribute and the corresponding physical values of a suprathreshold stimulus. It provides insight into the transmission of information through the sensory nervous system, including transduction of stimulus energy into neural impulses, encoding of neural impulses and judgmental process in the central nerve system.[21] In this study, the magnitude of the sensation produced by mechanical stimulation seemed to be lower at threshold, compared to that evoked by chemical stimulation, but sensation intensity accelerated with stronger stimulus intensity, leading to a greater power function exponent. The different psychophysical magnitude functions suggest that distinct sensory mechanisms are involved in processing mechanical and chemical stimulation of the cornea, and supports the parallel psychophysical channel hypothesis proposed for the cornea [32] and other components of the somatosensory system.[33, 34] This suggests, that the processing of mechanical stimulation generates greater unpleasant sensations soon after detection and that there, perhaps, is greater emphasis on noxious mechanical stimulation of the cornea than chemical stimulation.

Besides observations from psychophysical studies, pharmacological and physiological evidence also suggests that stimulus of submodalities may be conveyed by separate neural pathways.[8, 16, 19, 35] For example, topical capsaicin (an irritant substance with selective excitatory effects on polymodal nociceptors[36]) blocks impulses of corneal polymodal nociceptors stimulated using acid and heat but not mechanically.[8] Furthermore, the responses of corneal nociceptors to acidic solution were reduced by some Ca^{2+} antagonists without apparently affecting their responsiveness to mechanical stimuli.[35] These illustrate that mechanotransduction and chemotransduction involve separate mechanisms, i.e. the contributions of specific ion channels and other signalling molecules and their complex interactions with corneal nociceptors may be different.[37] Electrophysiological studies have

also shown that the cornea possesses functionally specific sensory receptors that are preferentially activated by different stimulus types.[13, 16, 19] The transduced information is processed by different classes of neurons that lead to distinct corneal sensations.[13] Mechanical stimulation activates mainly phasic mechanosensory and polymodal afferents, and strong stimulation presumably induces a sharp pain,[16] while chemical stimulation activates polymodal afferents evoking sustained duller irritation and pain.[16, 19]

There is evidence that in addition to the modality specificity in corneal primary sensory neurons, higher order processing of these separate mechanical and chemical inputs is different. It has been shown that the distribution of higher-order corneal neurons within the trigeminal complex are modality-specific.[38] Neurons excited by polymodal input (i.e., CO_2-responsive units) and activated by mechanical only (i.e., not responsive to CO_2) have been found in both the subnucleus interpolaris-caudalis transition and subnucleus caudalis-upper cervical spinal cord junction region.[39] The efferent projections of these neurons are either to the thalamic posterior nucleus/zona incerta or the superior salivatory/facial nucleus region, depending on the modality of the peripheral input.[39] In the present study, in addition to the differences between the power functions with stimulus types, the activation of distinct populations of corneal and higher order neurons when mechanical and chemical stimuli were used may also be inferred from the difference in intensities and characteristics of pain evoked.

In contrast to the differences in power functions with chemical and mechanical stimulation, we were unable to show difference between stimulus locations. There is recent evidence of neural density in human corneas and in living eyes observed using confocal microscopy,[5, 40, 41] that there is a previously unreported novel arrangement of the subbasal plexus with approximately equal density over a larger central and peripheral area.[5, 42] Studies have also demonstrated less variation of thresholds over the cornea using pneumatic cooling, mechanical and chemical stimuli,[26-28, 42] than previously evidenced using Cochet-Bonnet tactile stimulation.[43-45] This slight threshold variation was also confirmed in the current study. In addition, similar suprathreshold behaviour with noxious stimulation of central and peripheral cornea extends the description of the functioning of these neural mechanisms. Many lines of evidence are now converging to suggest that the channels that mediate corneal nociception behave similarly regardless of whether corneal stimulus location is central or peripheral.

Stevens[23] proposed that the exponent of the power functions he hypothesized characterized suprathreshold processing by sensory systems, partially reflected the potential danger to the organism.

The highest exponent reported, for example, was 3.5 when subjects received electric shock! In this light, the noxious stimuli used in this experiment were anticipated to generate high power function exponents (larger than 1.0), but they were not that high (one-sample t-tests showed that they were not statistically different than 1.0 [all $p \geq 0.07$]) and were generally similar to those reported by Millodot using tactile-mechanical corneal suprathreshold stimulation.[25] Applying the theory proposed by Stevens, that the exponent of the transducer function reflected potential danger to the organism, this would suggest that corneal irritation is not physiologically as harmful as electric shock. The exponents with chemical stimulation, were in addition, statistically lower than the mechanical ones (Table 4-1). As mentioned before, the implication is that the corneal sensory systems processing the different pneumatic stimuli are not the same – in channel theory, the chemical and mechanical channels are not the same.[32] One classification of pain processing comprises two subsystems, supported by the Aδ and C-fiber structural dichotomy.[46, 47] If this separation that includes the phenomonenological distinction into fast, sharp pricking pain and slow burning pain, is also manifest on the cornea, then the mechanical and chemical corneal stimuli each selectively stimulated one of these subcomponents. Importantly, though, the difference in power function exponents suggests that the C-fiber subsystem gain (indicated by the power function exponent) is lower than that of the Aδ subsystem.

In summary, the current study showed that transducer functions characterizing suprathreshold scaling of mechanical and chemical stimulation were different, whereas suprathreshold functioning was similar in the central and peripheral cornea. In addition to illustrating that, like other sensory systems studied in similar ways, Stevens' Power Law also applies to the processing of pneumatic mechanical and chemical corneal stimuli. These findings may provide useful information in eventually understanding and better managing symptoms arising from the ocular surface, as unpleasant corneal (or ocular surface) sensations that may be the commonest reasons for visits to ophthalmic clinicians.[48] Also, these sensations can be induced or exacerbated by refractive surgery[49, 50] contact lens wear,[51-54] and Dry Eye, a disease that impairs the functioning and quality of life of millions people with substantial social and economic impacts.[55-59]

Chapter 5
Interaction of corneal nociception and tear secretion

5.1 Overview

Purpose: To investigate the interaction between corneal stimuli at different positions and tear secretion, and establish relationships between nociceptive stimuli detection thresholds and stimulated tearing.

Methods: Using a computerized Belmonte-esthesiometer, mechanical and chemical stimuli, from 0 to 200% of the threshold in 50% steps, were delivered (in random order) to the central and peripheral (approximately 2-mm inside the limbus) cornea in four separate sessions to 15 subjects. Immediately following each stimulus, tear meniscus height (TMH) was measured using an Optical Coherence Tomographer (OCT) to quantify the amount of lacrimal secretion, and subjects reported whether they felt tears starting to accumulate in their eyes. Thresholds (50% detection) for detection of tearing were estimated.

Results: TMH increased with increasing stimulus intensity ($p<0.05$) and the overall increase was higher with central stimulation compared to the periphery ($p<0.05$). The changes in TMH with threshold-scaled stimulus intensity depended on test location ($p<0.05$) and stimulus modality ($p<0.05$). The maximum intensity of mechanical stimulation of the central cornea induced the greatest TMH (all $p<0.05$). For chemical stimulation, the stimulus intensity required to induce detectable tearing was higher than that to detect a stimulus and higher in the periphery than the center (all $p<0.05$).

Conclusions: Noxious mechanical and chemical stimuli evoked measurable tear secretion, with central corneal mechanical stimulation evoking the strongest lacrimation reflex. Central mechanical corneal stimulation is the most effective stimulus-position pairing and appears to be the major sensory driving force for reflex tear secretion by the lacrimal functional unit.

5.2 Introduction

Tear secretion is controlled by the integrated lacrimal functional unit that comprises the ocular surface (cornea, conjunctiva and meibomian glands), the lacrimal glands and the sensory afferent and autonomic efferent nerves that connect them.[1-3] The functional unit regulates the major components of the tear film[1, 4] and responds to environmental, endocrine and central neural influences,[2, 3, 5, 6] with overall function to protect the integrity of the tear film and the ocular surface and maintain the quality of the principle optical component of the eye.[7]

The sensory signals arising from the ocular surface are an important aspect of the lacrimal functional unit.[7] It has been considered that waking tear production is driven by a reflex circuit consisting of the sensory afferents coming mainly from the ocular surface, the synaptic integration at the spinal trigeminal nucleus (Vsp) and a relay to the superior salivatory nucleus (SSN) located in the brainstem, and the efferent fibers to parasympathetic and sympathetic ganglia and then to the lacrimal glands and other ocular surface tissue.[2, 6, 8, 9] During the steady state, without injury or pathology, the sensory nerves of the ocular surface provide low level input to the functional unit and operate with the efferent parasympathetic and sympathetic nerves to modulate resting tear flow.[5, 7] Stimulation of sensory nerves near or above injurious levels results in tear secretion and other reflexes to protect the eye from potential damage.[10]

The cornea is richly innervated by sensory nerves that serve important sensory and reflex functions.[11, 12] Corneal sensory nerves predominantly originate from neurons located in the ipsilateral trigeminal ganglion and can be classified as thin myelinated (Aδ type) and unmyelinated (C type) fibers.[10] The peripheral axons of the neurons, terminate throughout the corneal epithelium as “free nerve endings”,[12-15] although encapsulated neural terminals have been observed in the limbus and perilimbal bulbar conjunctiva.[16] Despite lack of morphological specialization at the endings, different functional sensory fibers including polymodal nociceptors, mechanonociceptors and cold receptors have been identified based on their electrophysiological properties.[17, 18] In addition, a small number of low-threshold mechanical receptors have been found in the limbus.[17] Recently, a study has proposed that the afferent pathways of basal and reflex tearing might involve a different subclass of sensory receptors.[19]

Although the peripheral and central neural pathway for lacrimal reflex induced by corneal stimulation has been partially elucidated, the relationship between the intensity of the sensory input and the outflow of the efferent autonomic nervous system in the lacrimal functional unit has not been

defined. It is unclear whether tear secretion induced by stimulation at the central and peripheral cornea has the same neural circuitry, although it has been suggested that the corneal distribution of functional types of receptors might be similar.[20] Additionally, the psychophysiological functions of stimulated lacrimation and how they relate to the stimulation of the central and peripheral cornea have not been explained.

In the present study we investigated the interaction between stimulation at various corneal positions and the efferent output, as determined by the tear secretion, and defined the psychophysiological function as the relationship between stimulus intensity and stimulated tearing. Systematically varied intensities of mechanical and chemical stimuli were delivered to different positions of the cornea using a Belmonte pneumatic esthesiometer. Tear volume expressed by tear meniscus height (TMH) was quantified using an Optical Coherence Tomographer (OCT)[21] and perceived lacrimation was quantified following each stimulus.

5.3 Methods and Materials

5.3.1 Subjects

Fifteen healthy subjects, consisting of 9 females and 6 males, aged from 18 to 47 (mean ± SD, 31.6 ±7.9), who were non-contact lens wearers and had no history of ocular or systemic diseases or surgery, participated in the study.

This study was conducted in accordance with the guidelines of the Declaration of Helsinki and received clearance from the University of Waterloo, Office of Research Ethics (Waterloo, Ontario, Canada). Informed consent was obtained from each subject.

5.3.2 Mechanical and chemical stimulation

A computer-controlled Belmonte esthesiometer that has been described in detail elsewhere[20, 22, 23] was used to deliver mechanical and chemical stimuli to the central and temporal peripheral cornea (about 2mm from limbus) of the left eye.

The mechanical stimuli consisted of a series of air pulses with flow rate varying from 0 to 200 ml/min. Chemical stimulation was induced by increasing the concentration of CO_2 in the air with the flow rate well below the mechanical threshold. The temperature of the stimulus was set at 50 ºC, which dropped to approximately 33 ºC at the ocular surface.

At the initial assessment, subjects received a training session using stimulation of the central cornea of the right eye. Initial detection thresholds to mechanical and chemical stimuli at both test locations were then estimated using the ascending method of limits (the average of three first reports of stimulus presence), in order to define the stimulus range. Mechanical and chemical stimuli were set at five levels, ranging from 0 to 200% of the initial detection threshold in 50% steps. Measurements were made during four separate sessions at approximately the same time of the day. All measurements were conducted at least four hours after subjects awoke to eliminate diurnal effects.[24] The order of the test location and the stimulus modality was randomly assigned and in each session, the intensity of the stimulus was presented in random order, with a minimum inter-stimulus interval of two minutes; each stimulus was delivered three times.

5.3.3 Tear meniscus height measurement

Prior to each measurement session and immediately following each stimulus, tear meniscus height (TMH)[21] at the mid-lower lid margin of the left eye was measured using a Humphrey Optical Coherence Tomographer (Model 2010, Zeiss Humphrey System, USA) to quantify the amount of lacrimal secretion. Custom software was used to measure TMH of the OCT images.

5.3.4 Estimation of detectable tearing thresholds

Subjects reported (yes or no) the presence of detectable tearing using a button box. Tearing threshold (midpoint of detectable tearing "psychometric function") and the slope were estimated using non-linear regression fits of the logistic function: Likelihood of reporting tearing = $1 - [1/ (1 + (\text{Stimulus intensity}/\text{Threshold})^{\text{Slope}})]$.

5.3.5 Data Analyses

Statistical analyses were performed using Statistica 8.0 (StatSoft Inc. Tulsa, OK, USA) and $p \leq 0.05$ was considered to be statistically significant. Repeated measures ANOVA and post hoc Tukey HSD tests were used to compare the differences in the TMH measurements between intensity levels, stimulus positions and modalities, and their interactions. The detectable tearing thresholds between locations for each modality and their interaction with the sensory thresholds were also compared.

5.4 Results

5.4.1 Effects of stimulus intensity, location and modality on TMH

Measurements of TMH at baseline (prior to each measurement session) and with mechanical and chemical stimulation of differing intensities stratified by modality and location are shown in Figure 5-1. There was a significant intensity effect regardless of stimulus modality and location as shown in Figure 5-2 (ANOVA $p<0.001$). TMH increased as a function of increased stimulus intensity. At 200% (2 x) threshold, TMH was significantly higher than all stimulus intensities except for 150% (1.5 x) threshold (Tukey HSD, all $p<0.05$). TMH without stimulation (0% threshold) was significantly lower than any given stimulus intensity (Tukey HSD, all $p<0.05$).

There was a significant location effect on TMH independent of stimulus modality (ANOVA $p<0.001$), with higher TMH for central stimulation. The overall increased TMH was similar between mechanical and chemical stimulation regardless of the stimulus locations (ANOVA $p>0.05$).

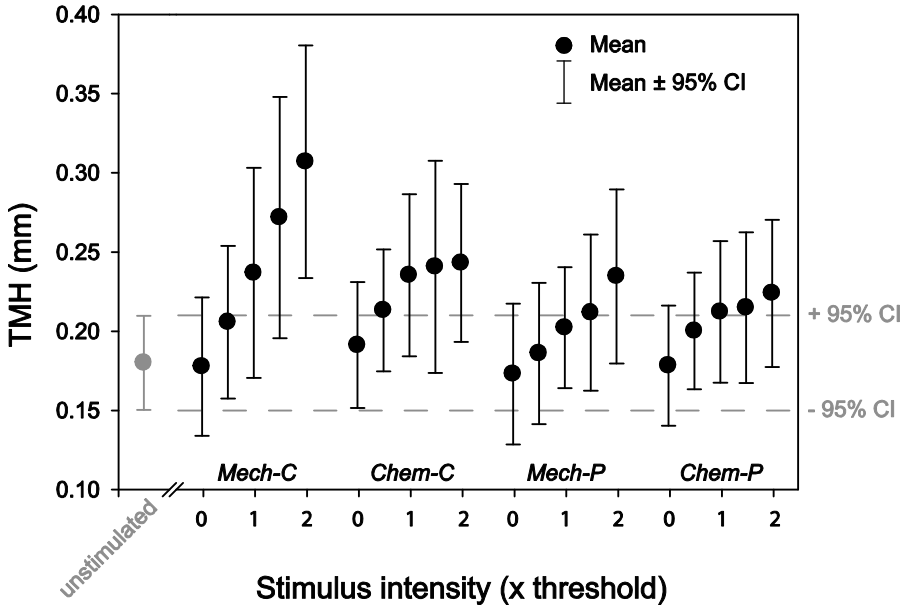

Figure 5-1 TMH at baseline (unstimulated condition) and with noxious stimulation

Note: The gray dashlines indicate the 95% confidence interval of unstimulated TMH. (Mech: mechanical stimulus; Chem: chemical stimulus; C: central; P: peripheral; CI: confidence interval).

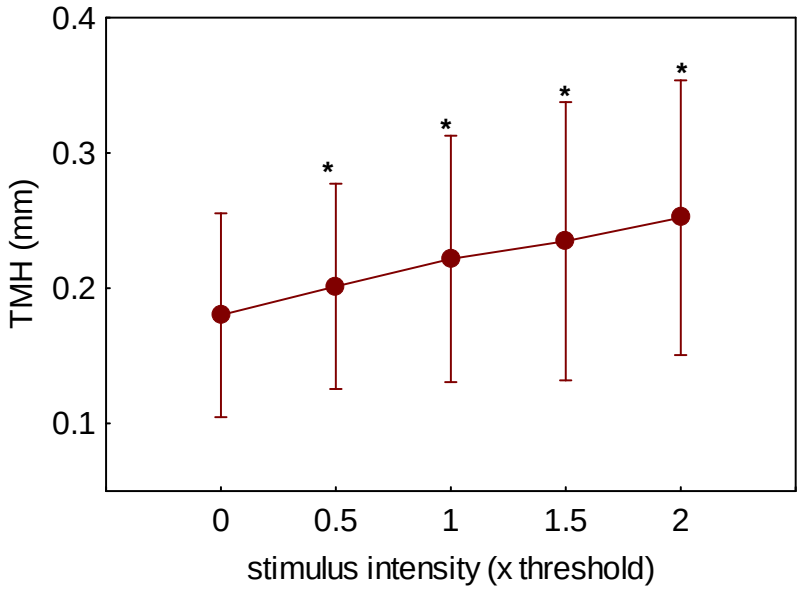

Figure 5-2 TMH (average of stimulus modalities and locations) versus stimulus intensity

Note: Vertical bars denote 95% CI. *Significant differences compared to 0 stimulus intensity (all $p<0.05$).

5.4.2 Interactions between stimulus intensity, location and modality

Significant two-way interactions were found between intensity and location, and intensity and modality, as demonstrated in Figures 5-3 and 5-4 (ANOVA $p=0.001$ and $p<0.001$ for location and modality, respectively). Independent of stimulus modality, intensity at and above the threshold resulted in greater TMH for the central stimulation than the periphery (Tukey HSD, all $p<0.05$). On the other hand, TMH induced by different modalities regardless of location were similar at most of the intensity levels except for the highest level (Tukey HSD, $p=0.001$). Although the overall three-way interaction between stimulus intensity, location and modality was not significant (ANOVA, $p=0.189$), post hoc pairwise comparison showed that the significant difference between modalities was at the highest suprathreshold level for central stimulation only (Tukey HSD $p=0.003$), with the greatest TMH induced by the maximum intensity of mechanical stimulus (Figure 5-5).

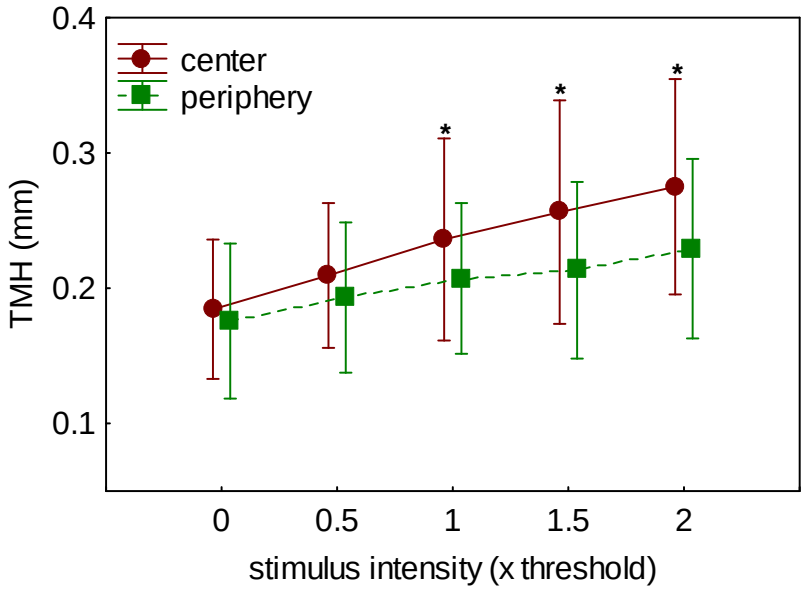

Figure 5-3 Interaction of TMH measurement and stimulus intensity regardless of stimulus modality at both positions

Note: Vertical bars denote 95% CI. * Significant differences compared to corresponding peripheral stimulation (all $p<0.05$).

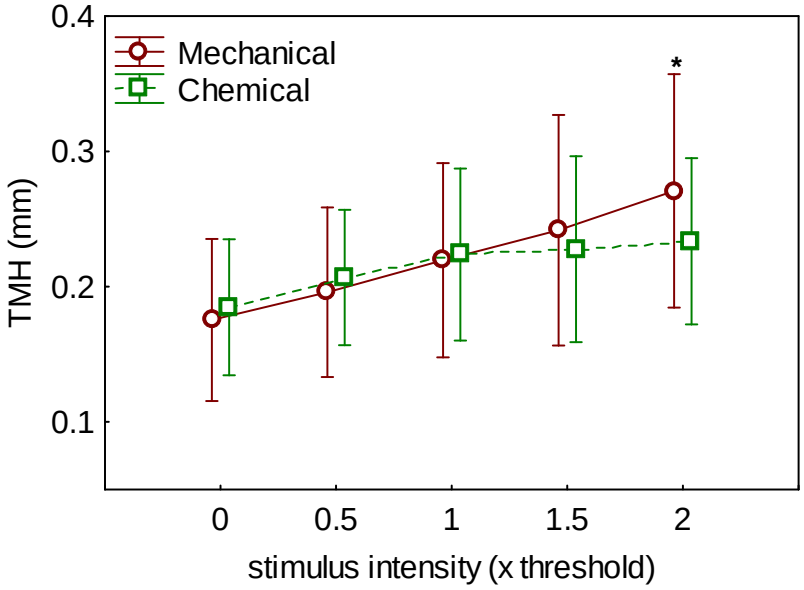

Figure 5-4 Interaction of TMH measurement and stimulus intensity regardless of stimulus position for both stimulus modalities

Note: Vertical bars denote 95% CI. *Significant difference compared to the chemical stimulation ($p=0.001$).

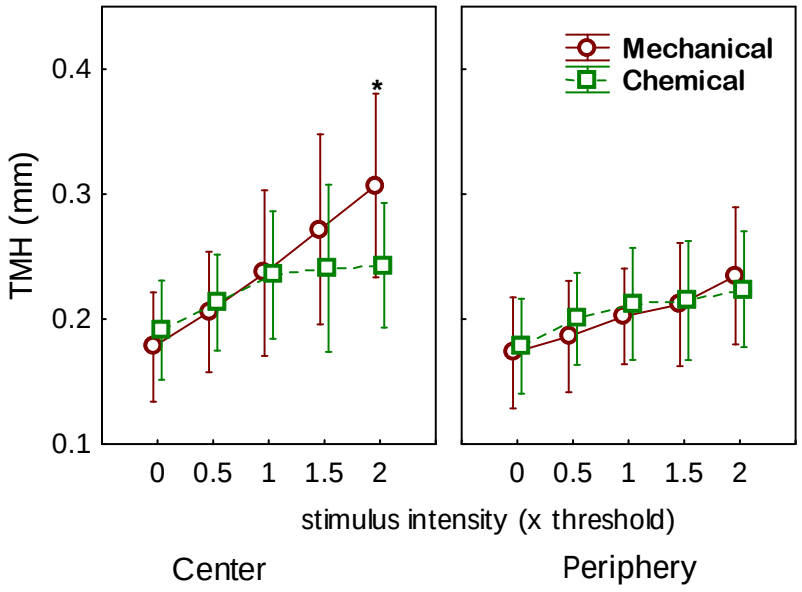

Figure 5-5 Interaction of TMH measurement and stimulus intensity for both stimulus modalities and position

Note: Vertical bars denote 95% CI.* Significant difference compared to the maximum intensity of chemical stimulation (p=0.003).

5.4.3 Effect of stimulus location on tearing thresholds and relation to stimulus detection

Thresholds to mechanical and chemical stimuli and the tearing thresholds at each location in response to different modality are presented in Table 5-1. Generally, thresholds to detect mechanical stimulation and detectable tearing were similar across locations and there was no significant interaction between threshold and location (ANOVA, both $p>0.05$). A significant difference was found between detectable tearing and detection threshold to the chemical stimulation; tearing thresholds were higher than the detection thresholds (ANOVA $p=0.043$) and this difference was dependent on the stimulus location (ANOVA $p=0.011$, Figure 5-6). Post hoc pairwise comparison showed that detectable tearing thresholds were higher at the periphery than at the center, and higher than the threshold of detecting a stimulus at each corresponding location (Tukey HSD, all $p\leq0.028$).

Table 5-1 Thresholds of detectable tearing and stimulus detection (Mean ± SEM)

Threshold	Mechanical (ml/min)		Chemical (%CO_2 added)	
	central	peripheral	central	peripheral
tearing	60.12 ± 11.09	65.25 ± 11.94	30.05 ± 6.61	38.95 ± 6.06
stimulus detection	54.36 ± 5.34	51.60 ± 6.01	21.84 ± 2.27	20.27 ± 2.29

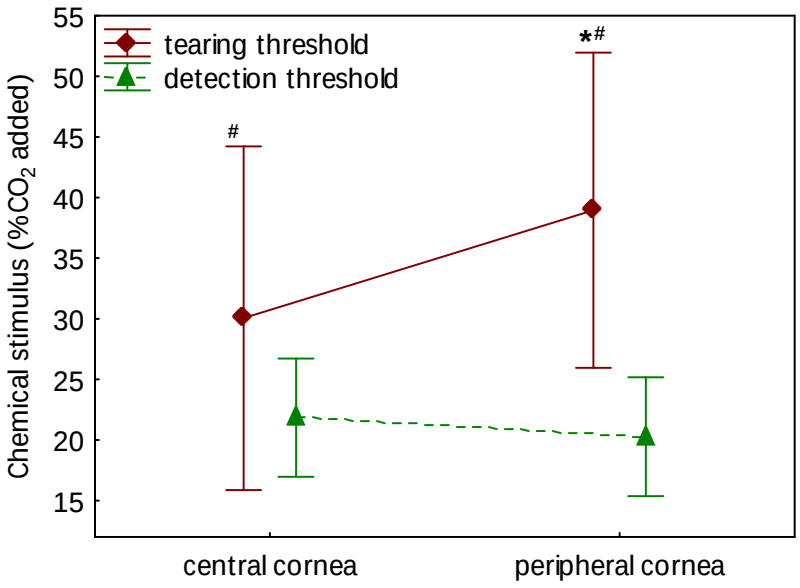

Figure 5-6 Detectable tearing and stimulus detection thresholds to chemical stimulation

Note: Vertical bars denote 95% CI. * Significant difference compared to the central cornea and # significant difference compared to the detection thresholds (all $p<0.05$)

5.5 Discussion

The primary objective of the study was to investigate the relationship between stimulation of corneal sensory nerves and the efferent output of the lacrimal functional unit determined by tear secretion. Tear secretion increases almost linearly with the increase of stimulus intensity in the present study.

It has been suggested that the sensory effects and hence the lacrimal functional unit operate differently depending on environmental conditions and pathology.[5] Without stressful stimulation, sensory nerves on the ocular surface provide sub-threshold sensory input to the functional unit modulating resting tear flow. When noxious stimuli activate sensory afferents in the functional unit, a

series of coordinated reflexes including reflex tearing are triggered in order to protect the eye from potential damage. The present study showed that suprathreshold nociceptive corneal stimuli evoke apparently reflex lacrimation, accompanied by the perception of increasing tear flow in the eye, while tear secretion following sub-threshold stimuli was the same as unstimulated tear flow (Figure 5-1). This physiological evidence supports the hypothesis that two states of neural control mechanism (i.e. basal-subconscious and augmented-conscious) are involved in regulating tear secretion, in order to protect the ocular surface from injury over a wide range of situations.[5]

Stimuli to the cornea are detected and encoded by the sensory receptors or transducers on the peripheral terminals of the corneal fibers. Corneal sensory fibers possess different functional types of receptors that are preferentially activated by different types of stimulation.[17, 18] The majority of the corneal fibres (about 70%) are polymodal nociceptors that are equally activated by near-noxious mechanical energy, chemical irritants, heat (above 39 ºC) and noxious cold.[17] Most of the polymodal nociceptor fibers are unmyelinated C type but some belong to the group of thin myelinated Aδ fibres.[17] About 15-20% of the corneal fibers are mechano-nociceptors which are fast-conducting, thin myelinated Aδ fibres and are activated exclusively by high magnitude mechanical force.[17] In the present study, mechanical and chemical stimulation generally produced similar effects on reflex tear secretion, consistent with the findings of Acosta et al,[19] although they used different methods to quantify tear volume. Studies in cat have shown that pneumatic mechanical stimuli to the cornea activated mainly the phasic mechano- and polymodal nociceptors, while gas mixtures of increasing CO_2 primarily excited polymodal nociceptors.[25, 26] This similarity between modalities suggests that both polymodal and mechano-nociceptors contribute to the afferent pathways of reflex tear secretion. The highest suprathreshold mechanical stimulation in our experiment might have activated not only the polymodal nociceptors but also the high threshold mechano-nociceptors resulting in neural summation thus producing greater tear reflex than the equivalent intensity of chemical stimulation.

The neural activities encoded by sensory receptors are carried centripetally by trigeminal ganglion neurons to higher levels in the central nervous system. The ocular surface is represented mainly in two spatially distinct regions of the spinal trigeminal nucleus in the lower brain stem: The trigeminal nucleus interpolaris-caudalis (Vi/Vc) transition and subnucleus caudalis-upper cervical spinal cord (Vc/C1) junction regions.[27-29] Because of this unique dual representation, it has been proposed that neurons at Vi/Vc and Vc/C1 transition regions mediate different aspects of corneal nociception and their efferent projection to supraspinal areas might also be different.[30, 31] The corneal neurons located

at the Vi/Vc transition region that project to superior salivatory nucleus (SSN) serve ocular-specific functions such as blink and tear reflexes, while those located within the superficial laminae (I-II) of Vc/C1 transition that project to posterior thalamic nucleus (PO) may play a prominent role in the sensory-discriminative aspects of corneal nociception.[32-34] The current study showed that at and above threshold, stimulation of the central cornea produced greater reflex tearing compared to the equivalent stimulus to the periphery, suggesting that reflex lacrimation responses vary depending on the stimulus location. Given that suprathreshold stimulation may potentially damage the cornea, and the ultimate goal of corneal reflexes is to protect the ocular surface and therefore the eye itself, it is plausible that the circuitry involved in reflex responses to noxious stimulation of the two locations may be different (for example, efferent projection to the SSN versus the PO), as the visual axis is situated within the central cornea.

In addition, it has been reported that a small number of thick, fast-conducting nerve fibers, innervate the perilimbal episclera.[17] These fibers possibly terminate at the limbal region with Krause-like corpuscular endings as described by Lawrenson and Ruskell,[16] and respond to gentle mechanical stimulation of the ocular surface (they are mechanoreceptors).[10] Activation of this type of receptor has been reported to be less effective compared to polymodal nociceptors in evoking the tearing reflex.[19] This may also contribute to the location differences in the study.

As expected, the intensity required to trigger detectable tearing is generally higher than that to detect a noxious stimulus, although for mechanical stimulation the differences between the two thresholds did not reach statistical significance. Additionally, we did not find a strong positional effect on thresholds to detect mechanical and chemical stimulation, similar to previous studies.[20, 35, 36] However, for suprathreshold chemical stimulation, the intensity required to induce subjectively detectable tearing was higher for the peripheral cornea compared to the center, consistent with the difference in amount of tear secretion between the two locations. It appears that chemical stimulation of central and peripheral cornea is similar in terms of mediating corneal sensation at threshold level but is different with respect to detecting stimulated tearing. This suggests that chemosensory information from central and peripheral cornea might be processed differently at the spinal trigeminal nucleus, depending on sensory-discriminative or tear reflex aspect.

In conclusion, the current study demonstrates that a systematic increase in tear volume as determined by TMH is monotonically related to the intensity of the sensory input from the cornea, in a dose-response way. This provides physiological evidence that sensory innervation of the cornea

(thus the ocular surface) is the major neural driving force for lacrimal gland secretion. Acting through areas of the central nervous system, the sensory nerves and efferent parasympathetic and sympathetic nerves of the lacrimal functional unit modulate tear secretion in order to ensure a healthy ocular surface and protect the eye under normal as well as environmentally challenging conditions (such as this experiment).

The components of the lacrimal functional unit are linked in a homeostatic loop by complex and precise sensory, parasympathetic and sympathetic neural control.[3] Establishing the relation between activation of the sensory nerves from the ocular surface and the graded output of the lacrimal gland secretion may help to further understand the neural mechanisms contributing to dry eye development, and ultimately to develop effective treatment for ocular surface diseases in which the functioning of the innervation of the lacrimal functional unit may be compromised.

Chapter 6

Ocular surface sensitivity in dry eye

Chapter 6 described the results of an investigation examining signs and symptoms in a group of 100 non-contact lens-wearing participants who presented with and without symptoms of ocular dryness and discomfort (dry eye vs. non-dry eye group). Part one was focused on corneal and conjuncitval sensitivity measured using a computerized Belmonte esthesiometer and its relationship with ocular surface tests and symptom severity in subjects with and without symptoms of ocular dryness. In part two the effects of symptoms of dryness, age and gender on corneal and conjunctival sensitivity to pneumatic cool stimuli were investigated.

Chapter 6 was published as follows:

Conjunctival and Corneal Pneumatic Sensitivity Is Associated with Signs and Symptoms of Ocular Dryness

Ping Situ, Trefford L. Simpson, Desmond Fonn, and Lyndon W. Jones

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Conjunctival and Corneal Hyperesthesia in Subjects with Dryness Symptoms

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	Situ	Simpson	Jones	Fonn
Experimental design	Y	Y	Y	-
Data collection	Y	-	-	-
Data analysis	Y	Y	-	-
Write-up publication	Y	Y	Y	Y

6.1 Part one

6.1.1 Overview

Purpose: To investigate the relationships between dry eye symptoms, corneal and conjunctival sensitivity to pneumatic stimulation, tear film stability and clinical ocular surface characteristics in symptomatic and asymptomatic subjects.

Methods: 97 subjects were enrolled and grouped by a questionnaire based single score for symptom of ocular dryness (none to trace= 'non-dry', mild to severe= 'symptomatic'); 43 were symptomatic and 54 were non-dry. Corneal (K) and conjunctival (C) sensitivity were measured using a computer controlled Belmonte pneumatic (room temperature) stimulus. Symptoms were assessed using Ocular Surface Disease Index (OSDI). Ocular surface staining with fluorescein (FL) and Lissamine Green (LG), non-invasive tear film break-up time (NIBUT), and the Phenol Red Thread test (PRT) were assessed.

Results: The symptomatic group showed lower K and C thresholds ($p<0.01$), greater corneal FL staining and conjunctival LG staining, and shorter NIBUT than the non-dry eye group (all others $p<0.05$). OSDI scores were higher in the symptomatic group ($p<0.001$). K and C thresholds, NIBUT were inversely correlated with OSDI scores and corneal and conjunctival staining (all $p<0.05$). K and C threshold and NIBUT (all $p<0.01$) were positively correlated. Stepwise multiple regression analysis showed that ocular surface sensitivity and NIBUT were significant predictors of OSDI scores.

Conclusion: Ocular irritation assessed using OSDI is associated with ocular surface hyperesthesia to cooling, corneal epitheliopathy and tear film instability. Although cause and effect are unclear, our analysis shows that altered corneal and conjunctival sensory processing and tear film attributes are essential aspects of what characterizes dry eye.

6.1.2 Introduction

Dry eye is characterized as an abnormality of the tears and ocular surface with a multi-factorial etiology, resulting in tear film instability, symptoms of discomfort and visual disturbance, and inflammation and potential damage to the ocular surface.[1] It is hypothesized that the ocular surface (including the cornea, conjunctiva, accessory lacrimal glands and meibomian glands), the main lacrimal glands and the afferent and efferent innervation that connects them comprise the lacrimal

functional unit.[1-3] This integrated functional unit regulates the major composition of the tear film[4, 5] and responds to environmental, endocrine and central neural influences.[2, 3, 6, 7] Dysfunction in any part of the functional unit potentiates alterations of the other components, thus compromising the ability of the ocular surface to respond to physiological and environmental challenges with one possible outcome being the development of symptoms associated with dry eye (provided this is not *the* precipitating factor).[2, 3, 6-8]

The presence of symptoms might be accounted for by the activation of sensory nerves at the ocular surface.[9, 10] Although the basis for symptoms in dry eye is not fully understood, a recent speculation has been that the dryness sensations following refractive surgery might be due to denervation-induced dysesthesia.[11] Measuring ocular surface sensitivity is one technique to evaluate the sensory nerve function and a few studies have measured corneal sensitivity in certain groups of dry eye patients.[12-16] However, studies evaluating corneal sensitivity and clinical tests such as ocular surface staining, tear film stability and symptoms of ocular irritation have found conflicting results. In addition, there is no report of whether conjunctival sensitivity is associated with the symptoms, or indeed to the clinical ocular and tear film tests commonly undertaken.

In the present study, we therefore measured corneal and conjunctival sensitivity using a computer-controlled modified Belmonte esthesiometer and investigated its relationship with ocular surface tests and symptom severity in subjects with and without ocular dryness symptoms.

6.1.3 Methods and Materials

This study was conducted in accord with the guidelines of Declaration of Helsinki and received clearance from the University of Waterloo, Office of Research Ethics (Waterloo, Ontario, Canada). Informed Consent was obtained from each subject.

A total of one hundred non-contact lens wearing subjects were enrolled. Of these, three subjects were excluded: Two were found to have corneal striae of unknown cause and one reported symptoms of dryness that were present only in the middle of the night. Each subject had no history of systemic or ocular disease and was not using any systemic or topical medication that would affect ocular health. Slit-lamp examination was initially performed to rule out lid, conjunctival or corneal abnormalities other than the clinical signs of dry eye. A questionnaire based single score for symptoms of ocular dryness (Simmons PA, et al. *IOVS* 2003;44: ARVO E-Abstract 2448), as described in Table 6-1, was used to classify “non-dry” (scores of “none” to “trace”) and

"symptomatic" (scores of "mild to severe") groups. This was administered by a trained ophthalmic assistant at study entry.

Table 6-1 Questionnaire based single score for symptoms of ocular dryness

Scores	None (0)	Trace (1)	Mild (2)	Moderate (3)	Severe (4)
Q1: Frequency of symptom	never	seldom	sometimes	frequent	always
Q2: Presence of discomfort	no	no	yes	yes	yes
Q3:Interference with activity	no	no	no	sometimes	usually

6.1.3.1 Symptom evaluation:

Subjects completed the Ocular Surface Disease Index© (OSDI) questionnaire, that consists of 12 items including questions related to visual function, ocular symptoms and environmental triggers.[17] The item scores were calculated according to the formula recommended by Schiffman et al.[17]

6.1.3.2 Clinical assessment:

Non-Invasive Tear Film Break-Up Time (NIBUT) was measured using an ALCON Eyemap® model EH-290 topography system (ALCON, Inc., Forth Worth, Texas, USA). A series of concentric Placido rings were projected onto the tear film and the amount of time before the first distortion of the tear film after a blink was recorded. The measurement was repeated three times and averaged. Tear volume was measured using the Phenol Red Thread (PRT) test (ZONE-QUICK, Showa Yakuhin Kako Co., Ltd. Tokyo, Japan) in a manner following manufacturer's recommendation.

Corneal flurorescein (KFL) and conjunctival lissamine green (CLG) staining were assessed with a biomicroscope using a cobalt blue excitation source with a yellow Wratten 12 filter and white light with Hoya 25A filter respectively. The intensity of staining was graded following the Oxford scheme.[18]

6.1.3.3 Sensitivity measurement:

Corneal and conjunctival sensitivity was estimated using a computer-controlled Belmonte pneumatic esthesiometer that has been described in detail elsewhere.[19, 20] Custom software was used to control stimulus duration and intensity and record subject responses from a button box.

Measurements of corneal and conjunctival sensitivity were performed on the right eye only, at least four hours after subjects awoke.[21] A training session was conducted using the central cornea of the left eye prior to the actual measurements being obtained. Subjects were instructed to view fixation targets at 3 meters and the tip of the esthesiometer was set 5 mm from the corneal and conjunctival surface and monitored using a calibrated video camera. The esthesiometer was rotated to ensure the stimulus was delivered perpendicularly to the surface.

The stimuli, consisting of a series of air pulses at room temperature (20°C), with air flow varying from 0 to 200 ml/min, were delivered to the central cornea and temporal conjunctiva (approximately 5 mm from the limbus). This stimulus is mechanical with an easily detectable non-noxious cooling effect.[22-27] The stimulus duration was one second, with a 15-second interval between the subject's response and the next stimulus. Two seconds prior to each stimulus, a short, computer-generated tone reminded the subject to blink and view the fixation target. The subjects were informed that they were to blink freely and also to close their eyes or look down between stimuli. Subjects could also interrupt the trials if necessary. Using the ascending method of limits,[28] corneal and conjunctival thresholds were determined by the average flow rate of three first reports of the stimulus presence.

A single observer (the first author) who was masked to the results of the subject's grouping, conducted the clinical and ocular surface sensitivity tests. Tests were performed in the following order: NIBUT, PRT, corneal and conjunctival staining evaluation, and esthesiometer measurements.

6.1.3.4 Data analyses:

Statistical analyses were performed using SAS 9.1 (SAS Institute Inc., Cary, NC, USA) and $p<0.05$ was considered to be statistically significant. Because of the characteristics of the data, corneal and conjunctival thresholds, and NIBUT were transformed by converting to the natural log in order to achieve normality of the data distributions. Unpaired t-tests were used to compare differences between the symptomatic and non-dry groups. For corneal and conjunctival staining, Mann-Whitney U tests were used for the comparison between groups. Pearson correlations were performed among transformed variables. Multiple-linear regression with stepwise selection was applied to assess the relationship between OSDI scores and measurements of ocular sensitivity and other clinical signs. To eliminate potentially harmful multicollinearity,[29] for highly correlated predictor variables, only one was included in model selection. In addition, response variable OSDI scores were transformed by

taking the natural log to satisfy the model assumption that variance of the residuals should be approximately constant.

6.1.4 Results

The demographic data of the two groups are reported in Table 6-2. The frequencies of each category classified by the symptom are presented in Figure 6-1. Scatterplots of individual corneal and conjunctival threshold versus age are shown in Figures 6-2A and 6-2B for symptomatic and non-dry group, respectively. Corneal and conjunctival thresholds for detection of pneumatic cool stimuli, NIBUT, PRT, corneal and conjunctival staining score, and OSDI scores for symptomatic and non-dry subjects are shown in Figures 6-3 to 6-6. The differences in all the variables except for PRT were significant between the two groups (all $p<0.05$).

Table 6-2 Demographic data of the two groups

	Non-dry	Dry eye symptomatic
Number of subjects	54	43
Age (range)	41 ± 18 (19 - 79)	49 ± 16 (19 - 80)
Gender: female/male	31/23	35/8

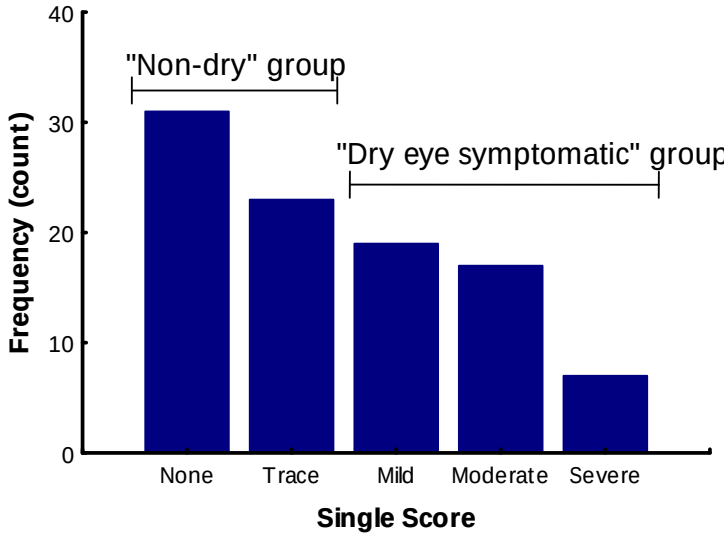


Figure 6-1The prevalence of the single score for symptom of ocular dryness and the grouping criteria

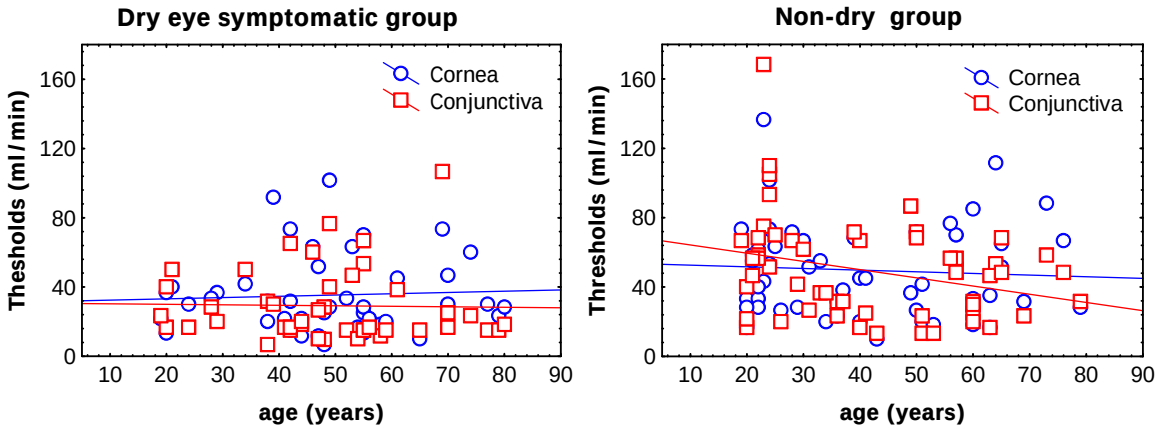


Figure 6-2 Scatterplots of corneal and conjunctival threshold vs. age for symptomatic (A, left) and non-dry group (B, right)

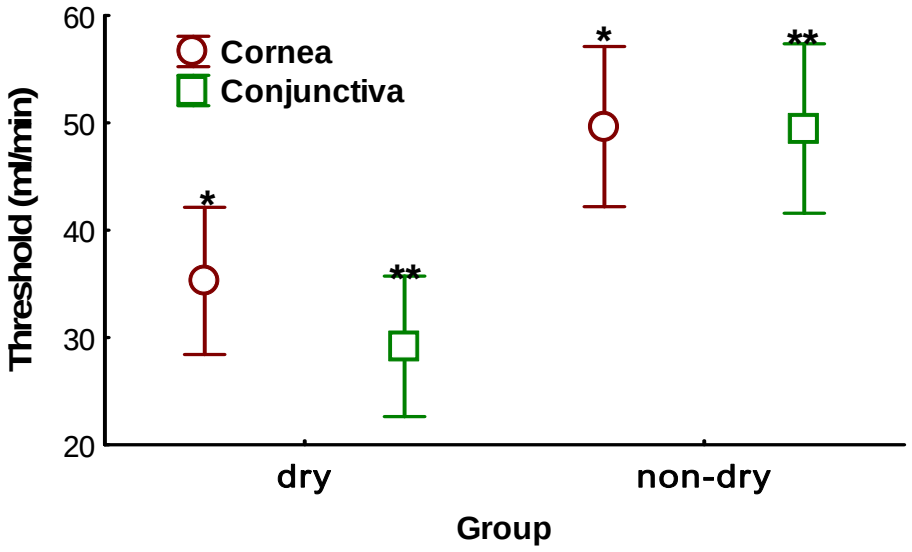


Figure 6-3 Box plots of corneal and conjunctival thresholds to pneumatic cool stimuli in dry eye symptomatic and non-dry group

Note: Values were mean ± 95% Confidence Interval. Both LN corneal and LN conjunctival thresholds (natural log transformed) were lower in the dry eye symptomatic group (p*=0.003 and p**<0.001).

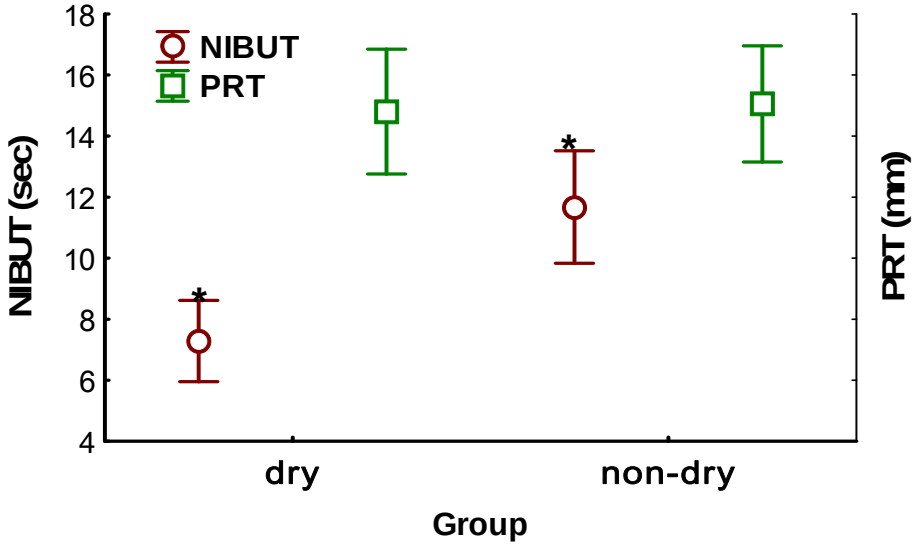


Figure 6-4 Box plots of NIBUT and PRT in dry eye symptomatic and non-dry group

Note: Values were mean ± 95% Confidence Interval. LN NIBUT in dry eye symptomatic group was significantly shorter than the non dry group (*p<0.001).

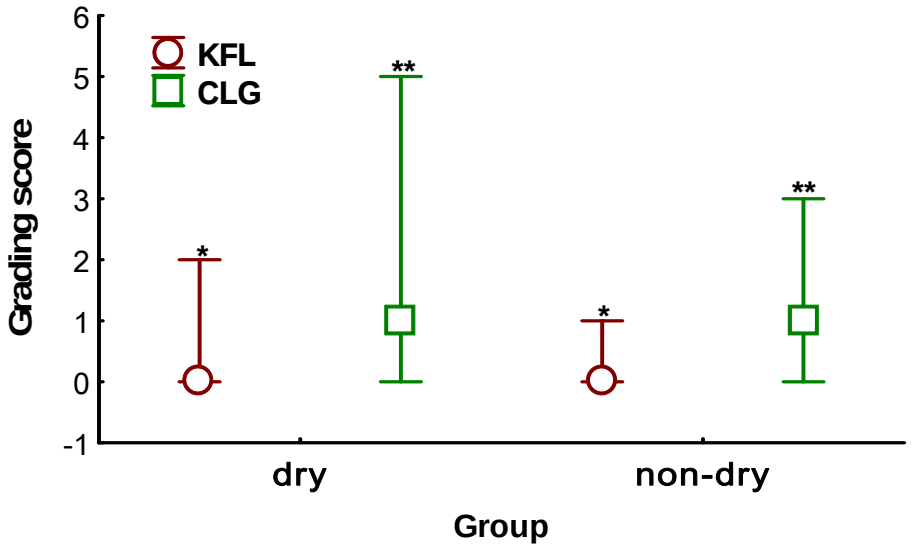


Figure 6-5 Grading scores of corneal fluorescein (KFL) and conjunctival lissamine green (CLG) in dry eye symptomatic and non-dry group

Note: Values were median and the 90 and 10 percentiles. Higher staining scores were found in dry eye symptomatic group (p*=0.031 and p**=0.048 for corneal and conjunctival staining, respectively).

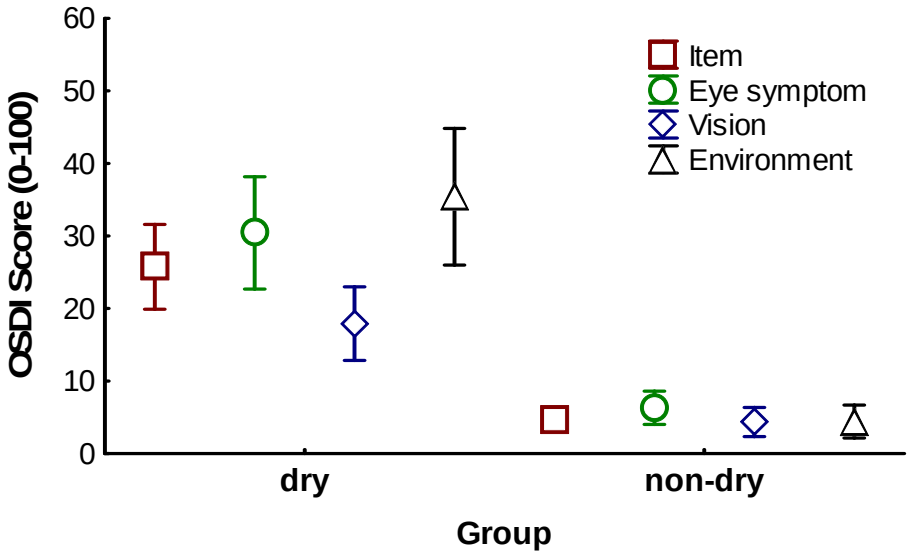


Figure 6-6 Box plots of OSDI score (total score= item) and the break down of each sub-score (visual function=vision, ocular symptoms= eye symptom, environmental triggers=environment)

Note: Values were mean ± 95% Confidence Interval. The differences in all LN disability scores between the dry eye symptomatic and non-dry group were significant (all p<0.001).

The correlations between corneal and conjunctival thresholds, clinical tests and symptom severity assessed by OSDI scores are presented in Table 6-3. Corneal and conjunctival thresholds to pneumatic cool stimuli were inversely correlated with staining and OSDI scores but positively correlated with NIBUT (all $p<0.05$). Inverse correlations were also found between NIBUT, staining and OSDI scores (all $p<0.05$).

Table 6-3 Correlations between thresholds, clinical tests and symptoms

	K threshold	C threshold	NIBUT	PRT	OSDI score	K FL staining	C LG staining
K threshold	------						
C threshold	0.78	-----					
NIBUT	0.31	0.40	-----				
PRT	NS	NS	0.32	-----			
OSDI score	-0.22	-0.40	-0.39	NS	-----		
K FL staining	-0.41	-0.29	-0.47	NS	0.31	-----	
C LG staining	-0.30	-0.27	-0.45	-0.28	NS	0.55	-----

Note: all $p<0.05$

The multiple regression model with OSDI as the outcome variable was statistically significant ($p<0.0001$). Conjunctival threshold, NIBUT and PRT accounted for 24% of the variation in OSDI score. With the other variables held constant, the item score was negatively related to conjunctival threshold and NIBUT and positively related to PRT. The effects of conjunctival threshold and NIBUT were significant (both $p<0.01$) in the stepwise regression analysis.

6.1.5 Discussion

Dysfunction of the ocular surface integrated functional unit may be manifest as dry eye with common features of symptoms of ocular discomfort, tear hyperosmolarity, interpalpebral ocular staining, reduced tear production and/or tear instability.[30] In the current study, the dry eye symptomatic group had corneal and conjunctival hypersensitivity (lower threshold), a shorter tear film break-up time, greater degree of ocular surface staining and a higher OSDI score than the non-dry eye subjects, suggesting alterations in various aspects of the lacrimal functional unit. In addition, ocular surface

hypersensitivity, tear film instability, epitheliopathy and symptoms of ocular discomfort were significantly correlated.

Neural control is one of the important aspects of the functional unit and links the components of the unit into a homeostatic loop with the primary function of protecting and maintaining the health of the ocular surface.[6] The sensory nerves of the ocular surface together with efferent sympathetic and parasympathetic innervation control the secretory activity of the lacrimal and meibomian glands and the conjunctival goblet cells.[5, 31, 32]

A few studies have evaluated the functioning of corneal sensory nerves by measuring corneal sensitivity in dry eye patients.[12-16] However, the part played by ocular surface sensitivity in relation to the natural history of different forms of dry eye is not fully understood. Tear film break-up in the interblink interval due to its instability could give rise to local drying and hyperosmolarity in the exposed surface, and to ocular surface damage and a disturbance of glycocalyx and goblet cells.[1] It has been postulated that ocular surface damage could produce reflex stimulation of the lacrimal glands in the initial stage of dry eye, in order to compensate for the tear film hyperosomolarity which arises as a result of excessive evaporation or/and insufficient aqueous tear flow.[1] Stimulation of the lacrimal functional unit in the absence of a protective tear film could result in neurogenic inflammation further damaging the ocular surface.[3] The present study revealed ocular surface hypersensitivity in the dry eye symptomatic group, indicating an alteration of the sensory nerve function. The altered sensory input was related to an index of tear film instability and epitheliopathy of the ocular surface, each part of a vicious circle of interacting mechanisms; the worse the quality of the tear film and the greater the degree of the epithelial staining, the higher the sensitivity (perhaps due to sensitization of sensory fibers).

The presence of symptoms of discomfort suggests nociception at the ocular surface evoked by the activation of sensory nerves.[10] It has been reported that the activities of corneal sensory fibers could be modified by injury and inflammation of the ocular surface.[9] Sensitization of corneal polymodal nociceptors, a decrease in threshold to one or more stimulus modalities and/or increased responsiveness and spontaneous activity to suprathreshold stimulation, can be induced by certain inflammatory mediators.[9, 33, 34] Interleukin 1 (IL-1), a pro-inflammatory cytokine found in the tears and conjunctiva (using impression cytology) in keratoconjunctivitis sicca (KCS) patients [35] has been reported to induce hyperesthesia.[36] The effect of sensitization increases the probability that a given

stimulus will activate the target receptor, i.e., sensitized nociceptors can be triggered by stimulation that would be insufficient to activate intact nerve endings under normal conditions.

On the other hand, the peptides and neurotransmitters, such as substance P and calcitonin-gene-related peptide released by activated nociceptors from peripheral nerve terminals, are able to facilitate production of the "inflammatory soup".[9, 37] Thus, it is also possible that the sensory changes themselves are the initiating factor and the resulting alterations in the tear film and surface follow due to neurogenic inflammation.[10]

In the current study, we found that ocular surface sensitivity and the index of the tear film stability were significant predictors for the severity of the symptoms. It may be possible that increased sensory input from sensitized receptors, resulting from inflammatory mediators and related to the aforementioned interacting mechanisms between tear film instability and disruption of the ocular surface, account for the symptoms in these subjects.

In addition to the activation of nociceptors at the ocular surface, the abnormal dryness sensation in dry eye may relate to altered sensation (dysesthesia). During sensory processing, the voltage-gated ion channels expressed by nociceptors contribute to the propagation of the signals detected by primary afferents.[38] The changes in expression of voltage-gated sodium channels play a key role in the pathogenesis of neuropathic pain[39, 40] such as dysesthesias,[41] and in the pain and hypersensitivity associated with tissue inflammation.[40] A recent study showed sensitized cold sensory receptors (non-nociceptors) in surgically lesioned guinea-pig corneas and the abnormal activities of sensitized cold receptors were attenuated by application of Lidocaine, suggesting that the cause of enhanced nerve ending activity of a cold receptor is due to increased expression of sodium channels (Belmonte C, et al. *IOVS* 2007;48: ARVO E-Abstract 3470). Evidence also suggests that, to a certain extent, the phenomena found in both inflammatory and neuropathic pain may share common physiologic mechanisms, including contributions from sodium channels.[40] Altered expression of ion channels, leads to an enhanced excitability of the membrane in primary sensory neurons and gives rise to abnormal neuronal activities and altered responsiveness to stimuli.

In the present study, the stimuli used to measure sensitivity consist of thermally cooling [23, 27] and mechanical [26] components and usually produce a non-noxious "cooling sensation" at threshold.[22] The enhanced sensitivity to this "non-noxious" stimulation in the symptomatic group suggests that altered sensation, possible due to increased expression of ion channels, may play a role in the symptoms of dry eye and contribute to the greater disability to environmental triggers such as air

conditioning, windy and low humidity reported by the dry eye symptomatic group. Pharmacological block of these ion channels may have therapeutic potential to be a complement treatment for dry eye.

The reports in the literature on corneal sensitivity to pneumatic mechanical stimuli in dry eye patients are contradictory.[12-14] ***Hyperesthesia*** has been found in dry eye patients and post-LASIK patients with dry eye and this was positively correlated to corneal epithelial barrier function.[14] Others reported ***hypoesthesia*** in dry eye patients and suggested that the decreased corneal sensitivity in dry eye was due to the damage of sensory innervation.[12, 13, 42] We also found corneal and conjunctival hypersensitivity in dry eye symptomatic subjects, supporting the findings reported by de Paiva and Pflugfelder.[14] The reconciliation of these apparently discrepant results remains to be elucidated. If, as discussed earlier, corneal and conjunctival sensitivity represent the functioning of the sensory nerves of the ocular surface, hypersensitivity (sensitization of the sensory nerves) and hyposensitivity (damage of the sensory nerves) may not be contradictory, but rather, may be indicators of different states of compensation during the continuum of the condition (including a failure to compensate) in the integrated functional unit.

In our study, tear volume measured using PRT was not different between the dry eye symptomatic and non-dry eye group. In addition, most of the subjects in the dry eye symptomatic group fell into the categories of "mild" and "moderate". Also, some symptomatic subjects in the sample had none of the signs of dry eye assessed in this experiment. It is plausible that increased sensory input from the ocular surface may have produced an augmented tear secretion in these patients, in order to compensate for tear hyperosmolarity resulting from a compromised functional unit (regardless of the etiology). This augmented secretion could be a possible factor contributing to the weak correlation between corneal and conjunctival sensitivity and staining. This correlation might be expected to be higher in a sample that included more subjects with severe disease who are unable to adapt to the disrupted ocular surface functional unit.

Many studies have reported that the associations between symptoms and clinical dry eye tests were sometimes statistically significant, but typically not strongly so.[13, 14, 16, 43-45] In the present study, we developed a model that included sensitivity, NIBUT and PRT to predict the severity of ocular discomfort measured by OSDI score accounted for about one fourth of the variance. This may reflect the diverse etiologies and interacting mechanisms related to dry eye development giving rise of a disease with a complex profile.

In conclusion, the symptoms of ocular irritation consistent with dry eye disease assessed using OSDI appeared to be associated with ocular surface (particularly conjunctival) hyperesthesia to cooling stimulation, corneal epitheliopathy and tear film instability. The interacting mechanisms of disruption of the barrier function, tear film instability, and altered sensory processing associate to neurogenic inflammation within the lacrimal functional unit may lead to or be influenced by the dry eye symptoms. Additionally, changes in expression and function of ion channels isoforms in the peripheral and probably the central nervous system may play a role in the abnormal dryness sensation in dry eye.

6.2 Part two

6.2.1 Overview

Purpose: To compare conjunctival and corneal sensitivity in non-contact lens wearing subjects with and without symptoms of ocular dryness, stratified by age and gender.

Methods: 97 subjects were enrolled, 54 of whom were asymptomatic and 43 of whom were symptomatic of ocular dryness. A single score for the symptom of dryness was used to classify non-dry eye (scores of none to trace) and dry eye symptomatic (scores of mild to severe) groups. The subjects were further stratified into "younger" (19-49 years) and "older" age groups (50-80 years). Conjunctival and corneal sensitivity of the right eye was measured at the central cornea and temporal conjunctiva, using a computer-controlled pneumatic esthesiometer with stimulus temperature set at 20ºC. The ascending method of limits was used to determine the thresholds.

Results: Conjunctival and corneal thresholds were significantly lower in the dry eye symptomatic than in the non-dry eye group (both $p<0.01$). The conjunctival threshold was lower than the corneal threshold in the dry eye symptomatic group ($p<0.01$) but not in the non-dry eye group ($p>0.05$). Conjunctival threshold in the non-dry eye females was lower than the males ($p<0.05$). No difference between age groups was found for conjunctival and corneal thresholds in this study (all $p>0.05$).

Conclusions: Conjunctival and corneal sensitivity to pneumatic cool stimulation is increased in subjects with symptoms of ocular dryness. This hyperesthesia seems to be more significant in the conjunctiva.

6.2.2 Introduction

Dry eye is a common condition and refers to a variety of characteristics with multi-factorial aetiologies which affect the tear film and/or ocular surface.[1] The prevalence of dry eye increases with aging and among women.[2-4] It has been hypothesized that the ocular surface (including the cornea, conjunctiva, and meibomian glands), the lacrimal glands (both main and accessory lacrimal glands) and the afferent and efferent innervation act as a functional unit.[5-9] Alterations in any portion of this functional unit is hypothesized to affect the quantity and composition of the tear film and impair the integrity of the ocular surface,[10,11] thus compromising the ability of the ocular surface to respond to environmental changes and perhaps leading to symptoms of dry eye.[1,5,8]

The sensory axons terminate as free nerve endings in the epithelium of the cornea and conjunctiva,[12] although special types of terminals have been identified in the limbal conjunctiva.[13] Electrophysiological studies of rabbit and cat corneas have revealed different functional types of receptors that are activated by specific types of stimuli. For example, mechanical stimuli activate both mechano-nociceptors and polymodal nociceptors, while chemical and thermal stimuli activate polymodal nociceptors. Cold-sensitive receptors signal downward temperature variation of the ocular surface in the non-noxious range.[14-18] The activities of corneal sensory fibres can also be modified as a result of injury and inflammation of the ocular surface.[15] Functioning of corneal and conjunctival nerves can be evaluated by measuring ocular surface sensitivity. The Cochet-Bonnet esthesiometer has been the most commonly used instrument to measure corneal sensitivity. The recent development of pneumatic esthesiometers[19-21] has allowed measurements of corneal and conjunctival sensitivity over a wider range of stimulus type, namely mechanical, chemical and thermal.

Very little empirical work has been done investigating ocular sensitivity in dry eye patients.[22-27] The details of neuronal processing in the lacrimal gland - ocular surface feedback loop remain largely unknown. Moreover, there are no reports of conjunctival sensitivity in symptomatic patients. In this study, we compared conjunctival and corneal sensitivity to pneumatic stimuli at room temperature in subjects with and without symptoms of ocular dryness. The second purpose of the study was to investigate the effect of two predictor variables, age and gender, on conjunctival and corneal sensitivity measured using a modified Belmonte esthesiometer.

6.2.3 Methods and Materials

This study was conducted in accord with the guidelines of Declaration of Helsinki and received clearance from the University of Waterloo, Office of Research Ethics (Waterloo, Ontario, Canada). Informed Consent was obtained from each subject.

Subjects were recruited from the local community. Ninety-seven non-contact lens wearing subjects who had no history of systemic and ocular diseases and/or were not using any systemic or topical medication that would affect ocular health were enrolled in the study. Slit-lamp examination was conducted to rule out clinically significant lid, conjunctival or corneal abnormalities. Fluorescein and Lissamine green staining were evaluated following clinical standard procedures.[28] Tear volume (phenol red thread test) (Zone-Quick, Showa yakuhin Kako Co.,LTD, Tokyo Japan) was measured according to manufacturer's recommendation and tear stability (non-invasive tear break up time) was assessed using a modified Alcon EyeMap topographic system (Alcon Laboratories, Fort Worth, TX).

A questionnaire-based single score for symptom of ocular dryness,[29] as described in Table 6-4, was used to classify non-dry eye (scores of none to trace) and dry eye symptomatic (scores of mild to severe) groups. Subjects' grouping and demographic data are listed in Table 6-5.

Table 6-4 Definition of single score for symptoms of dryness and subjects grouping criteria

Questions	**Non-dry eye**		**Dry eye symptomatic**		
Q1: Frequency of symptom	never	seldom	sometimes	frequent	always
Q2: Presence of discomfort	no		yes		
Q3:Interference with activity	no		no	sometimes	usually
Single score for symptom of dryness	None (0)	Trace (1)	mild (2)	moderate (3)	severe (4)

Table 6-5 Subjects' demographic distribution

	Non-dry eye		**Dry eye symptomatic**	
Sub-group	younger	older	younger	older
Number of subjects	33	21	24	19
Age (mean±SD ,range)	29 ± 8 (19-49)	61 ± 8 (50-79)	38 ± 10 (19-49)	63 ± 10 (52-80)
Gender (female/male)	17/16	14/7	18/6	17/2

Corneal and conjunctival sensitivity was estimated using a computer-controlled pneumatic Belmonte esthesiometer [19] that has been described in detail elsewhere.[30] A video camera continuously monitored the distance between and orthogonal alignment of the tip of the esthesiometer and the ocular surface. Custom software was used to select stimulus duration and record subject responses from a button box.

Measurements of corneal and conjunctival sensitivity were performed on the right eye only, at least four hours after subjects awoke.[31] A training session was conducted using the central cornea of the left eye prior to the actual measurements. Subjects were instructed to view fixation targets at 3 meters and the tip of the esthesiometer was set 5 mm from the corneal and conjunctival surface using the

video camera. The tip of the esthesiometer was rotated to ensure the stimulus was delivered perpendicularly to the surface.

The stimuli, consisting of a series of air pulses at room temperature (20°C, a mechanical stimulus with a cooling effect[18,21,32-34]) with air flow varying from 0 to 200 ml/min, were delivered to the central cornea and temporal conjunctiva (approximately 5 mm from the limbus). The stimulus duration was one second, with a 15-second interval between the subject's response and the next stimulus. Two seconds prior to each stimulus, a short, computer-generated tone reminded the subject to blink and view the fixation target. The subjects were informed that they were to blink freely and also to close their eyes or look down between stimuli. Subjects could also interrupt the trials if necessary. Using the ascending method of limits[35] with a randomly selected initial level of stimulus and a step of increment in flow rate of 5 ml/min, corneal and conjunctival thresholds were determined by the average flow rate of three first reports of the stimulus presence.

Statistical analyses were performed using Statistica 7.0 (StatSoft Inc. Tulsa, OK USA) and $p<0.05$ was considered to be statistically significant. Because of the characteristics of the data, Mann-Whitney U tests were used to compare the corneal and conjunctival thresholds between symptom, gender and age groups. Wilcoxon Matched Pair tests were used to compare the differences in thresholds between cornea and conjunctiva. Spearman Rank correlation was used to examine the association between corneal and conjunctival threshold.

6.2.4 Results

Conjunctival and corneal thresholds for detection of pneumatic stimuli at room temperature are presented in Figure 6-7. Both conjunctival and corneal thresholds were lower in the dry eye symptomatic group than in the non-dry eye subjects. The differences between the two groups were significant ($p<0.001$ and $p<0.01$ for conjunctival and corneal threshold, respectively). Conjunctival thresholds were significantly different to corneal thresholds in the dry eye symptomatic group ($p<0.01$) but they were not significantly different from the non-dry eye group ($p>0.05$). In addition, conjunctival and corneal thresholds were correlated significantly in both groups ($Rho=0.77$, $p<0.001$).

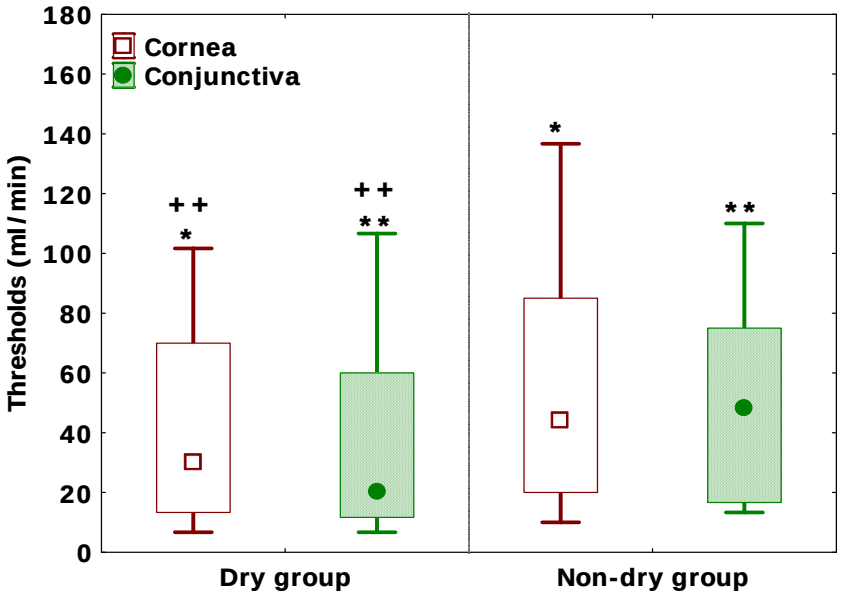


Figure 6-7 Box plot of the medians for corneal and conjuncitval thresholds in the dry and non-dry groups

Note: Values were: medians, box: 10%-90%; Whisker: non-outlier range.

The medians for corneal and conjuncitval thresholds in the dry and non-dry groups, characterized by gender are shown in Figure 6-8. The gender difference in conjunctival threshold was significant in the non-dry eye group ($p=0.02$) but not in the dry eye symptomatic group ($p=0.06$). Corneal thresholds were similar in females and males in both symptomatic and asymptomatic subjects ($p>0.05$ for both dry eye symptomatic and non-dry eye group), though thresholds were generally lower in females than males.

There were no significant differences in conjunctival and corneal thresholds between the younger and older group ($p>0.05$ for both cornea and conjunctiva). This pattern remained the same when the age groups were further stratified by symptom, as shown in Figure 6-9 (all $p>0.05$).

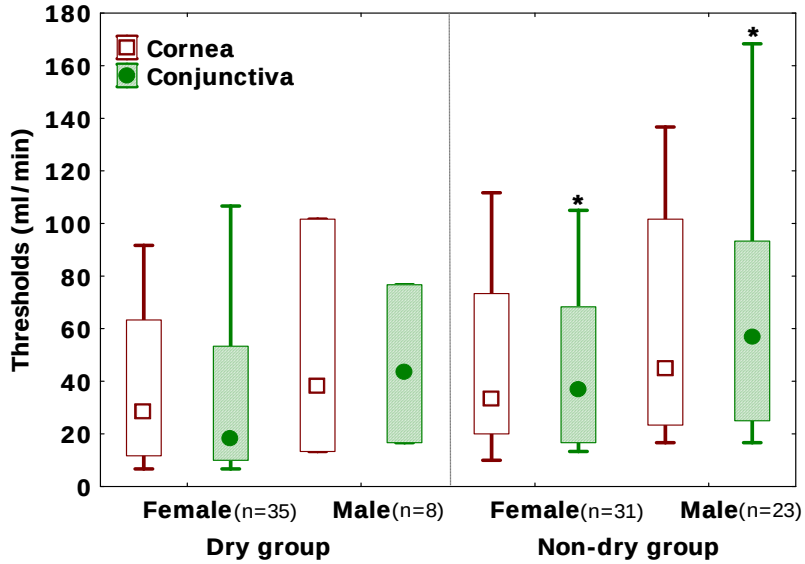


Figure 6-8 Box plot of the medians for corneal and conjuncitval thresholds in the dry and non-dry groups, subdivide by gender

Note: Values were: medians, box: 10%-90%; Whisker: non-outlier range.

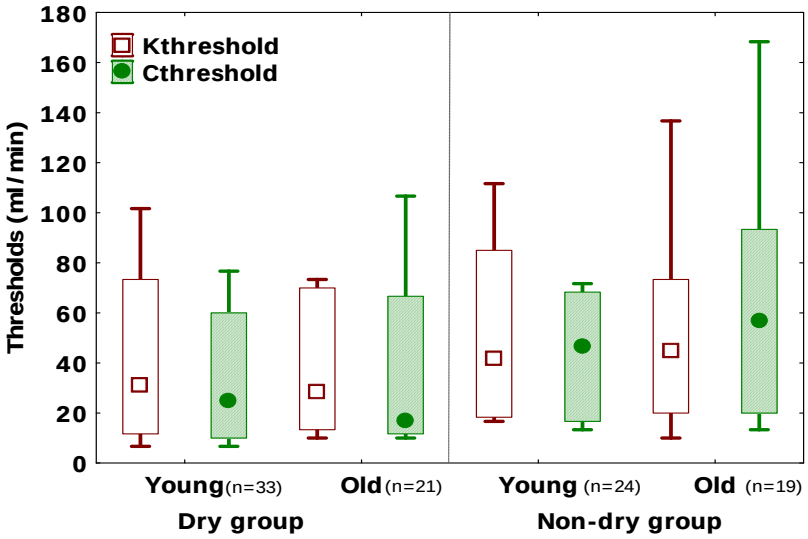


Figure 6-9 Box plot of the medians for corneal and conjunctival thresholds in the dry and non-dry groups, subdivide by age

Note: young (19-49 years) and old (50-80 years). Values were: medians, box: 10%-90%; Whisker: non-outlier range.

6.2.5 Discussion

This study investigated conjunctival and corneal sensitivity to pneumatic stimuli at room temperature in subjects with and without symptoms of ocular dryness. The corneal thresholds obtained in this study were similar to those found in previously published studies using the same type of stimuli.[32,36] Conjunctival thresholds in the present study were slightly lower than the threshold of the inferior conjunctiva reported by Stapleton and colleagues.[36] This could be due to a number of differences in the methods, including the ocular site where measurements were made in the two studies.

Neuronal regulation is hypothesized to be one of the important mechanisms contributing to the pathophysiology of dry eye.[10] The components of the lacrimal functional unit are linked by the sensory, sympathetic, and parasympathetic nerves to a homeostatic loop.[5] The neuronal pathways in the lacrimal functional unit interact through reflex mechanisms and play complex excitatory or inhibitory roles in regulating the production and function of the mucin and aqueous components of the tears and perhaps also the lipid phase in the tear film.[5,6,37,38] These nerves are also involved in maintaining the health of the ocular surface.[39,40] Previous studies investigated the relationship between corneal sensitivity and tear secretion measured with the Cochet-Bonnet esthesiometer [26,27] and demonstrated that some dry eye patients had decreased corneal tactile sensitivity. Reduced afferent stimulation resulting from decreased corneal sensitivity has been hypothesized to account for the reduction in tear production in dry eye patients.[7] Recently, a study reported hyperesthesia to pneumatic mechanical stimulation in dry eye patients as well as post-LASIK patients with symptoms of dryness (measured using a Belmonte pneumatic esthesiometer),[25] whereas another studies showed hypoesthesia in dry eye patients.[23,24] In the present study, we found that both conjunctival and corneal sensitivity to pneumatic stimulation at room temperature was higher in the symptomatic subjects, which is consistent with the findings of de Paiva and Pflugfelder. [25] This points to altered sensory processing and disruption of the homeostatic neural control mechanism in the dry eye symptomatic subjects.

It has been reported that the activities of corneal sensory fibres could be modified by injury and inflammation of the ocular surface.[15] Sensitization, the decrease in threshold and enhanced responsiveness and spontaneous activity caused by repeated noxious stimuli, is a feature of polymodal nociceptors, including corneal polymodal neurons. Sensitized nociceptors can be triggered by local inflammatory mediators and mechanical stimulation that would have been insufficient to activate intact nerve endings under normal conditions. Endogenous inflammatory mediators from

cells related to tissue injury and inflammation are believed to contribute to sensitization.[15] These mediators alter the permeability of the ion channels of the receptors, as well as trigger a variety of second and third messenger pathways and ultimately affect the membrane potential and the excitability of the nociceptors.[15] Certain inflammatory mediators such as prostaglandin E2 and bradykinin have been reported[41] to induce sensitization of corneal polymodal nociceptors. Interleukin 1 (IL-1), a pro-inflammatory cytokine found in the tears and conjunctiva (using impression cytology) in KCS patients[42] has been reported to induce hyperesthesia.[43] Since inflammation has been hypothesized to be a common pathway for dryness related ocular surface diseases,[8-10] the inflammatory mediators may contribute to the altered activity of sensory receptors and result in the hyperesthesia in symptomatic patients we found in this experiment.

The conjunctiva is not only supplied by free nerve endings, as in the cornea, but also is a highly reactive tissue, richly supplied by blood vessels, connected to the lymphatic system, and filled with immunocompetent cells.[44] Conjunctival epithelial stratified squamous cells secrete electrolytes and water into the aqueous layer and also absorb them from the tear film, thereby modifying it.[37] Conjunctival epithelial goblet cells secrete mucin into the mucous layer and these cells have specific innervation.[37,45] Sensory nerves also serve as afferent input for the neural regulation of, and interaction with, the parasympathetic and sympathetic nervous systems in the conjunctiva. In the present study, conjunctival sensitivity to pneumatic stimuli at room temperature was significantly greater than corneal sensitivity in the symptomatic patients. It is therefore likely that the sensory processing of the conjunctiva is more impaired in symptomatic subjects. Considering that the conjunctiva is directly involved in the regulation of aqueous and mucin phases of the tear film and has a large area covering the ocular surface, this conjunctival hypersensitivity may have important clinical implication in dry eye diseases. In addition, we also found that conjunctival sensitivity was positively correlated with corneal sensitivity; on this basis we propose that the previously often overlooked conjunctiva also plays an important sensory role in the functional unit. Perhaps corneal and conjunctival hyperesthesia may also account for the reported symptoms of ocular irritation being aggravated by environmental challenges such as smoke, low humidity, and air conditioning in dry eye patients.[46,47]

The prevalence of dry eye seems to increase with age and to be greater among women.[2-4] It is unknown whether age and gender would be confounding factors contributing to the difference in sensitivity found in the symptomatic and asymptomatic groups. Decreased corneal sensitivity to

tactile stimuli has been reported in people over 50-60 years of age and the development of arcus senilis was suggested to partly account for this decline with age.[48,49] Recently, a study found that corneal sensitivity decreased in the older age group (age ≥60 years) when comparing to the younger group (age <60 years) and suggested the reduction of sensitivity was due to the decreased subbasal nerves density.[23] Bourcier et al. found that corneal sensitivity (measured with a Belmonte pneumatic esthesiometer) to thermal stimulation (heat and cold) did not correlate with age.[24] In our study, the stimuli produced a mainly thermal (cooling) effect.[15;26] We also found that corneal sensitivity was similar in subjects below and above 50 years of age, which is consistent with findings reported by Bourcier et al.[24] However, females and males in the non-dry eye group had different levels of conjunctival sensitivity. The reason for this gender-based variation in conjunctival sensitivity remains to be elucidated. We failed to find a difference between genders in the dry eye symptomatic group and this could be partly due to insufficient statistical power, as only a small proportion of participants in this group were male.

The findings in the current study using a pneumatic esthesiometer conflict with previous reports of decreased corneal sensitivity to tactile stimulation with the Cochet-Bonnet esthesiometer in dry eye patients.[22,26,27] The stimulus used in our study produced a pneumatic cooling effect. This is not the same as a noxious tactile stimulus, and these different stimuli are processed by different ocular surface sensory channels.[32] On the other hand, limited or no differences in corneal and conjunctival sensitivity to thermally cooling stimuli using pneumatic esthesiometer have been reported previously,[21,50,51] which is also observed in the non-dry eye group in the present study. Therefore, our findings provide additional support for the hypothesis that Cochet-Bonnet and pneumatic room temperature stimuli are fundamentally different.[14,30,33,50]

The hypersensitivity to pneumatic stimulation at room temperature is also different to recent reports of decreased corneal sensitivity to noxious mechanical, and chemical stimulation in dry eye patients.[23,24] Once again, there were differences between the two studies with regard to stimulus types. The pneumatic cool stimuli used in the current study, consisting of thermal (cool) and mechanical components,[18,21,34,36] may activate both cold and polymodal receptors of the ocular surface. Perhaps the responsiveness from the cold receptors in the dry eye symptomatic group (i.e. enhanced sensitivity to cold stimulus) contributes to this discrepancy between studies. One more possibility might account for this difference reported by both our group and de Paiva & Pflugfelder. If there are differences in the tear film of symptomatic and asymptomatic subjects that affect how the pneumatic

stimulus interacts with the ocular surface, the results could differ between groups. This hypothesis cannot reconcile the differences between studies or account for such observations as lack of an age effect, but does highlight the complexity of, and our lack of, a thorough understanding of the direct effect of this stimulus on the corneal and conjunctival surface and nerves. This deficiency in knowledge deserves further research.

In summary, this study found that symptomatic subjects showed corneal and conjunctival hypersensitivity to pneumatic stimuli at room temperature, indicating alteration of sensory processing in these patients. This was more prominent in the conjunctiva than in the cornea suggesting that the change in conjunctival sensitivity may be an important characteristic of dryness-related ocular surface diseases.

6.3 Summary of Part one and two

These studies showed that conjunctival and corneal sensitivity to pneumatic cool stimulation was increased in subjects with symptoms of ocular dryness and the symptomatic group manifest greater corneal and conjuncitval staining and shorter tear film break-up time. The hyperesthesia to cooling was associated with symptoms of ocular irritation and seemed to be greater on the conjunctiva. Also, in the non-dry eye group, conjunctival sensitivity was higher in female subjects than the males.

Chapter 7

Effect of SH lens wear on ocular surface sensitivity

Chapter 7 has been submitted to *Investigative Ophthalmology & Visual Science* for publication and is currently under review.

Chapter 7 was submitted as follows:

Effects of silicone hydrogel contact lens wear on ocular surface sensitivity to tactile, pneumatic mechanical and chemical stimulation

P Situ, T.L. Simpson, L.W. Jones, D. Fonn

	Situ	Simpson	Jones	Fonn
Experimental design	Y	Y	-	Y
Data collection	Y	-	-	-
Data analysis	Y	Y	-	-
Write-up publication	Y	Y	Y	Y

7.1 Overview

Purpose: To determine the effects of silicone hydrogel lens wear and lens-solution interactions on ocular surface sensitivity.

Methods: Forty-eight adapted lens-wearers completed the study, which comprised two phases. Phase 1 included habitual lens, no-lens wear (7±3 days) and balafilcon A lenses (PV) with a hydrogen peroxide-based regimen for 2 weeks; Phase 2 included wear of PV with multipurpose solution preserved with either polyhexamethylene-biguanide (PHMB) or Polyquad/Aldox (each for 1 week) with a 2-week washout period between solutions. Tactile and pneumatic (mechanical and chemical) stimuli were delivered to determine thresholds by a Cochet-Bonnet and Belmonte pneumatic esthesiometer respectively. Corneal and conjunctival thresholds and staining scores were assessed at baseline, after 2- and 8-hour of lens wear on day-1 and at the end of each wearing cycle (after 2 hours).

Results: In phase 1, compared to no-lens baselines, corneal tactile thresholds increased at the 1-day 8-hour and 2-week visits ($p<0.05$) while conjunctival mechanical thresholds decreased at the 1-day 2-hour and 2-week visits ($p<0.05$). In phase 2, the chemical thresholds were lower with PHMB-preserved solution compared to the Polyquad/Aldox system at 1-day 2-hour and 1-week visits ($p<0.05$). Staining scores were inversely correlated to conjunctival chemical thresholds (all $p<0.05$).

Conclusions: Ocular surface sensitivity changed in adapted lens-wearers after lenses were refit following a no-lens interval, and during lens wear with different care regimens. Corneal staining occurring with certain lens-solution combinations is accompanied by sensory alteration of the ocular surface, i.e., higher levels of staining are correlated with increased conjunctival chemical sensitivity.

7.2 Introduction

Highly oxygen permeable silicone hydrogel (SH) lenses offer certain advantages over traditional hydrogel lenses for lens wearers, by eliminating lens-induced hypoxia and producing less detrimental effects on corneal homeostasis.[1] Despite these ocular health benefits, SH lens wear is generally similar to hydrogel lens wear with respect to its mechanical interaction with the ocular surface and the effects on structure and physiology of the tear film.[2]

The contact lens interacts with the cornea and conjunctiva; both are innervated by sensory nerve endings[3] that are functionally heterogeneous.[4] For example, mechano-nociceptors respond to mechanical stimuli only, cold receptors signal downward temperature changes in the non-noxious

range, while polymodal-nociceptors respond to mechanical, chemical and thermal stimuli.[4] The cornea and conjunctiva provide sensory input to the functional unit comprising the ocular surface (cornea, conjunctiva and meibomian glands), the lacrimal glands and the sensory and motor nerves that connect them.[5] Through a complex network, the afferent and efferent nerves link the components of the integrated unit into a homeostatic loop, with the primary function to protect and maintain the health of the ocular surface and the tear film.[6]

The measurement of ocular surface sensitivity is one way to assess the functioning of the sensory nerves and has been a useful clinical indicator of corneal health, in contact lens wear and corneal disease and during the healing process following various corneal injuries and refractive surgery.[7-9] In the past, sensitivity has been measured mainly using Cochet-Bonnet type esthesiometers[10, 11] in which hair or nylon filaments of variable diameter and length were used to deliver tactile stimuli to the ocular surface. The more recently developed pneumatic esthesiometers[12-14] deliver controlled air pulses at various temperature and air-CO_2 mixtures to the ocular surface and allow measurements of ocular surface sensitivity over a range of thermal, mechanical and chemical stimuli.

Reduced corneal sensitivity has been reported in contact lens wear.[15-23] The reduction in sensitivity seems to be eliminated after the cessation of lens wear.[16, 17] Metabolic impairment (i.e. hypoxia) has been considered to be the principal cause of reduced sensory nerve function in traditional low oxygen permeability (Dk) hydrogel lens wear.[20, 24] Other factors contributing to this sensitivity loss include sensory adaptation[25, 26] and acidosis-suppressed corneal nerve function.[7] Most previous reports have been based on wearing earlier generation low Dk lens materials and the sensitivity measurements were limited to tactile stimulation. It is largely unknown whether SH lens wear affects ocular surface sensitivity differently, particularly when lens-induced hypoxia has been reduced or eliminated. Also, there have been no reports on how tactile, mechanical and chemical sensitivity changes with SH lens wear over time. Moreover, perhaps due to the unique nature of SH lens materials, there is accumulating evidence that the interaction between certain SH lenses and preserved multipurpose solutions (MPSs) affects the ocular surface, manifested as corneal fluorescein staining.[27, 28] It is unclear whether the interaction between the lens material and care regimens would have an impact on ocular surface sensitivity, although one study with a small sample has intimated that this may be the case.[23]

The present study investigated the effects of SH lens wear and lens-solution interactions on ocular surface sensitivity. We used different stimuli delivered by Cochet-Bonnet and Belmonte

esthesiometers and measured corneal and conjunctival thresholds in a group of adapted contact lens wearers, before and during a short period of cessation of lens wear and after refitting with SH lenses disinfected with a variety of care regimens.

7.3 Methods and Materials

This study was conducted in accord with the guidelines of the Declaration of Helsinki and received clearance from the University of Waterloo, Office of Research Ethics (Waterloo, Ontario, Canada). Informed Consent was obtained from each subject. It was registered with the National Clinical Trial Registry (NCT00455455).

7.3.1 Subjects

Sample size calculations to detect significant differences were derived from unpublished data (to achieve power of 0.80, with effect size of 0.4 at 5% significance level, required sample size was 50). Fifty subjects, consisting of 35 females and 15 males (mean age 25.2 ± 7.4 years, ranged from18 to 45 years), who were adapted contact lens wearers and asymptomatic during at least 8 hours of lens wear, participated in the study. Each subject had no history of eye surgery, systemic or ocular disease and was not using any systemic or topical medication that would affect ocular health. Two subjects did not complete the study; one was due to burning during lens insertion and the other one was for personal reasons.

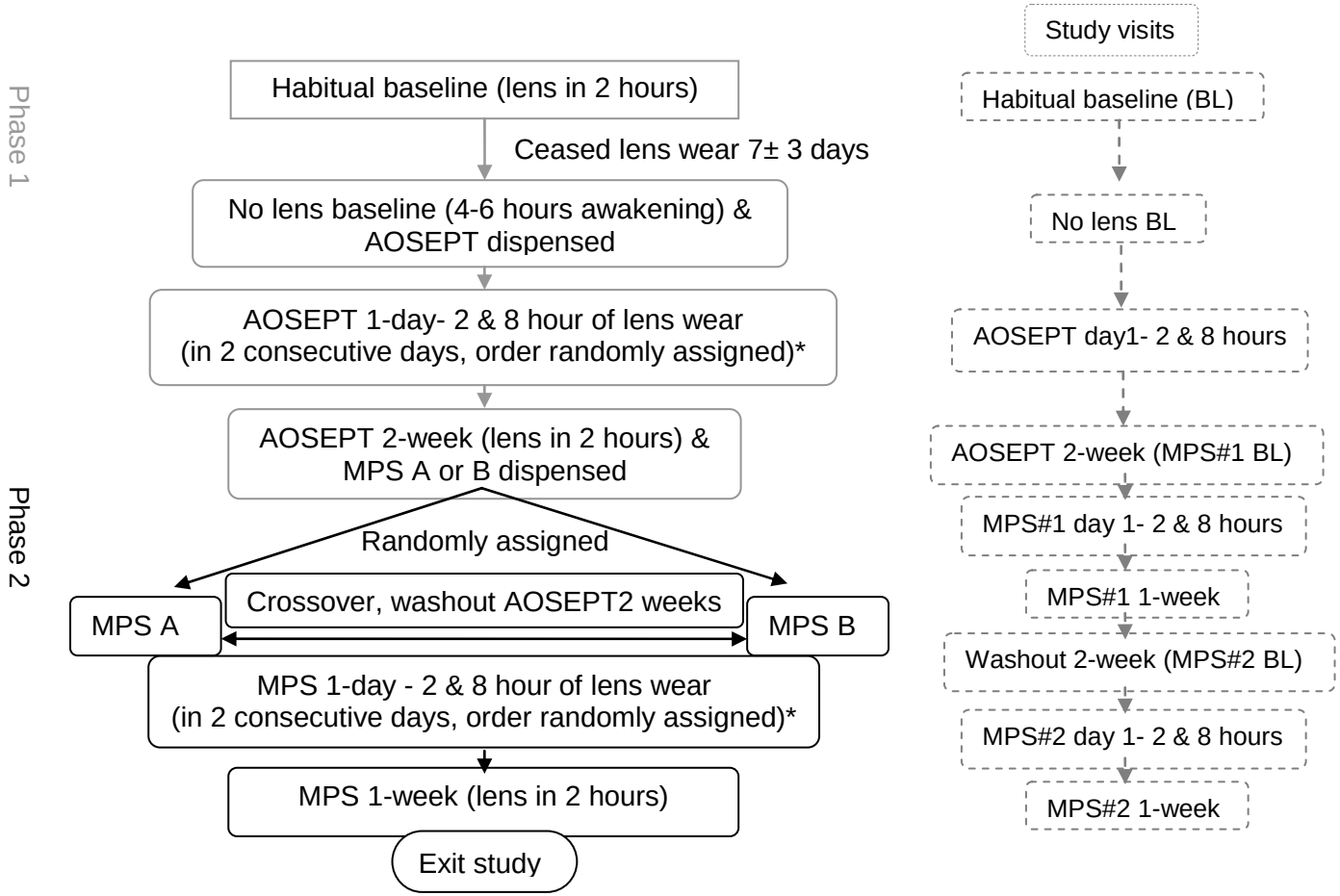
7.3.2 Study lens and care regimens

Balafilcon A SH lenses (PureVision, Bausch & Lomb, Rochester, NY) and three lens care regimens were used in the study. A hydrogen peroxide-based regimen (AOSEPT, CIBA Vision, Duluth, GA) was used in Phase I and in a wash-out period in Phase II, to eliminate potential confounding effects of surfactants and preservatives. Two multipurpose solutions (MPSs), the polyhexamethylene biguanide (PHMB)-based (ReNu MultiPlus, Bausch & Lomb, Rochester, NY) and a solution preserved with Polyquad(polyquaternium-1) and Aldox (myristamidopropyl dimethylamine) (OPTI-FREE RepleniSH, Alcon Laboratories, Fort Worth, TX), were used in Phase II. Subjects wore balafilcon A lenses throughout the study, fit according to the manufacturer's guidelines, on a daily wear basis. Each subject received a new pair of lenses upon commencing each lens care regimen period. Lenses were cleaned and disinfected after each wearing period (i.e. after each day of lens wear), using the lens care regimen assigned to each particular phase. Subjects were not permitted to use rewetting drops while wearing the study lenses.

7.3.3 Study protocol

The study had a randomized, single (experimenter) masked and bilateral design with crossover as illustrated in Figure 7-1. It consisted of two phases: Phase I included habitual lens wear, no lens wear and use of PureVision lenses (PV) with AOSEPT for two weeks, and Phase II included PV with the two MPS care regimens (each for one week), with a two-week washout period during which PV with AOSEPT were used. The order of the MPS solutions used in Phase II was randomly assigned and the investigator was masked with respect to the solution that each subject was using.

Following slit-lamp biomicroscopy to examine the lens and ocular surface, corneal and conjunctival thresholds to tactile, pneumatic mechanical and chemical stimulation were measured on one eye at each visit (Figure 7-1). The test eye was determined randomly and the same eye was used throughout the study.



*The two- and eight-hour measurements were taken on consecutive days to ensure that the lenses would be worn for eight consecutive hours prior to the eight-hour measurement. To eliminate the time effect introduced by the order of the measurement sessions, they were conducted in a random order at two and eight hours after lens insertion.

Figure 7-1 Flowchart of study design and visits

7.3.4 Primary outcome measures

7.3.4.1 Tactile stimulation thresholds

Tactile thresholds were measured using a Cochet-Bonnet esthesiometer (Luneau France, France). A nylon thread (0.12 mm diameter) was used to deliver tactile stimulation to the cornea and conjunctiva. The length of the thread was decreased in 5-mm steps until the participant reported feeling it, and the length of this stimulus detected was converted into pressure using the table provided by the manufacturer.

7.3.4.2 Pneumatic mechanical and chemical stimulation thresholds

A computerized Belmonte pneumatic esthesiometer that has been described in detail elsewhere[12] was used to measure mechanical and chemical thresholds, using an ascending method of limits. The tip of the esthesiometer was set 5 mm from the corneal and conjunctival surface and monitored using a calibrated video camera. The mechanical stimuli consisted of a series of air pulses with flow rates varying from 0 to 200 ml/min. Chemical stimulation was induced by increasing the concentration of CO_2 in the air (with the stimulus flow rate fixed at half of the initially estimated mechanical threshold). For both mechanical and chemical threshold measures, the stimulus temperature was at 50°C, which was approximately 33°C at the ocular surface.[12, 29, 30] The stimulus duration was two seconds, with a 20-second and 45-second intervals between the subject's response and the next stimulus for mechanical and chemical measures, respectively.

Tactile, pneumatic mechanical and chemical thresholds were estimated on the temporal mid-peripheral cornea (approximately 3 mm from the apex) and temporal conjunctiva (approximately 5 mm from the limbus). A training session was conducted using the temporal mid-peripheral cornea of the contralateral eye prior to measurement. Subjects were instructed to view fixation targets at about three meters and to blink freely between stimuli. Subjects could also interrupt the trials if necessary. Corneal and conjunctival detection thresholds were the average of three intensities at stimulus detection for each modality of stimulation. The measures were performed in the following order: Pneumatic mechanical, chemical and tactile thresholds.

7.3.5 Secondary outcome measure

7.3.5.1 Corneal and conjunctival staining

Five regions of the cornea (central, inferior, temporal, superior and nasal) were evaluated for staining two to three minutes following sodium fluorescein instillation (FUL-GLO® fluorescein strip wetted by Minims® single-dose saline), using cobalt light and a #12 Wratten filter. A "percent-staining-area" was calculated based on the average staining area (extent) measured across all five regions. Conjunctival staining was measured using Lissamine green (Rose Stone Enterprises) with white light and a Hoya 25A filter and graded based on the severity of each region (inferior, temporal, superior and nasal) on a scale of 0 (none) to 4 (severe).

7.3.6 Statistical analyses

Statistical analyses were performed with Statistica 8.0 (StatSoft Inc. Tulsa, OK, USA) and $p \leq 0.05$ was considered to be statistically significant. Repeated measures analyses of variance (ANOVA) and post hoc Tukey HSD tests were carried out on the thresholds and corneal staining data. The difference in thresholds in Phase I was compared between visits. For Phase II data, the main effects of MPS solution and visit and their interaction were examined. In addition, Friedman ANOVA and post hoc Wilcoxon with Bonferroni correction was used to test the differences in conjunctival staining between MPS solutions and visits. Pearson correlations were performed between thresholds and corneal staining scores.

Although all analyses of esthesiometry data were done on thresholds, since sensitivity is the reciprocal of the threshold and the most commonly used term, this term is used in discussing these outcomes.

7.4 Results

7.4.1 Effects of SH lens wear on corneal and conjunctival sensitivity

Corneal and conjunctival thresholds for tactile, pneumatic mechanical and chemical stimulation for Phase I are presented in Table 7-1.

Table 7-1 Corneal and conjunctival tactile, pneumatic mechanical and chemical thresholds (mean ± SD) in Phase I

	Tactile (mm/grs/S)		Pneumatic mechanical (air flow ml/min)		Chemical (%CO2 added)	
	cornea	conjunctiva	cornea	conjunctiva	cornea	conjunctiva
Habitual lens	31.0 ± 18.2	116.0 ± 45.1	56.8 ± 30.3	60.3 ± 33.2	24.1 ± 9.2	47.8 ± 16.7
No lens	28.7 ± 16.0	117.1 ± 37.5	53.4 ± 26.2	64.1 ± 30.6	27.0 ± 11.7	47.0 ± 15.9
Dispensing PV lens and AOSEPT solution						
Day 1 – 2hrs	32.4 ± 15.9	122.5 ± 33.5	47.9 ± 23.5	50.7 ± 24.0	25.3 ± 11.0	49.2 ± 18.3
Day 1 - 8hrs	35.1 ± 20.5	126.0 ± 42.0	47.4 ± 23.8	54.3 ± 29.7	25.8 ± 11.2	49.9 ± 17.9
Week 2 - 2hrs	34.5 ± 19.0	121.4 ± 39.6	49.6 ± 24.6	53.6 ± 33.5	26.2 ± 13.3	49.0 ± 18.8

There was a significant difference in corneal tactile threshold between visits, as shown in Figure 7-2 (p=0.003, ANOVA). At the 8-hour visit on day one and two weeks after the study lens was dispensed, the tactile threshold was higher compared to that at the end of the no lens-wear period (Tukey HSD, p=0.003 and 0.012 for 1-day 8-hour and 2-week, respectively). There was no significant change in conjunctival tactile thresholds between visits (p>0.05, ANOVA).

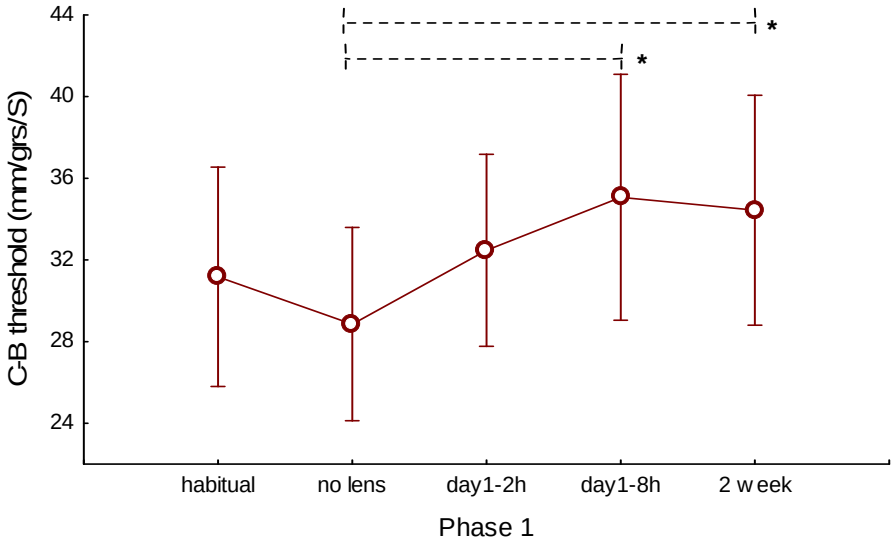


Figure 7-2 Changes of corneal threshold to tactile stimulation in Phase 1

Note: Data are the mean ± 95% CI. Tactile thresholds at 1-day 8 hours and 2-week visits were increased from no-lens baseline. *p<0.05.

For pneumatic mechanical thresholds, there were significant differences in both corneal and conjunctival thresholds between visits, as seen in Figures 7-3 and 7-4 (p=0.013 and 0.002 for cornea and conjunctiva, respectively, ANOVA). Corneal thresholds at the 2 and 8-hour visits on day one were lower than those with habitual lens wear (Tukey HSD, p=0.036 and 0.022 for 2- and 8-hour, respectively). The conjunctival threshold at the end of the no lens-wear period was higher than measurements taken after two hours of PV lens wear at the day one and 2-week visits (Tukey HSD, p=0.003 and 0.038 for 1-day 2-hour and 2-week, respectively).

For corneal and conjunctival chemical thresholds, there were no significant differences between visits in Phase I (both p>0.05, ANOVA).

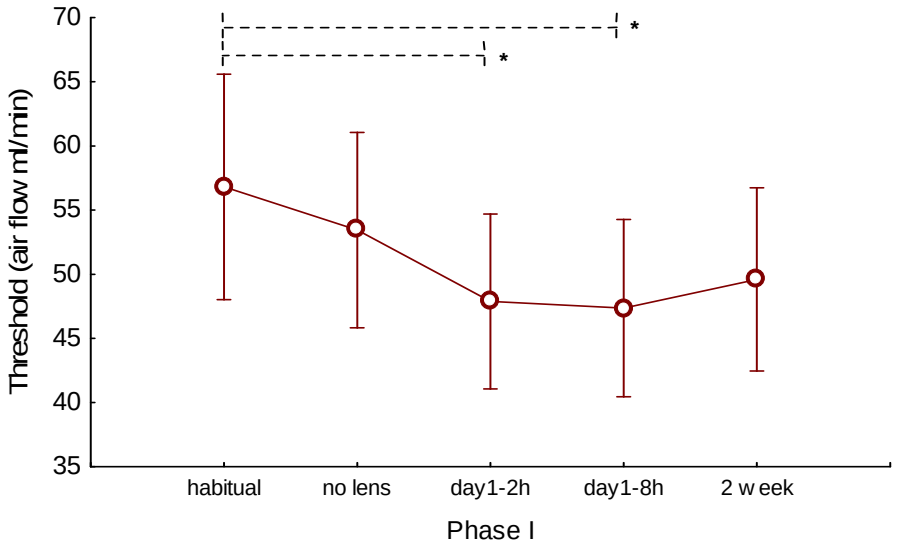


Figure 7-3 Changes of corneal threshold to pneumatic mechanical stimulation in Phase 1

Note: Data are the mean ± 95% CI. Compared to habitual lens wear, threshold was lower at the one-day visits after refitting with the study lenses following a short period of no lens-wear. *p<0.05.

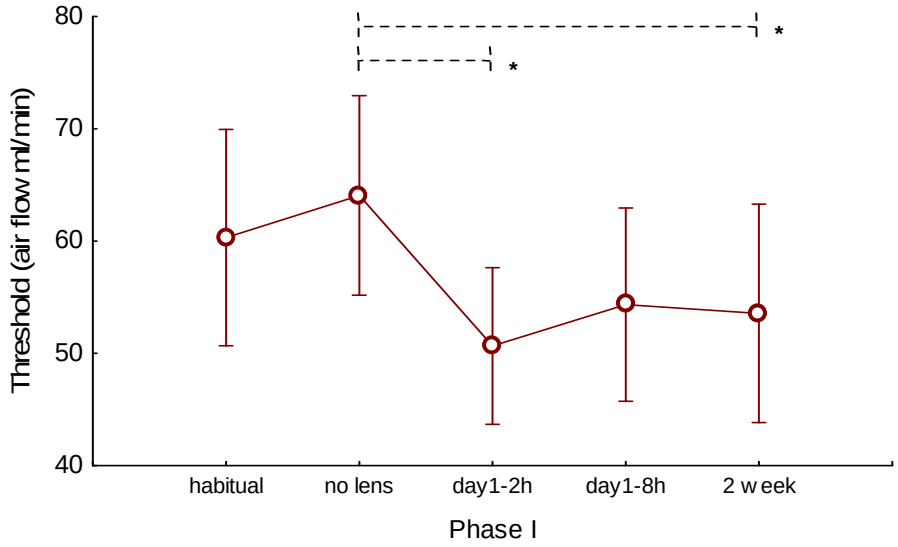


Figure 7-4 Changes of conjunctival threshold to pneumatic mechanical stimulation in Phase 1

Note: Data are the mean ± 95% CI. Threshold was decreased at both 2-hour visits compared to no lens-wear. *p<0.05.

7.4.2 Effect of lens-solution interactions on corneal and conjunctival sensitivity

Corneal and conjunctival thresholds to tactile, pneumatic mechanical and chemical stimulation for Phase II are summarized in Table 7-2. The statistical results for Phase II are described in the following order: chemical, pneumatic mechanical and tactile thresholds.

Table 7-2 Corneal and conjunctival tactile, pneumatic mechanical and chemical thresholds (mean ± SD) in Phase II

	Tactile (mm/grs/S)		Pneumatic mechanical (air flow ml/min)		Chemical (%CO2 added)	
	cornea	conjunctiva	cornea	conjunctiva	cornea	conjunctiva
PV + Polyquad/Aldox system period						
Baseline (AOSEPT)	35.5 ± 19.0	123.9 ± 33.3	50.4 ± 25.9	53.1 ± 32.3	26.9 ± 13.0	48.7 ± 20.2
Day1 – 2hrs	35.1 ± 17.2	125.6 ± 31.2	46.6 ± 25.7	51.4 ± 28.7	26.0 ± 12.9	51.2 ± 20.1
Day1-8hrs	34.2 ± 20.3	126.6 ± 32.6	45.0 ± 24.8	49.4 ± 28.6	25.6 ± 14.1	50.6 ± 21.3
Week 1-2hrs	33.9 ± 18.1	120.7 ± 27.5	44.3 ± 21.7	50.1 ± 32.0	25.5 ± 13.4	50.5 ± 20.2
PV + PHMB-preserved system period						
Baseline (AOSEPT)	34.2 ± 16.5	121.9 ± 36.7	46.2 ± 26.5	48.5 ± 30.0	25.5 ± 12.9	49.6 ± 18.7
Day1 – 2hrs	34.6 ± 16.3	122.6 ± 35.1	44.6 ± 28.5	46.5 ± 27.5	22.9 ± 12.8	46.8 ± 21.2
Day1-8hrs	36.9 ± 18.6	121.8 ± 33.7	43.5 ± 24.8	45.5 ± 27.7	24.8 ± 12.8	49.0 ± 21.3
Week 1-2hrs	36.0 ± 18.7	124.2 ± 33.4	45.6 ± 25.8	50.5 ± 35.5	22.1 ± 13.8	46.2 ± 22.4

Corneal chemical threshold was significantly different between the MPSs regardless of visits (p=0.049, ANOVA). The threshold with the PHMB-preserved solution was lower compared to the Polyquad/Aldox system. Figure 7-5 shows that there was a significant difference in corneal chemical thresholds between visits averaged across solutions (p=0.035, ANOVA). Threshold at one week was lower than baseline (with AOSEPT) (Tukey HSD p=0.025). However, the interaction between solution and visit was not significant, as seen in Figure 7-6A (p>0.05, ANOVA).

Conjunctival chemical threshold in Phase II appeared to be lower with the PHMB-preserved solution than with the Polyquad/Aldox system. However, the difference was not statistically significant (p>0.05, ANOVA). There was a significant interaction between solution and visit as shown in Figure 7-6B (p= 0.028, ANOVA). At the 2-hour visit on day one and the 1-week visit, there were significant differences between the two MPSs (Tukey HSD, both p=0.049); lower threshold with the use of PHMB-preserved solution.

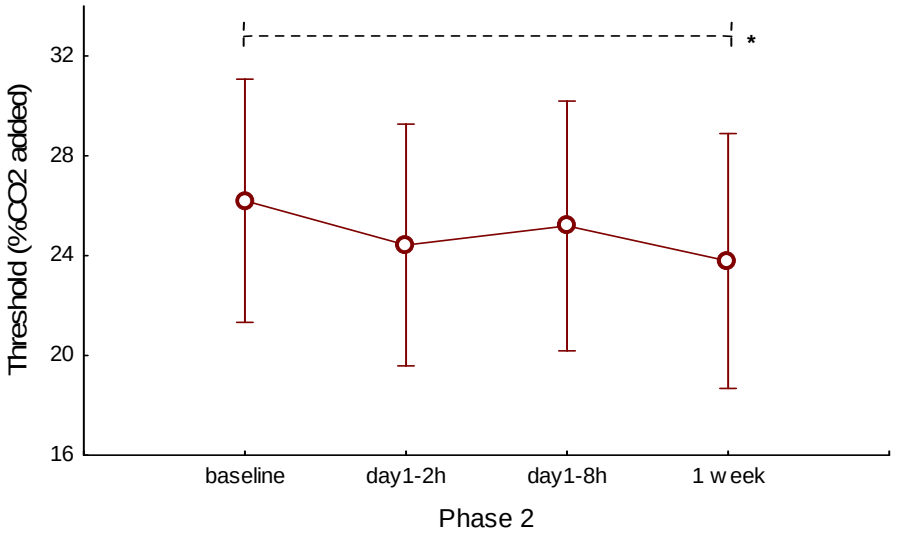


Figure 7-5 Changes of corneal chemical thresholds over time in Phase 2

Note: Data are the mean ± 95% CI. Chemical threshold at 1-week visit regardless of MPS used was lower compared to baseline (with use of AOSEPT). *p<0.05.

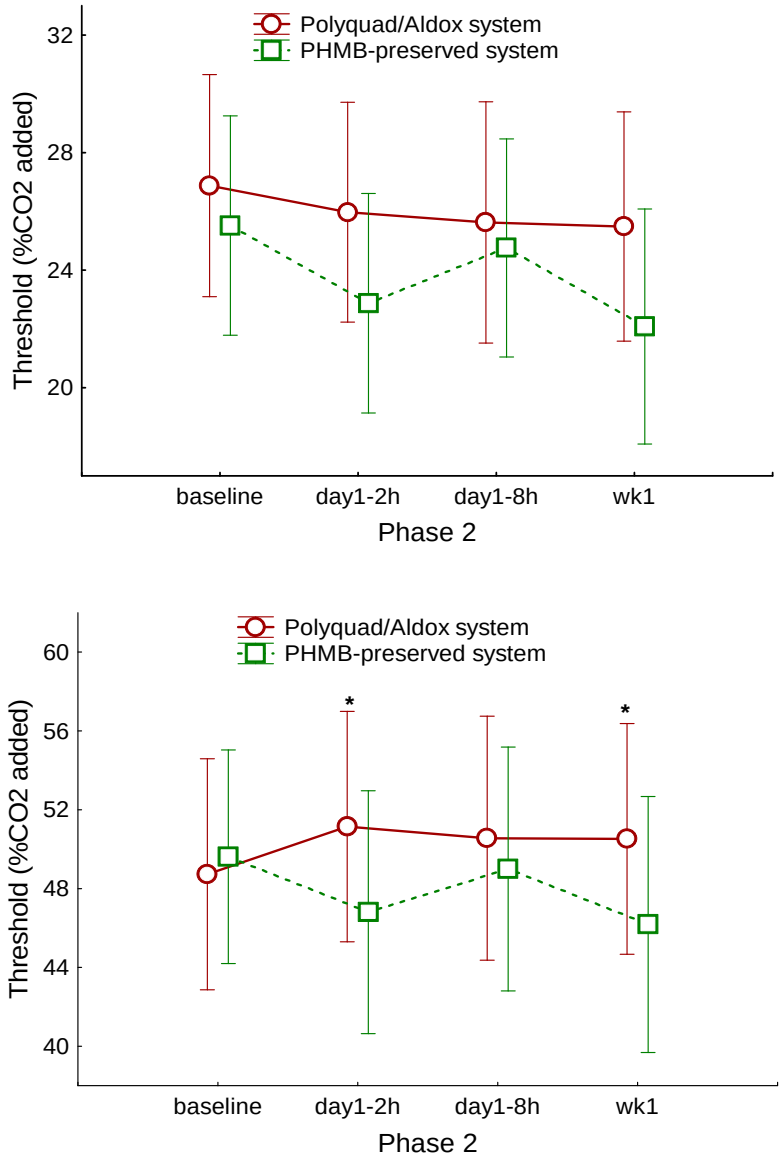


Figure 7-6 Interactions between solution and visit for corneal (A, top) and conjunctival (B, bottom) chemical thresholds in Phase 2

Note: Data are the mean ± 95% CI. (A, top) Corneal thresholds with the use of PHMB-preserved solution tended to be lower than that with the Polyquad/Aldox system at the 2-hour visits although the interaction was not statistically significant (ANOVA, $p>0.05$). (B, bottom) At the 2-hour visits, conjunctival chemical thresholds were significantly different between solutions (*$p<0.05$).

For pneumatic mechanical thresholds (Table 7-2), there was a significant difference between visits regardless of solution used (p=0.049, ANOVA). Thresholds with the use of MPS tended to be lower than the baseline (AOSEPT). However, the differences between each paired comparison did not reach statistical significance (Tukey HSD, all p>0.05).

There were no significant differences in tactile thresholds between solutions and visits and no significant interactions (all p>0.05, ANOVA).

7.4.3 Lens-solution-induced staining and its association with sensitivity

In Phase I with the use of AOSEPT, staining was minimal for all the visits and the data were not reported here because of the scope of the study. Grading of corneal and conjunctival staining for Phase II (stratified by MPS solution) is presented in Table 7-3.

Table 7-3 Grading of corneal and conjunctival staining

	Cornea (mean % area ± SD)		Conjunctiva (median; 10, 90 percentile)	
	Polyquad/Aldox system	PHMB-preserved system	Polyquad/Aldox system	PHMB-preserved system
Baseline	1.3 ± 2.0	1.8 ± 3.0	2.0 (0, 6)	3.0 (0, 6)
Day1-2hrs	1.6 ± 2.4	29.5 ± 32.5	2.0 (0, 5)	5.0 (1, 9)
Day1-8hrs	5.7 ± 9.9	20.3 ± 25.1	3.0 (1, 5)	4.5 (1, 8)
Week1-2hrs	4.7 ± 7.5	49.9 ± 39.9	3.0 (1, 6)	6.0 (2, 10)

There were significant differences in corneal staining between solutions and visits (both p<0.0001, ANOVA). Figure 7-7 demonstrates that the differences between visits were dependent on the solution type (p<0.0001, ANOVA). With the Polyquad/Aldox system, corneal staining at all follow up visits was similar to the baseline (with AOSEPT) (Tukey HSD all p>0.05), whereas corneal staining with the PHMB-preserved solution increased at all follow up visits compared to the baseline. The highest score was at the 1-week visit after two hours of lens wear (Tukey HSD all p<0.003).

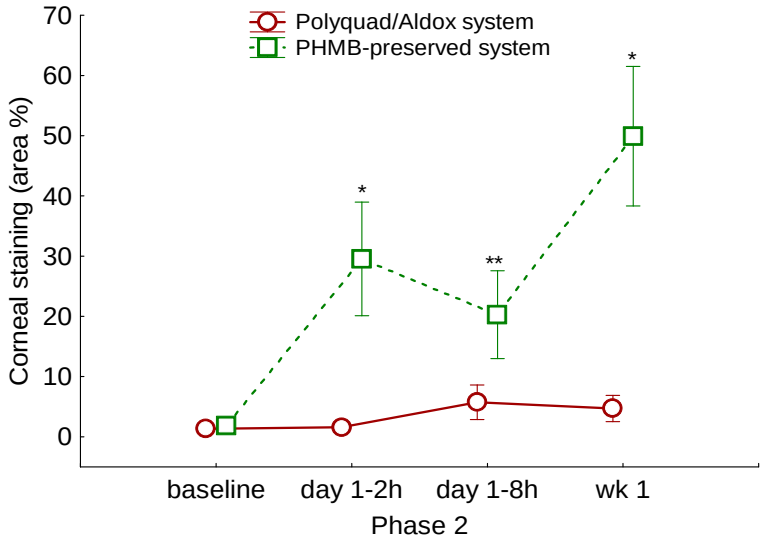


Figure 7-7 Interactions between solution and visit for corneal staining scores in Phase 2

Note: Data are the mean ± 95% CI. At all visits, the levels of corneal staining were higher with the use of PHMB-preserved solution compared to use of the solution preserved with Polyquad/Aldox. *p<0.001, **p<0.003.

With the PHMB-preserved solution, conjunctival staining increased over time (Friedman p<0.001) and was higher than that with the Polyquad/Aldox system at all visits except for baseline (Wilcoxon with Bonferroni correction all p<0.001). In addition, corneal staining was inversely correlated to conjunctival chemical thresholds at the 2- and 8-hour visits on day one (Pearson r= -0.34 and -0.37 for the 2- and 8-hour visits, respectively, both p<0.05).

7.5 Discussion

Contact lens wear affects the ocular surface in various ways,[2, 31, 32] and one of the effects has been to change the functioning of the sensory nerves of the ocular surface, as reflected in the response to corneal and conjunctival stimulations. Sensory nerves not only provide information about the relationship between the body and the external environment (e.g. potential dangers or injuries), but also signal the physiological condition of the body such as local metabolism (e.g. acidic pH, hypoxia), immune and hormonal activity.[33] The sensory impulses arising from the ocular surface are one of the

important aspects of the integrated functional unit and play a part in maintaining the homeostatic environment of the ocular surface.[6]

In the current study, conjunctival sensitivity to pneumatic mechanical stimulation increased from the no-lens baseline after refitting with the SH lenses and a similar trend but smaller magnitude was observed in the cornea, suggesting an altered sensory processing of the ocular surface. This increase in sensitivity appeared to be transient i.e. greater at the 2-hour and day-1 visits for conjunctiva and cornea, respectively. Stapleton et al[29] compared the effects of short-term SH and hydrogel lens wear on ocular surface sensitivity in neophytes and found that conjunctival sensitivity increased after the wear of SH lenses. The mechanism which leads to this increase in sensitivity to mechanical stimuli is unclear. Contact lenses applied to the eye can introduce stimulation due to friction on the ocular surface, particularly during the initial phase of wear.[34] This mechanical effect might be greater with SH lens materials due to their relative high elastic modulus.[34] In addition, the structure and physiology of the tear film can be altered during lens wear.[2, 31, 32, 35] The combination of these factors may lead to an increase in sensory input from the ocular surface, signalling the temporary disequilibrium between the components of the functional unit. Moreover, subclinical conjunctival inflammation that has been detected in asymptomatic contact lens wearers[36] is likely to be another contributing factor, as the activities of the sensory nerve could be modified by injury and inflammatory mediators.[4]

The change in mechanical sensitivity with SH lens wear in the current study was more pronounced in the conjunctiva. The conjunctiva is not only supplied by free nerve endings to provide sensory input to the functional unit, as in the cornea, but is also a highly reactive tissue.[37] It is richly supplied by blood vessels, connected to the lymphatic system, and filled with immunocompetent cells.[37] In addition, the conjunctiva is directly involved in regulating the secretion of components of the tear film such as electrolytes, water and mucin,[38, 39] and has a large area covering the ocular surface. It is conceivable that the conjunctiva will be sensitive to any changes occurring on the ocular surface, including the effects of contact lens wear.

In comparison, corneal tactile sensitivity decreased after wearing the SH lenses from the no-lens wear baseline, which is similar to previous reports with hydrogel lens wear.[15, 20, 22] The reduction in corneal sensitivity with low DK hydrogel lens wear has been attributed to metabolic impairment resulting from lens-induced hypoxia.[20, 24] SH lens materials have diminished the lens-induced hypoxic impact on corneal physiology, with resulting improvement of a number of physiological

markers, especially when lenses are worn overnight.[40-42] If hypoxia and mechanical stimulation are responsible for lens-induced alteration in sensitivity but hypoxia effects have been minimized, the mechanism reducing tactile sensitivity with SH lens wear must be due to mechanical stimulation. Adaptation, the reduction in sensitivity after repeated suprathreshold stimulation, is a phenomenon which occurs in a variety of forms in sensory systems.[43-47] Functionally, adaptation may help to optimize the dynamic range of encoding in a neural system by shifting the sensitivity,[48-50] and it develops and recovers depending on the time course of the stimulation.[51, 52] Although subjects in the current study were adapted daily contact lens wearers, temporary cessation of lens wear and therefore the withdrawal of mechanical stimulus might have allowed some recovery and therefore no adaptation to the lenses, as seen in Figure 7-2. After refitting with SH lens, the close interaction between the lens and the cornea produced a sustained stimulation of the surface and adaptation, resulting in a shift in corneal sensitivity to the tactile stimuli.

In addition to the changes in pneumatic mechanical and tactile sensitivity with SH lens wear, ocular surface chemical sensitivity seemed to reflect the interaction between SH lens materials and MPS in this study. The higher levels of ocular surface staining induced by the solution preserved with PHMB were accompanied by an increased chemical sensitivity. This is different to the decreased tactile sensitivity in solution-induced staining reported by Epstein.[23] Comparison between the two studies is difficult to make due to differences in study design, lens materials and stimulus modality used. Even though the exact mechanism causing lens-solution interaction induced staining remains unclear, the presence of this type of staining indicates an alteration in the ocular surface (Muya L, et al. *IOVS* 2008;49: ARVO E-Abstract 4869) that may have an impact on the sensory input (or vice versa) to the functional unit. This increased chemical sensitivity suggests sensitization of the polymodal-nociceptors (or chemoreceptors[53]) of the ocular surface. Sensitization, a reduction in threshold to one or more stimulus modalities and/or the development of lower-frequency spontaneous activity,[54] can be caused by repeated noxious stimuli such as inflammatory mediators on the ocular surface.[4, 55] This sensitization makes the polymodal receptors excitable to non-noxious stimuli and enhances the magnitude of their response to noxious stimuli.[54] Many components in ophthalmic solutions have the potential to induce inflammatory changes on the ocular surface,[56] and in the current study, the combination of lens and solution might have acted as exogenous chemical stimuli of the ocular surface, triggering a cascade of events, and resulting in enhanced responsiveness of the polymodal receptors. More studies are warranted to explore the pathway and mechanism of sensitivity changes induced by this lens-solution interaction.

In the present study, we found different profiles of the changes in ocular surface sensitivity with lens wear and lens-solution combinations, when measuring with different stimulae from the two esthesiometers used. The basis for the discrepancies between the two types of sensitivity measurement is not fully understood. It has been suggested that the stimulus delivered by Cochet-Bonnet esthesiometer may selectively activate mechano-nociceptors that are responsible for acute, sharp pain induced by direct mechanical contact with the ocular surface.[4, 57] On the other hand, the pneumatic stimulation may activate both mechano- and polymodal-nociceptors. More recently, it has been proposed that a series of labelled lines consisting of neurons in the spino-thalamic tract (the pain-signalling pathway to the brain) signal the homeostatic state of the body (interoception), whereas the pathways processing exteroceptive information (i.e. potentially dangerous stimuli impinging upon our body) associated with pain might be different.[58] Additionally, studies have suggested that cornea-responsive neurons in the spinal trigeminal nucleus, the subnucleus interpolaris/caudalis transition (Vi/Vc) and the subnucleus caudalis/upper cervical cord transition (Vc/C1), process corneal input differently and perhaps serve different ocular nociception functions.[59-61] Therefore, in addition to being related to differences between the two esthesiometers such as stimulus modality etc., this dissimilarity between tactile and pneumatic mechanical sensitivity may reflect the contribution of different nociceptive signalling pathways; the tactile sensitivity might represent the alarm-altering functions to exteroceptive stimuli while pneumatic mechanical and chemical sensitivity might provide additional interoceptive information such as changes in the ocular surface and tear film.

In summary, despite the advances of SH lens materials in eliminating lens-induced hypoxia, this study has demonstrated that SH lens wear and lens-solution interactions affect ocular surface sensitivity. The effects of lens wear on the sensory nerve function appear to be more complex than previously thought. Given that the sensory input arising from the ocular surface plays a critical role in maintaining the optimally balanced state of the lacrimal functional unit, sensitivity measures may be considered as an indicator of the subclinical changes induced by SH lens wear. However, the current study has some limitation partially because it examined lens wear for a relatively short time. Questions, such as how ocular surface sensitivity, particularly conjunctival sensitivity, varied after longer-term SH lens wear, whether sensory aspects of the ocular surface contribute to the end-of-day discomfort which is commonly reported by lens wearers including SH lenses wear, and what the clinical implications of the changes in sensitivity are, remain unanswered. The Belmonte pneumatic esthesiometer does however provide uniquely useful information when assessing different aspects of sensory functioning in contact lens wearers.

Chapter 8
General discussion

The series of experiments and clinical studies in this thesis examine corneal and conjunctival sensitivity across the ocular surface and the sensations and reflex responses to suprathreshold stimuli. The intent was to gain insight into the basic mechanisms that contribute to the sensitivity and sensation of the ocular surface and to relate these findings to the sensitivity changes occurring in DED and with contact lens wear.

Using a computerized Belmonte esthesiometer it was found that sensitivity with pneumatic mechanical, cool and chemical stimulation at different corneal positions varied only slightly. This is unlike previous reports[1-3] that touch sensitivity measured using Cochet-Bonnet esthesiometry declined towards the peripheral cornea (Chapter 3). The small variation with position was consistent with the finding of a relatively constant corneal cooling threshold.[4, 5] The pattern appeared to be in accord with the newly described parallel arrangement of sub-basal nerves observed in human corneas[6] and results reported using *in vivo* confocal microscopy.[7] This more homogeneous nerve fibres distribution and therefore invariant sensitivity across the human cornea, differ from that seen in the rabbit corneas.[8]

Based on the finding of slight variation in pneumatic sensitivity between central and peripheral cornea, sensations evoked by suprathreshold mechanical and chemical stimulation at different corneal position were investigated (Chapter 4). It was demonstrated that the transducer function to suprathreshold stimuli was similar at the central and peripheral cornea but was different between the modalities (i.e. mechanical versus chemical). This similarity in suprathreshold behaviour with noxious stimulation of central and peripheral cornea extended the sensitivity results found in Chapter 3 and broadened the description of the underlying neural mechanisms responsible for corneal nociception. It seems that the functioning of the corneal sensory system is more homogeneous at different positions than previously thought. On the other hand, the modality specificity that exists at various levels of the processing pathways may contribute to the different qualities of pain between the two modalities.[9-14] The evidence suggested that different mechanisms may be responsible for various aspects of corneal nociception, although the functions of sensory nerves as reflected in their sensitivity and suprathreshold responses to corneal stimulation were similar across the cornea. Mechanisms that mediate the sensory-discriminative aspect of corneal nociception seemed to be

similar regardless of stimulus location, whereas the mechanisms that underlie the different qualities of pain and irritation evoked by mechanical and chemical stimuli may not be the same.

This regional variation in the functioning of corneal innervation was further explored by investigating the relationship between the sensory input at different locations of the cornea and the efferent outputs determined by tear secretion in the lacrimal functional unit (Chapter 5). This study demonstrated that both polymodal and mechano-nociceptors contribute to the afferent pathways of reflex tear secretion, a result in agreement with those of Acosta et al.[15] Interestingly, reflex tearing was greater with central stimulation compared to peripheral stimuli, suggesting that the responses were position dependent. Presumably, this greater reflex response to central stimuli is intended to protect the eye from any external stimulation that may potentially impair vision as the central cornea is situated along the visual axis. The reflex tearing evoked by noxious stimulation of the central and peripheral cornea may be driven by different neural circuitries at higher levels of the processing pathway.

It has been proposed that sensory input from the cornea (thus the ocular surface) is an important aspect of the integrated lacrimal functional unit and plays a part in regulating tear secretion. The study in Chapter 5 showed a nearly linear relationship between tear secretion volume and the stimulus intensity, but tear secretion following sub-threshold stimuli was similar to the unstimulated tear flow, while suprathreshold nociceptive corneal stimuli evoked reflex lacrimation. These results support the hypothesis that sensory innervation of the cornea (thus the ocular surface) is the systematic neural driving force for lacrimal gland secretion[16, 17] and regulates tear secretion under normal (unstimulated) conditions and after physiological and environmental challenges.[18, 19]

Additionally, the sensory and the autonomic nerves link the components of the lacrimal functional unit into a homeostatic loop, and dysfunction of any part of the functional unit could result in alteration of the other components and manifest as DED.[19] It was found that the dry eye symptomatic group had corneal and conjunctival hypersensitivity, shorter tear film break-up time, greater degree of ocular surface staining and higher OSDI score compared to the non-dry eye group, indicating alterations in various aspects of the lacrimal functional unit (Chapter 6). The corneal and conjunctival hypersensitivity appeared to be associated with symptom severity, tear film instability and corneal epitheliopathy. Despite the reported changes of corneal sensitivity in DED remaining controversial,[20-25] evidence from this study suggests that altered sensory processing may be one of the essential factors that characterises DED.

It is important to note that the hypersensitivity was more prominent in the conjunctiva than in the cornea, although corneal and conjunctival sensitivity was highly correlated in this study. Since cornea and conjunctiva have similar types of sensory receptors and their epithelia have the same embryological derivation, it is likely that they share roles in providing sensory signals to the lacrimal functional unit for the purpose of maintaining the integrity of the tear film and the health of the ocular surface.

This notion was confirmed in the study investigating the effect of SH contact lens wear on corneal and conjunctival sensitivity (Chapter 7). It was demonstrated that conjuncitval pneumatic mechanical sensitivity was increased after refitting with SH lenses following a no-lens wear interval. This increase in conjunctival sensitivity with SH lens wear was consistent with the results reported by Stapleton et al.[26] In addition, it appeared that corneal staining induced by certain lens-solution combinations was accompanied by altered ocular surface chemical sensitivity.

Unfortunately, past studies on sensitivity in DED and contact lens wear have mainly focused on the cornea, and the conjunctiva has received little attention. Given that conjunctiva is not only directly involved in regulating mucin production and water and electrolytes secretion into the tear film but is also a highly active tissue that is richly supplied by blood vessels and connected to the lymphatic system, it is plausible that previously overlooked conjunctival sensitivity may also have an important clinical role in conditions where the homeostatic state of the lacrimal functional unit has been disrupted.

Another interesting finding was the difference in sensitivity across the cornea when measured using Cochet-Bonnet and pneumatic (Belmonte) esthesiometers. Besides the difference in the variation of sensitivity across the cornea, the changes in corneal sensitivity after refitting SH lenses following a short-period of no-lens wear were different between the two instruments. A previous study has shown that central corneal sensitivity measured using Cochet-Bonnet and non-contact pneumatic esthesiometers was not correlated.[27] It was proposed that the stimuli used in these two types of instruments might have activated different subclasses of functional type receptors although how this is possible is unclear.[27, 28]

The results from the studies in this thesis suggest that two esthesiometers may measure different aspects of sensory nerve function that serves to protect the cornea (thus the ocular surface) from external and internal challenges. It has been recognized that the nociceptive system have exteroceptive and interoceptive properties, serving important roles in signalling potentially dangerous

stimuli impinging on our body (exteroception) and also signalling injured or dysfunctional tissue that disrupts homeostasis (interoception).[29] Recently, a series of specific sensory channels (labelled lines) consisting of neurons in the spino-thalamic tract (the pain-signalling pathways to the brain) that receive interoceptive information have been proposed.[30] On this basis (among other things), it was hypothesised that the pathways processing interoception and exteroception may be different.[31] The tactile sensitivity of the ocular sensory nerves might represent the alarm-altering function of the exteroceptive nociceptive system to escape/avoidance of external threats, while pneumatic sensitivity might provide additional interoceptive information such as changes in the physiological condition of the ocular surface and tear film.

Despite the advances of Belmonte esthesiometry enabling measurement of corneal and conjunctival sensitivity over a wider range of stimuli type, there are a few limitations of the instrument. Similar to the other types of pneumatic esthesiometry,[32, 33] the stimulated area is larger than that of a Cochet-Bonnet esthesiometer. Also, how the air column interacts with the tear film is not understood. The tear film itself (and thus its interaction with the pneumatic stimulus) might have an effect on the measurement. Plus, the instrument used in these studies is a prototype and there is little room to refine its design in order to better control the consistency and range of the stimuli. Also, some stimuli operate over the maximum dynamic range (i.e. 0-100% added CO_2).

In addition to the limitations in the instrumentation, the psychophysical methods used to estimate thresholds are based on classical "yes-no" design and absence of control over the subject's decision criterion.[34, 35] This deficit, to some degree, may introduce bias in the measurement. Lastly, unlike the visual system, the sensory system of the ocular surface varies substantially based upon physiological and environmental challenges. Furthermore, pain (including corneal nociception induced by suprathreshold stimuli) is an experience involving emotion and awareness.[30, 36] Perhaps, these factors may potentially add the noise to the sensory system and increase the variability of the measurements.

Chapter 9
Conclusions and future direction

In conclusion, sensitivity to pneumatic cool and mechanical stimuli varies only slightly across the cornea while chemical sensitivity is almost constant. This pattern of variation is different to the highest sensitivity in the center and marked decline in the periphery described previously with tactile stimuli. The distribution of the human corneal sensory nerve endings may be more homogenous than previously hypothesised.

In addition, the transducer function is similar in the central and peripheral cornea, and therefore the threshold and suprathreshold mechanisms mediating the sensory-discriminative aspect of corneal nociception may be similar across the cornea. However, the reflex tearing response to suprathreshold stimuli appears to be related to stimulus position, with greater emphasis on the corneal apex, perhaps because of the importance of the visual axis. Also, the transducer functions obtained using mechanical and chemical stimuli are different, suggesting that the neural mechanisms responsible for the nociceptive sensation evoked by mechanical and chemical stimulation may be different. This modality specificity that is retained at various levels of the processing pathway may contribute to the different nociceptive sensory experiences evoked by mechanical and chemical stimuli.

Sensory input from the cornea is an important aspect of the lacrimal functional unit and a major driving force for tear secretion. Corneal and conjunctival sensitivity is altered in DED and the alteration in sensory processing is associated with the severity of the symptoms. SH contact lens wear produces a decrease in corneal tactile sensitivity and increases conjunctival sensitivity to pneumatic mechanical stimuli, while corneal staining associated with lens-solution interaction co-occurs with altered corneal and conjunctival sensitivity to chemical stimuli. The changes in sensitivity in DED and contact lens wear seem to be more prominent in the conjunctiva than in the cornea.

The profiles of “mechanical” sensitivity measured using Cochet-Bonnet and pneumatic (Belmonte) esthesiometers are dissimilar. The two measurements may reflect the different functional aspects of sensory nerves (interoception and exteroception).

There remain many unanswered questions in the field related to the sensory processing by the ocular surface and the clinical relevance of these neural mechanisms. It is largely unknown how the natural history of different forms of DED relates to ocular surface sensitivity. It is not understood at

all what the clinical implications of the changes in ocular surface sensitivity accompanying contact lens wear are, particular in SH lens wearers. Future studies are required to explore the pathway and mechanism involved in sensitivity changes induced by the SH lens and lens-care regimen interaction. In addition, exploring the basic science examining the interaction between tear film and the pneumatic mechanical and chemical stimuli of the esthesiometer may help to better understand some controversial results reported in the literature.

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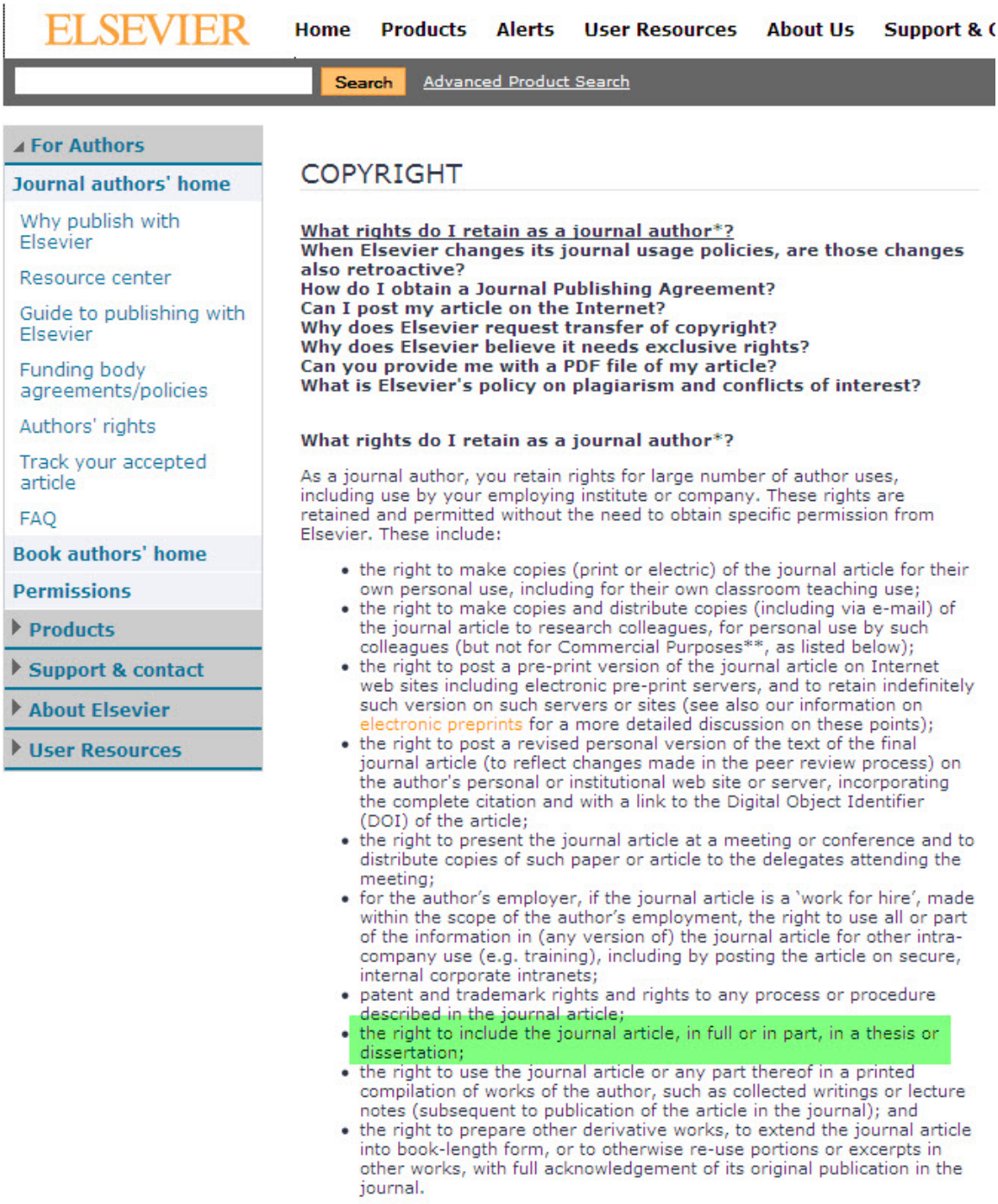

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