The Appearance of Hyper-Reflective Superficial Epithelial Cells Observed Using *in vivo* Confocal Microscopy

by

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Authors 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.

I understand that my thesis may be made electronically available to the public.

Abstract

Purpose

Hyper-reflective superficial cells were an unexpected finding while examining the corneal epithelium using confocal microscopy (CM), during an MSc thesis[1] conducted in 2006 at the University of Waterloo, Canada. The author[1] suggested that the appearance of these hyper-reflective cells could be associated with solution induced corneal staining (SICS) that was also observed in those participants who had manifested these hyper-reflective cells. However, this hypothesis has not been reported in the literature. This thesis aimed to investigate variables that could possibly predict the appearance of hyper-reflective superficial cells. These investigated variables were the effect of: contact lenses, contact lens solutions, lens/solution combinations, long-term use of certain contact lenses and solutions, age, dry eye symptom, topical anaesthetics and sodium fluorescein. In addition to this, the normal superficial epithelium of controls was defined.

Methods

CM images of the superficial epithelium were obtained during the various experiments from: 32 non-contact lens wearing participants, 18 post-menopausal participants symptomatic of dry eye and 18 post-menopausal age-matched asymptomatic women and 147 adapted soft contact lens wearers. For one experiment CM was performed with the contact lens *in situ*, making the use of a topical anaesthetic unnecessary. Superficial cellular appearance of CM images was graded using a custom grading scale. Hyper-reflective cells were counted. Corneal staining was assessed using sodium fluorescein.

Results

Results obtained during the various experiments revealed that hyper-reflective cells predominately appeared with the use of a specific lens/solution combination. Also, the number of hyper-reflective cells peaked after two hours of lens wear. It was also shown that when hyper-reflective cells occurred during an experiment, not every participant who was exposed to that specific lens/solution combination manifested hyper-reflective cells. Also, a great deal of inter-subject variability in observed numbers of hyper-reflective cells was noted.

Conclusion

In conclusion, this thesis established that the hyper-reflective cells that were observed by Harvey[1] were reproducible and may co-occur with corneal staining induced by a specific lens/solution interaction.

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Dedication

To my family and everyone else who helped and supported me along this journey.

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List of Symbols and Abbreviations

µm	micrometer
Advance	ACUVUE® Advance™ (Vistakon® Johnson & Johnson Vision Care Inc., Jacksonville, FL, USA)
AFM	Atomic Force Microscopy
Aldox	Myristamidopropyl dimethylamine
BAK	benzalkonium chloride
CCLR	Centre for Contact Lens Research
CCD	charged coupled device
CL	contact lens
Clear Care	Clear Care™ (CIBA Vision Corp., Duluth, GA, USA)
CM	confocal microscopy
CS	corneal staining
D	diopters
Dk	oxygen permeability
Dk/t	oxygen transmissibility
EDTA	ethylenediamine tetraacetic acid
EWC	equilibrium water content
FDA	United States Food and Drug Administration
hr	hour
HRT	Heidelberg retina tomograph
Hydranate	hydroxyalkylphosphonate
LSCM	Laser-scanning confocal microscope
min	minute
mm	millimeter
mmHg	millimeters of mercury
MA	methyl methacrylate
MAA	methacrylic acid
MPS	Multipurpose solution
NA	numerical aperture

Night&Day	Focus Night & Day™ (CIBA Vision Corp., Duluth, GA, USA)
nm	nanometer
NVP	N-vinyl pyrrolidone
Oasys	ACUVUE® OASYS™ (Vistakon® Johnson & Johnson Vision Care Inc., Jacksonville, FL, USA)
OD	right eye
OS	left eye
OU	both eyes
OptiFree Express	OPTI-FREE® Express® MPS (Alcon® Laboratories, Fort Worth, TX, USA)
O_2Optix	O_2 Optix™ (CIBA Vision Corp., Duluth, GA, USA)
PHMB	polyhexamethylene biguanide
PMMA	polymethyl methacrylate
polyHEMA	2-hydroxyethyl methacrylate
polyquad	polyquaternium-1
ppm	parts per million
PQ-1	polyquaternium-1
PureVision	PureVision™ (Bausch & Lomb, Inc., Rochester, NY, USA)
RCM	Rostock corneal module
ReNu	ReNu MultiPlus® MPS (Bausch & Lomb, Inc., Rochester, NY, USA)
ReplenisH	OPTI-FREE® RepleniSH® MPS (Alcon® Laboratories, Fort Worth, TX, USA)
RGP	rigid gas permeable contact lenses
SD	standard deviation
SEAL	Superior epithelial arcuate lesion
SICS	Solution induced corneal staining
SiHy	Silicone hydrogel contact lenses
SoloCare Aqua	SoloCare Aqua™ (CIBA Vision Corp., Duluth, GA, USA)
SSCM	Slit-scanning confocal microscope
t	thickness
TRIS	trimethyl-siloxy)-γ-methacryloxy-propylsilane
TSCM	Tandem-scanning confocal microscope
USAN	United States adopted name
WC	water content

Chapter 1

Introduction and Background

Introduction

Currently, approximately 130 million people worldwide wear contact lenses primarily to correct refractive errors.[2] Out of these, around 90% use soft contact lenses, including both conventional hydrogels and silicone hydrogels (SiHy). Only 10% still use rigid gas permeable (RGP) contact lenses.[2] Due to the relatively low oxygen transmissibility of conventional hydrogel lenses, the main complications with these lenses are chronic and acute signs of hypoxia,[3-13] followed by responses that are of inflammatory and infective nature.[14-19] The introduction of high oxygen permeable SiHy lenses in 1999 intended to maintain and enhance ocular health while wearing contact lenses virtually eliminated the hypoxic problems.[6;7;20-24] However, the goal that inflammatory and infective complications would also decrease was not met. SiHy lenses produce almost the same numbers and amounts of these complications.[22;25-28] Another setback that followed the introduction of SiHy lenses was that corneal staining (CS) increased notably.[29-32] Certain lens/solution combinations resulted in relatively dense superficial punctate CS with an annular pattern.[29;33] This type of CS is referred to as solution induced corneal staining (SICS), and is suggested to be due to a toxic reaction.[29;30;34]

An MSc thesis[1] with the title "The effects of contact lens care solutions on the corneal epithelium: a comparative investigation using confocal microscopy (CM)" was presented in 2006 at the University of Waterloo, Canada. This thesis was conducted to further investigate the higher amounts of SICS when PureVision (balafilcon) lenses were used in combination with ReNu MultiPlus solution than when this lens was used with OptiFree Express, as reported by Jones et al.[29] The emphasis in this project was to examine if no rub contact lens solutions had an effect on the corneal epithelium, especially in SiHy contact lens wearers, when the corneal epithelium was imaged using a confocal microscope (CM). The conclusion drawn from this study was that in SiHy contact lens wearers, contact lens care solutions do affect the corneal epithelium and that the SICS may be the result of a contact lens/solution interaction. However, an unanticipated alteration to the superficial epithelium in a group of participants was an interesting ancillary finding of this study. CM revealed brightly reflective superficial cells (Figure 1) in certain study participants and the author[1] suggested that their appearance may be associated with a contact lens/solution interaction.

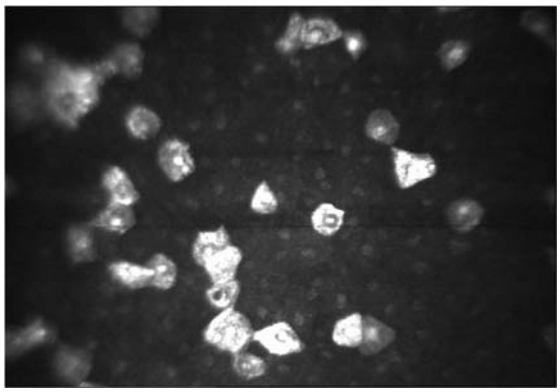


Figure 1: Hyper-reflective superficial epithelial cells (Courtesy of CCLR)

Hyper-reflective superficial cells in association with SICS have not been reported in the literature and a detailed literature review on the appearance of the superficial epithelial cells will follow later. Therefore the experiments in this thesis were designed to investigate the presence of and examine predictor variables associated with the appearance of hyper-reflective superficial epithelial cells. These included: contact lens wear, use of contact lens solution, or a combination of both, use of sodium fluorescein and/or anaesthetics. Table 1 lists the possible predictor variables influencing the appearance of hyper-reflective cells that were examined in this thesis.

Table 1: Possible predictor variables influencing the appearance of hyper-reflective cells, examined in this thesis

Variables that may cause appearance of hyper-reflective cells	Conclusion
Contact lens solution	?
Lens/solution combinations	?
Specific lens/solution combinations that induce SICS	?
Contact lens wear	?
Age	?
Dry eye	?
Sodium fluorescein	?
Anaesthetics	?
Long-term use of same type of contact lens and solution	?

CM was the instrument chosen to examine the epithelium in these experiments. The advantage of it is that it provides images of the cornea *in vivo* with relatively high magnification (approx. 500x), and therefore changes to the epithelium on a cellular level might be visible.

Basic structure and function of the cornea

The cornea represents the front, transparent part of the eye and can have a diameter that ranges for individuals between approximately 9.5 and 13.5 mm.[35] Corneal thickness is reported to be approximately 530-563 µm centrally[36;37] and about 670 µm peripherally.[38;39] The curvature of the normal cornea is steeper centrally and flatter in the periphery.[40] The cornea has several important functions, such as maintaining tissue transparency to ensure that visible light reaches the retina, providing approximately 2/3 of the total ocular refracting power, and sustaining a mechanical and chemical barrier between the eye and the environment.[40] Being an avascular tissue, oxygen for corneal metabolism is largely drawn from the atmosphere and from the precorneal tear film.

As a structure (Figure 2), the cornea can be divided into five distinct layers and the precorneal tear film that is covering the anterior layer. From anterior to posterior the layers are: the epithelium, Bowman's membrane, stroma, Descement's membrane and the endothelium.[40]

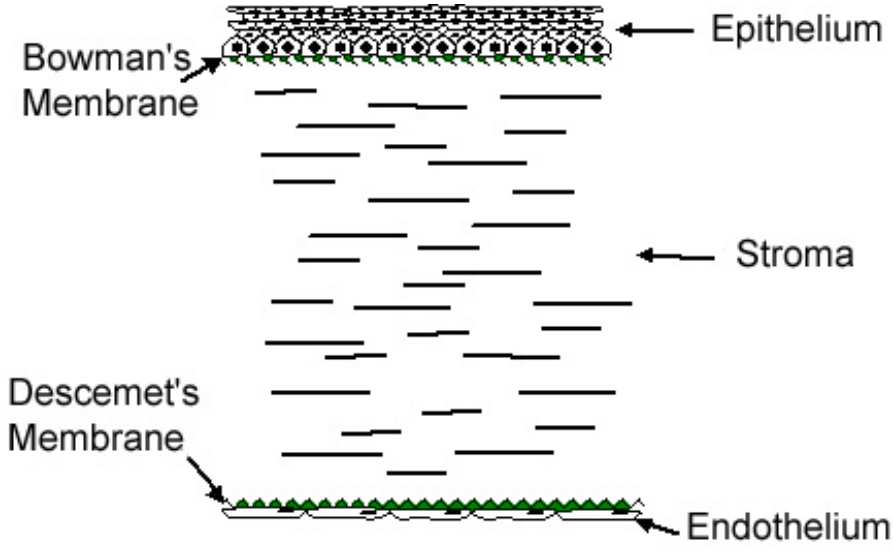


Figure 2: Cross-section of corneal layers, showing five distinct layers (with permission from SPIE and the author James Schwiegerling, reproduced from "Field Guide to Visual and Ophthalmic Optics[41])

The epithelium

The epithelium is the outermost surface of the cornea and consists of five to six nucleated cell layers, is stratified, squamous, and non-keratinized.[40] It is composed of a superficial cell layer, middle wing cell layers and a basal cell layer. The epithelial thickness comprises approximately 10 percent of the total corneal thickness, which represents about 50 µm.[37]

As the corneal epithelium is the focus of this thesis, it will be discussed in greater detail, later in the text.

Bowman's membrane

Bowman's membrane or layer lies just below the basal epithelium, is between 8 to 12 µm thick and consists of acelluar collagen fibrils.[40] It is attached to the anterior stroma by collagen fibrils that insert into Bowman's membrane and become a part of the anterior stromal lamellae. The actual function of the Bowman's membrane is not completely defined; it may contribute to maintain the overall corneal shape and is also important in separating the basal epithelial cells from stromal cells. In the case of trauma, Bowman's membrane cannot be regenerated.[40]

Stroma

The stroma lies beneath Bowman's membrane and is about 90 percent of the total corneal thickness. It mainly consists of an extracellular matrix, including stacked lamellae of collagen fibrils of uniform diameter, and a proteoglycan ground substance.[42] The layered arrangement is more regular in the posterior stroma than anterior, where the lamellae are narrower and interwoven. The special shape, arrangement and spacing of the collagen fibrils are essential to keep the cornea transparent.[42] The main stromal cells are keratocytes. These are large and flat cells and are scattered between the collagen fibrils.[43] Keratocytes are connected with each other through gap junctions, often in both anterior-posterior and lateral direction.[44;45] The anterior stroma contains the highest density of keratocytes with a decline towards the posterior. Close to the Descement's border keratocyte density increases slightly.[46;47] Reports on the human full-thickness keratocyte density range from 18,336±4,277 to 23,043±3,692 cells/mm^2.[3;48-50] The functions of the keratocytes are to repair and maintain the stromal collagen fibrils and they play a role during wound healing. After injury, some cooperation and communication exist for the wound healing process between the epithelium and the stroma.Stromal wound healing is more effective if the epithelium is intact during the healing process.[51] Also, the modification of the stromal ground substance is adjusted by the presence of the epithelium. After injury, epithelial cells migrate into the stromal defect and remain until the healing process is completed.[52]

Descement's membrane

Essentially, the acellular Descement's membrane is the basement membrane of the corneal endothelium that lies posterior to the stroma, and is composed of an anterior banded layer and a posterior non-banded layer. It increases in thickness during life, to as much as 10 μm for some individuals.[40] The endothelium regenerates this layer if any damage occurs.[40;53] If endothelial cells are stimulated to produce excess amounts of material, guttae (focal thickenings of Descement's membrane) can arise.[40] It is thought that Descement's membrane also helps to maintain corneal curvature.[52]

The endothelium

The endothelium is the innermost corneal layer. It is a monolayer of hexagonal endothelial cells, approximately 5 μm thick, and normally of similar size and shape.[40] The cell density is highest at birth and gradually decreases over time. The primary task of the endothelium is to maintain corneal hydration by pumping fluid out of the cornea.[54] Disturbance of this endothelial function can lead to loss of corneal transparency.[42;54;55]

Pre-corneal tear film

The tear film is a specialized moist structure that covers the conjunctivae (bulbar and palpebral) and the cornea. Traditionally, its average thickness is reported to range between approximately 9 μm immediately after a blink and 4 μm just before a blink.[56;57] More recently however, using reflectance spectra, tear film thickness between 1.2 and 7.3 μm has been demonstrated.[58;59] Historically, the normal tear film was divided into three layers. The outer layer is the thin lipid layer (approx. 0.1- 0.5 μm), the main tasks of which are to act as a barrier and to prevent evaporation during blinks and to lower the surface tension.[35] Underneath the lipid layer lies the aqueous or lacrimal layer (6.5 – 7.5 μm)[35] containing dissolved ions, proteins, enzymes and electrolytes. This intermediate watery layer is hypothesized to be responsible for wetting, supplying nutrients, controlling infectious agents, regulating osmolality, and washing away debris and toxins.[35] The deepest layer of the tear film was reported to consist of mucin. It is a gel-like layer with main functions hypothesized to be wetting and lubricating the ocular surface and anchoring the tear film to the corneal surface.[35;60] More recent observations,[61;62] however, have suggested that the tear film model should be revised as it reflects a more complex system. Instead of having the three separate layers, it is thought that the tear film is a dynamic gradient, with the lipid, aqueous and mucin layers, mixing and interacting throughout. And even more recently, the tear film has been explained as a bi-layered structure consisting of a superficial lipid layer overlying an aqueous/mucinous layer.[63]

A healthy and well-balanced tear film fulfils four important roles: (1) wetting the corneal epithelium in order to prevent any damage due to dryness, (2) creating a smooth anterior optical surface by filling in small surface irregularities, (3) acting as a path for oxygen and other nutrients to the avascular cornea, and lastly (4) it is the prime defence source against ocular surface infection as it contains proteins and enzymes that act as antibacterial agents.[35]

The normal functioning and integrity of a healthy tear film is maintained by a complex physiological mechanism that includes adequate production of the different components by various glands, the stability of the different layers on the ocular surface and appropriate drainage provided through the lacrimal ducts.[35] During contact lens wear and with lenses on the front surface of the cornea, the lenses are surrounded with the pre-ocular tear film. Therefore, to make contact lens wear successful, the lens material, the cornea and the tear film should be compatible. Reports[64-66] however show that the main reasons for contact lens drop out are dry eye problems. This is hypothesized to be in part due to the disruption of the tear film with the contact lens on the eye as that the tear film's orderly structure and function cannot be maintained. It is reported[67] that contact lenses do change the structure, composition, physiochemical properties and the dynamic behaviour of the normal tear film.[35]

Corneal defence mechanisms

There are several lines of defence mechanisms to prevent ocular insult or infections. Perhaps the simplest, yet very effective process is the sweeping motion of the eyelids in order to protect the ocular surface against potential pathogens.[40] The tear film is also involved in the defence mechanism as it flushes away foreign particles from the surface and protects the cornea from drying out. In addition, the tears contain a range of antimicrobial proteins, such as lysozyme, lactoferrin, lipocalin, β-lysin, defensins, as well as immunoglobulins.[35] Corneal protection also includes various cell types, for instance epithelial cells, keratocytes, phagocytic cells, and Langerhans cells.[40]

The Epithelium

Anatomy, Physiology and Function

Anatomy and Ultrastrucural features

The corneal epithelium is a multilayered structure and is stratified, squamous and non-keratinized. The five to six layers of nucleated cells are continuous with the epithelium of the bulbar conjunctiva at the

limbus.[40] Based on morphological organization, the epithelium can be divided into three layers: the superficial cell layer, the wing cell layer, and the basal cell layer (Figure 3).

Epithelial thickness is approximately 50 µm[43;68] and thickens in the periphery forming a continuation with the conjunctival epithelium at the limbus.[69-72] CM has showed central corneal thicknesses of humans of 48.6 ± 5.1 µm[47] and 50.7 ± 7.4 µm.[73] Feng and Simpson[69] using Optical Coherence Tomography (OCT), reported thicknesses of the central human epithelium, limbal epithelium and bulbar conjunctival epithelium of 54.9 ± 1.9 µm, 74.6 ± 7.4 µm, and 44.9 ± 3.4 µm, respectively.

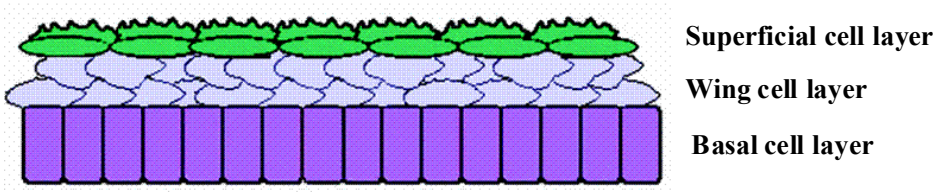


Figure 3: Layers of the corneal epithelium

Basal cells

The basal cells, positioned directly above Bowman's membrane, are the deepest epithelial cells. The cells are perfectly aligned in a palisade manner, are columnar shaped with rounded heads and flat bases and measure approximately 10 µm in width and 15 µm in height.[40] The basal cells are connected to each other by desmosomes and also by gap junctions. However those connections are not as numerous as in the wing cell layer. Basal cells are connected to the underlying Bowman's Membrane via hemidesmosomes.[39] So-called anchoring filaments pass through those hemidesmosomes and are inserted into Bowman's Membrane and provide a very strong attachment.[39] Mitosis only occurs in the basal cell layer.[40;73;74] Large numbers of glycogen granules are found in the cytoplasm of the basal cells, which indicates a source of stored metabolic energy that can be used during epithelial stress (e.g. wound healing).[74] This metabolic activity is at a higher level in basal cells than the more differentiated wing or superficial cells. Also, these cells have a prominent Golgi apparatus.[40] Basal epithelial cell density, measured using CM, ranges from 3601.38 ± 408.19 cells/mm^2 to 6188 ± 427 cells/mm^2. [75,76]

Wing cells

The wing cell layer, the middle layer, consists of polygonal cells with a convex anterior, capping the underlying basal cells and sending processes between them.[40] Desmosomes and gap junctions join wing cells together and desmosomes also attach wing cells to superficial and basal cells.[77] Mitochondria are present in the wing cells, however not in large numbers. Wing cells are post mitotic and migrate towards the surface epithelium.

Superficial cells

The outermost epithelial cell layer is the superficial cell layer. It usually consists of two to three layers of polygonal, thin and squamous cells that are 40 to 60 µm in diameter and have in the normal state , a large cytoplasm/nucleus ratio.[39] Their density at the central human cornea has been reported[76] to be approximately 1,213±370 cells/mm^2. Towards the periphery the cells get larger and therefore the density decreases.[78] The surface cells have the largest surface area, ranging from 590±93 µm^2 to 628±13 µm^2 measured with specular microscopy[78;78-80] and 789±95 µm^2 to 913±326 µm^2 measured with CM.[76;81] Measured at the nucleus, the superficial cells are 4 to 6 µm thick in the centre and around 2 µm in the periphery. The plasma membrane of the superficial cells is thought to secrete a glycocalyx adjoining the mucin layer of the tear film.[82-84] The increased surface area of the superficial cells is a result of the many projections that are located at their apical surface. These fingerlike projections (microvilli) and the ridge-like projections (microplicae) are hypothesized to enhance the stability of the tear film.[35] The surface cells are tightly joined together by tight junctions along their lateral walls. The purpose of these tight junctions is to provide a barrier to intercellular movement of substances from the tear film and also to prevent the uptake of excess fluid from the tear film.[40] However, a very effective, semi-permeable membrane is present which allows the passage of fluid and molecules through the cells.[35;35;40] Numerous desmosomes offer an additional adhesion between the cells.[39] Different shades of superficial cells, light to dark appearance, have been identified by scanning electron microscopy as well as CM, believed to indicate the amount and pattern of microvilli and microplicae.[80;85-88] Dark cells possess fewer surface features; it is assumed that there is loss of microvilli and microplicae and their surface is less rough than that of light cells.[80;86] Also, it has been suggested that the light cells are the youngest of the superficial cells, having just arrived at the surface. Therefore, the dark cells would represent mature cells that are in process of being desquamated.[80;86]

Other cells in the corneal epithelium

Langerhans cells, part of the immune system, are mainly present in the conjunctival, limbal and peripheral corneal epithelium.[40] Their purpose is it to produce more white blood cells or stimulate

antibody production in the event of threatening micro-organisms.[39] Also they can release cytokines and other mediators of inflammation.[87]

Physiology

A main function of the corneal epithelium is to act as a barrier to prevent the entrance of substances from the tear film and the uptake of excess fluid from the tear film.[40] Corneal ion transport systems have been studied since it was recognized that metabolism-linked processes are responsible for the control of corneal hydration. For details on the complexity of the corneal ion transport, the reader is referred to a number of texts.[89-93]

The barrier properties of the corneal epithelium can be assessed by the topical application of sodium fluorescein.[89;91;94;95] Under normal conditions, the epithelium is impermeable to this anionic molecule. The result is that normal areas stain little or not at all, whereas areas with epithelial defects stain intensely.[40]

The effectiveness of the tight junctions as an ionic diffusion barrier have been measured in microelectrode experiments in which the cellular resistance to ions has been measured.[96] The outer membrane of the superficial cells, in absence of factors that stimulate ion transport, is twice as good a barrier to ion flow as tight junctions. Therefore, considering the actual surface areas of membranes and junctions, the ratio for resistance increases by at least 100 times. Hence, the tight junctions offer a significant barrier to ion transport, but considering their area they are more permeable than the cell membranes.[97]

The corneal epithelium is rich in glycogen, which serves as an energy store during aerobic conditions. With open eyes, the tear P_{O2} is 155 mmHg; this will drop to approximately 55 mmHg during closed eye conditions.[39] During hypoxic conditions e.g. induced by a tight contact lens, the glycogen level drops. Mindel and Mittag[96] showed that in the rabbit epithelium, prolonged lid closure caused an acute fall in choline acetylase levels. Acetylcholine has also been proposed to be important in regulating epithelial cell mitosis.[98]

Epithelial renewal and repair

Renewal

The corneal epithelium is a self-renewing tissue and normally replaces itself approximately every 10 days. Mitosis, only occurring in the basal cells, is responsible for this replacement. The germinative region of the corneal epithelium, represented in the presence of stem cells, lies predominately at the limbus (Palisades of Vogt).[99] The stem cells divide and differentiate into basal cells and migrate slowly from the periphery towards the centre of the cornea and then upwards in the direction of the corneal surface.[100] During this upward movement the basal cells transform into more differentiated wing cells. Wing cells then move further towards the corneal surface while turning into squamous superficial cells. At the end of this life cycle of the cells, the majorities of the superficial cells leave the surface in an orderly manner and are shed or sloughed off by blinking into the tear film. Every time a surface cell is sloughed off, a new epithelial cell is needed in order to compensate for the loss and to ensure overall corneal epithelial structure and integrity. The velocity of the cells, after the initial division and differentiation, is estimated to be about 100 µm per week.[40;98] Throft and Friend[100] have proposed the X, Y, Z hypothesis (Figure 4) where the maintenance and renewal rate of the epithelium is also dependent on a centripetal movement of epithelial cells. They explained that epithelial cells born in the periphery slowly migrate centrally, but some of these cells move upwards and become post-mitotic wing cells. A recent report however, indicated that besides the limbal region, stem cells are also present throughout the corneal epithelium.[101] What the authors[101] have suggested is, that the stem cells residing in the corneal epithelium would be responsible for smaller corneal repairs that would occur to "normal wear and tear" of the cornea everyday, and that for more "serious repair jobs" the involvement of the limbal stem cells is required.

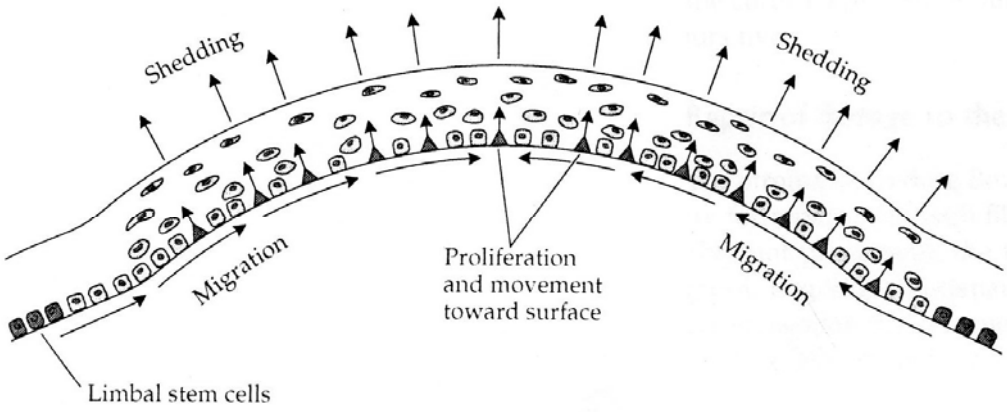


Figure 4: Renewal and Replacement of the Epithelium, after Throft and Friend 1983 (with permission from Sinnauer Associates, Inc)

Cells undergo different transformational steps when changing from cuboidal basal cells to squamous superficial cells. Sloughing off the surface involves programmed cell death.[39] The mechanism to trigger corneal epithelial apoptosis is not understood. For the corneal epithelium, apoptosis begins when basal cells move out or the basal layer and wing layer. Wilson et al.[102] suggested that in case of ocular tissues, amongst others, the death signal is FasL (Fas ligand) binding to the Fas receptor (death) protein. FasL is a surface protein component of ocular cells.[102] The presence of dying or dead cells at the corneal surface is not necessarily always due to apoptosis. Sometimes cell death can occur due to trauma or infection.[40] Cells then do not shrivel and expire in a normal way, they rather undergo necrosis while swelling, bursting or spilling out their intracellular contents. Inflammation may get activated after the release of intercellular materials from necrotic cells.[68] Apoptosis, as a normal part of life, is a common phenomenon which occurs in most of the body epithelial and endothelial cells, as well as many other cell types.[68]

Epithelial repair

As long as the limbus is intact, damage to the corneal epithelium can be repaired quickly.[40] The normal replacement activities will then be accelerated. Damaged cells send out signals and these trigger a local response around the injured area and this results in a rapid end to local mitosis, and repair takes place by centripetal migration of cells into the injured area.[40] As basal cells are normally attached to their

basement membrane by hemidesmosomes, these attachments have to be broken in order to allow migration into the injured area. After migration stops, the attachments are re-formed and mitosis then resumes until epithelial thickness is re-established.[39] However, before completing this process, superficial cell tight junction are re-created in order to ensure the permeability properties of the epithelium.[103] If the basement membrane is intact during the injury, this detachment and re-attachment of migrating basal cells is the same as during the normal cell renewal procedure. The presence of the basement membrane is not necessary, as basal cells will also migrate into the stroma and produce a basement membrane to which they can attach. A new basement membrane begins to appear within 6 weeks. Therefore, destroying the basement membrane, e.g. during refractive surgery, will prolong the healing process.[68] In the case of total epithelial loss, including the limbus, the adjacent conjunctival epithelium is capable of resurfacing the cornea. However, this will happen at a reduced rate,[39] and the cornea will get covered with vascularized, conjunctival epithelium that contains goblet cells.[40]

Contact lenses and Solutions

Contact lens development

Modern contact lenses, used to correct refractive error, have been in use for more than 50 years. The initial requirements for contact lenses were to have good optical properties and be physiological inactive. During the 1940s PMMA (polymethyl methacrylate) a carbon-based polymer, replaced glass as a contact lens material. The main disadvantage of PMMA is that it is oxygen impermeable and this interferes with corneal metabolism, as it creates chronic hypoxic conditions. Therefore the search for other contact lens materials continued, with special emphasis on enhanced oxygen permeability, dimensional stability and adequate wetting properties. Attempts were made to integrate silicone rubber, a synthetic elastomer, into PMMA. The big advantage of silicone rubber is its high oxygen permeability, approximately a thousand times greater than PMMA, which arises from the backbone of alternating silicone and oxygen atoms. On the other hand, one major disadvantage is that it is hydrophobic. However, it was not until the silicone-oxygen backbone could be successfully copolymerized with monomers used for contact lens materials, that a big step forward was made. Gaylord's patents in 1974 and 1978 provided the impetus for the development of two different types of contact lens materials: soft contact lenses that contain water (hydrogels) and rigid gas permeable contact lenses (RGP) that do not contain water.[104] The two major outcomes of his work were the development of what is known as TRIS, the siloxy-methacrylate monomer, tris (trimethyl-siloxy)-γ-

methacryloxy-propylsilane, and the "recognition of the value of incorporating fluoroalkyl methacrylates" in order to additionally enhance oxygen permeability.[105]

With their introduction in the early 1970s, more flexible and softer contact lenses have taken over the market from the less comfortable RGPs. Approximately 90% of people worldwide wear this type of contact lens.[106] Even though hydrogel lenses are more comfortable and cause less symptoms of dryness, discomfort and dryness sensations are still the main reasons for drop out of approximately 3 million contact lens wearers per year worldwide.[65;107;108]

Conventional hydrogels

Hydrogels form the largest group of contact lens materials. The first hydrogel material was 2-hydroxyethyl methacrylate (polyHEMA) developed by Otto Wichterle.[108] The term conventional hydrogels refers to the family of polyHEMA-based materials. PolyHEMA, in the absence of water, is a hard glassy material and after hydration, rigid polymer is transformed into a soft contact lens material. The advantage of these lenses is that a change in hydration does typically not affect its dimensional stability. Factors that could affect hydration are changes in pH, tonicity and temperature. Another major plus of these lenses is that they are relatively inexpensive to produce.[105]

The physical behaviour of a hydrogel is predominantly controlled by its water content (EWC = equilibrium water content) which is approximately 38% in polyHEMA. Variations in EWC can be induced by copolymerizing polyHEMA with different monomers. Adding a hydrophobic monomer, such as methyl methacrylate (MA) will result in a reduction of EWC, whereas adding a more hydrophilic monomer, such as N-vinyl pyrrolidone (NVP) or methacrylic acid (MAA) will cause the EWC to increase.[105;109] As the EWC of polyHEMA is strongly linked with its oxygen transmissibility an increase in EWC will result in increase in oxygen transmissibility and vice versa. Oxygen transmission through a contact lens is based on the amount of oxygen getting through the thickness of the lens; therefore the term "Dk/t" refers to the oxygen transmissibility of a lens. "D" is the diffusion coefficient, how fast dissolved oxygen is moving through a given material, "k" is the constant of dissolved oxygen molecules within the material, while "t" represents the thickness of the material.[104]

A factor that contributes to the interaction of the hydrogel surface and the surface of the eye is wettability. The interaction between lens and tear film is partly determined by the bulk properties of the lens as well as how the lens is produced, using either the lathing technique or the molding technique.[104] Stability, safety, and the interaction with the tear film are some of the numerous factors that contribute

to choosing the different monomers used in the hydrogels. One of the most important factors is the ionic charge of the monomer, as this directly affects the behaviour of the material on the eye. Monomers that have a neutral charge tend to attract low amounts of tear film proteins, whereas those of a higher charge attract materials of lower isolectric point.[110] Amongst others, components of the tear film that can attach to the contact lens material are lysozyme,[110-113] lactoferrin and albumin.[114;115]

The United States Food and Drug Administration (FDA) has introduced a grading system to distinguish between the different polyHEMA based materials, by dividing them into four categories based on their water content and their ionic charge. The ionic charge is dependent on the quantity of the hydrophilic monomers (especially MAA) in the material. A content of >0.2% causes the material to have a negative surface charge. Table 2 shows the FDA grading system.

Table 2: FDA-Classification of Conventional Hydrogels*

FDA-Classification	Group I	Group II	Group III	Group IV
Water Content	Low	High	Low	High
Charge	Non-ionic	Non-ionic	Ionic	Ionic

*Low = < 50% water; High = > 50% water: Ionic = charged; Non-ionic = No charge

Also, a material name (USAN) is given for particular combination of monomers and crosslinking agents in order to identify different hydrogel lenses (e.g. etafilcon A). The name used is specific for a certain material.[105]

PolyHEMA-based contact lenses are still widely used and fitted today. However, the main disadvantage of this material is that the oxygen transmissibility is still quite low and perhaps causes chronic hypoxic conditions such as neovascularisation, slowing of mitosis and the occurrence of epithelial microcysts.[6;7;116]

Silicone hydrogels (SiHy)

Theoretically, water dissolves oxygen. Therefore, the assumption is that if more water is incorporated into contact lens materials, the increasing levels of water would also increase the oxygen permeability of the material. This water-oxygen relationship is still used as primary attribute when inventing new conventional hydrogel lens materials. However, there is a limit to how much water can be incorporated into a hydrogel lens before, for example, the handling of the lens is difficult. Therefore, other

chemicals, having greater oxygen solubility than water have been explored. If silicone rubber could be incorporated into a hydrogel, an increase in oxygen permeability might be achieved. As the proportion of silicone-based polymers decreases so does the water content resulting in an increase of the Dk. In 1998 when the first SiHy lenses were introduced to the market, they were marketed to be extended or continuous wear lenses because it was suggested that they were able to provide sufficient oxygen to the cornea in order to prevent hypoxic conditions.[117;118]

To overcome the hydrophobicity problem, manufacturers used different methods to change the surface of the lens materials. CIBA Vision treats their lenses (lotrafilcon A and lotrafilcon B) in a gas plasma reactive chamber to apply a permanent, ultrathin, high refractive index, continuous hydrophilic surface[119-121] Figure 5A and B show the surfaces of lotrafilcon A and lotrafilcon B imaged using Atomic Force Microscopy (AFM). The surface imaged with AFM of a balafilcon A (Bausch & Lomb, Inc., Rochester, NY) lens can be seen in Figure 5C. As is apparent, a different surface exists in these lenses. Silicone components form silicate islands after plasma oxidation and are more hydrophilic. The aim is to modify these groups in order to place more wettable and polar groups at the surface, trying to hide the hydrophobic elements underneath.[120]

Figure 6A and B, show the surfaces, of galyfilcon A and senofilcon A lenses (both Johnson & Johnson, Jacksonville, FL), obtained using AFM. Instead of surface modifications or treatments, the manufacturer added an internal wetting agent, Hydraclear to the lens matrix. This wetting agent is based on a long chain, high molecular weight molecule that is slowly released from the lens surface, "hiding" the silicone and creating a hydrophilic environment.[120] The surface of comfilcon A (CooperVision Inc., Pleasanton, CA) is shown in Figure 6C has no surface treatments and no internal wetting agent.

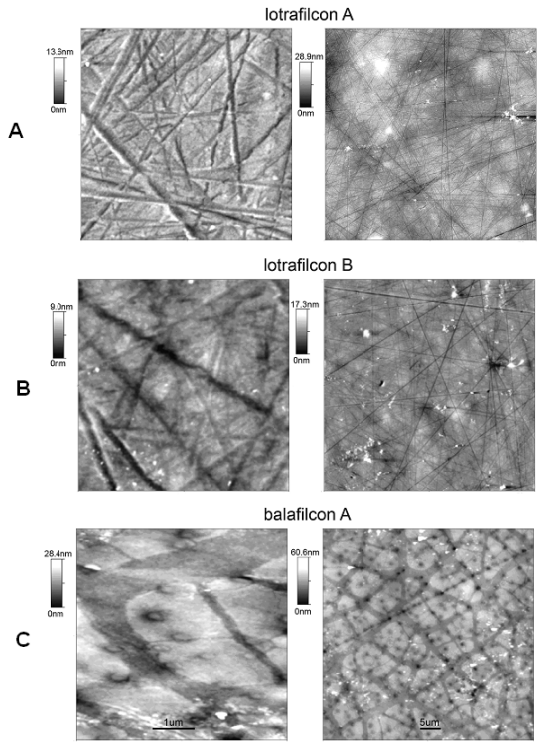


Figure 5: Atomic Force Microscopy (AFM) of the surface of rinsed, unworn (A) lotrafilcon A, (B) lotrafilcon B, and (C) balafilcon A lenses. Left images represent 5 µm and right images represent 50 µm (with permission from Optometry and Vision Science, reproduced from Teichroeb et al.[121])

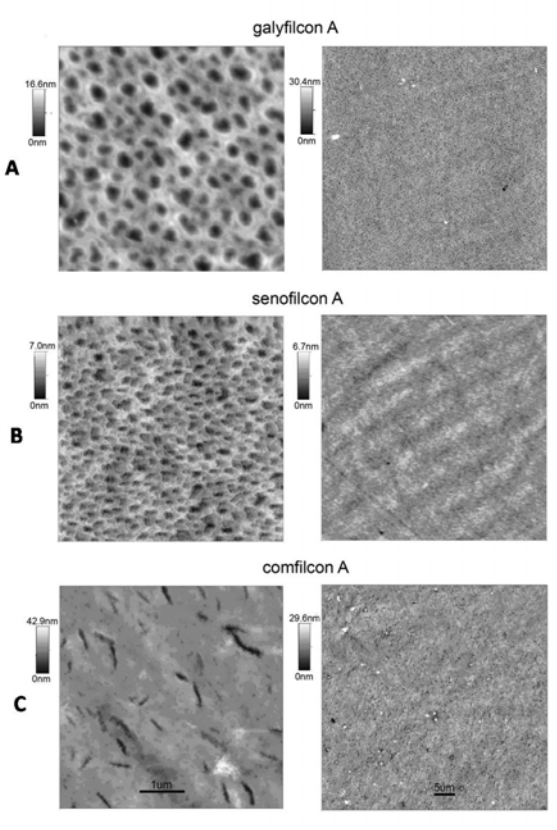


Figure 6: Atomic Force Microscopy (AFM) of the surface of rinsed, unworn (A) galyfilcon A, (B) senofilcon A, and (C) comfilcon A lenses. Left images represent 5 µm and right images represent 50 µm (with permission from Optometry and Vision Science, reproduced from Teichroeb et al.[121])

The first generation SiHy lenses were Focus Night&Day (lotrafilcon A) and PureVision (balafilcon A). These two lenses have a considerable higher modulus when compared to conventional hydrogels, which is a result of the higher amounts of silicone and the therefore lower water content.[117;122] The newer SiHys have typically moved to higher water content, in an attempt to produce better clinical outcomes.[105] Table 3 lists the currently available SiHy lenses and some of their specific properties.

Table 3: Characteristics and properties of currently available SiHy lenses

Proprietary name	**Night & Day™**	**O_2 Optix™**	**PureVision™**	**ACUVUE® Advance™**	**ACUVUE® OASYS™**	**Biofinity®**	**PremiO**	***1-DAY* ACUVUE® TruEye™**
USAN	Lotrafilcon A	Lotrafilcon B	Balafilcon A	Galyfilcon A	Senofilcon A	ComfilconA	Asmofilcon A	Narafilcon A
Manufacturer	CIBA Vision	CIBA Vision	Bausch & Lomb	Johnson & Johnson	Johnson & Johnson	CooperVision	Menicon	Johnson & Johnson
Water content (%)	24	33	36	47	38	48	40	46
Oxygen Permeability (Dk)	140	110	91	60	103	128	129	100
Centre thickness (mm) -3.00D	0.08	0.08	0.09	0.07	0.07	0.08	0.08	0.08
Oxygen Transmissibility (Dk/t)	175	138	101	86	147	160	161	118
FDA group	I	I	III	I	I	I	I	I
Modulus (MPa)	1.5	1.0	1.1	0.4	0.7	0.75	0.90	0.66
Surface Treatment	25nm plasma coating with high refractive index	25nm plasma coating with high refractive index	Plasma oxidation process	No surface treatment. Internal wetting agent.	No surface treatment. Internal wetting agent.	No surface treatment	Nanogloss surface treatment	No surface treatment. Internal wetting agent
Principal monomers	DMA+TRIS+ Siloxane monomer	DMA+TRIS+Siloxane monomer	NVP+TPVC+NVA+PBVC	mPDMS+DMA+HEMA+siloxane macromer+ PVP+EGDMA	mPDMS+ DMA+HEMA +siloxane macromer+ PVP+TEGDMA	FM0411M; HOB; IBM; M3U; NVP; TAIC; VMA	Not disclosed	Not disclosed

DMA (N,N-dimethylacrylamide); EGDMA (ethyleneglycol dimethacrylate); HEMA (poly-2-hydroxyethyl methacrylate); mPDMS (monofunctional polydimethylsiloxane) NVP (N-vinyl pyrrolidone); TEGDMA (tetraethyleneglycol dimethacrylate); TPVC (tris-(trimethylsiloxysilyl) propylvinyl carbamate); TRIS (trimethylsiloxy silane); NVA (N-vinyl aminobutyric acid); PBVC (poly[dimethysiloxy] di [silylbutanol] bis[vinyl carbamate]); PVP (polyvinyl pyrrolidone); HOB (2-Hydroxybutyl methacrylate); IBM (Isobornyl methacrylate); FM0411M (α-Methacryloyxyethyl iminocarboxyethyloxypropyl-poly(dimethylsiloxy)-butyldimethylsilane); M3U (α ώ-Bis(methacryloyloxyethyl iminocarboxy ethyloxypropyl)-poly(dimethylsiloxane)-poly(trifluoropropylmethylsiloxane)-poly(ώ-methoxypoly(ethyleneglycol)propyl methylsiloane)); TAIC (1,3,5-Triallyl-1,3,5-triazine-2,4,5(1H,3H,5H)-trione); VMA (N-Vinyl-N-methylacetamide).

Contact lens care solutions

Overall care of contact lenses is dependent on contact lens care solutions, as well as how they are used (e.g. rinsing, rubbing, soaking, etc.).[123;124] The main purpose of a contact lens solution is to disinfect the lens. [123;124] Contact lens solutions have been on the market since the late 1940s and with the appearance of PMMA contact lenses, a primary purpose of these early solutions was to enhance wettability.[124] With the introduction of novel contact lens materials, such as polyHEMA in the 1970s, more emphasis was put on optimizing and improving the solutions in order to make them compatible with the specific material (e.g. SiHy lenses).[124] Therefore, the "ideal" contact lens solution was intended to combine adequate disinfection efficiency, lens material and ocular surface compatibility, enhanced comfort, simple usage as well as being inexpensive.[123]

For a detailed description of the composition and clinical performance of modern contact lens solutions, the reader is referred to the literature.[29;113;123-127]

Disinfectants

The main elements of a contact lens solution are the disinfectants or antimicrobial agents. The activity of disinfectants includes the capacity to maintain the contact lens solution activity while providing an efficient kill of pathogens (disinfection) without harming the ocular tissue. Modern contact lens care preservation and disinfection is based on the chemical preservative/disinfectant killing any contaminating organism but not binding to the contact lens or being inactivated by debris on the contact lens surface.[123] Contact lens solution manufacturers are required to show that their solution disinfects against Pseudomonas aeruginosa, Staphylococcus aureus, Serratia marcescens, Candida albicans and Fusarium solani (International Standards Organisation). Theoretically, a broad-spectrum preservative would be beneficial, as each chemical agent is typically only effective against a limited spectrum of microbes. In order to achieve this, the required concentration may be too high and would lead to a toxic reaction on the cornea.

The first generation of disinfectants, used for hydrogel lenses included chlorhexidine and thimersoal. However, they caused a significant allergic reactions, and were replaced.[33;128] There are currently five disinfectants that are primarily used in contact lens solutions.

Hydrogen peroxide (CH_2O_2)

Hydrogen peroxide has been successfully used for many years and is considered the "gold standard" for disinfection.[123] Its typical concentration in contact lens solutions is 3% (30,000 ppm), but to prevent a toxic reaction to the cornea by lens insertion, hydrogen peroxide has to be neutralized to less than 100 ppm.[124;129-131] So-called "two-step systems" provide a more effective disinfecting process than "one-step systems" as the lens is disinfected for longer.[123;124] The neutralization in a "one-step system" begins immediately after placing the lens into the solution, whereas for a "two-step system", neutralization is manual, either by rinsing the lens or by adding a dilution agent to the solution.[123]

Polyhexamethylene biguanide (PHMB)

PHMB has been available for many years and is used under various synonyms in different contact lens solution manufacturers. The long-chained polymetric PHMB binds to negatively charged microbial plasma membranes causing membrane disruption and cellular lysis.[123;132]

Polyquaternium-1

This high-molecular-weight antimicrobial agent is known under the trade name "polyquad". It is only used in the Alcon products OptiFree Express and OptiFree RepleniSH. The mode of action is similar to PHMB, as it attacks the plasma membrane of pathogens.[133] The advantages of its greater molecular weight and size compared to the first generation of preservatives are that it does not penetrate the hydrogel as easily and is more effective at lower concentrations, resulting in possibly reducing the risk of toxicity and hypersensitivity.[123]

Other available antimicrobial agents

In addition to polyquad, Alcon uses Aldox (Myristamidopropyl dimethylamine), another antimicrobial agent in both of its OptiFree products (OptiFree Express and OptiFree RepleniSH). Studies have shown that this cationic agent possesses both antifungal and antiamoebic activity.[123;124;134]

Alexidine is a novel ophthalmic preservative (cationic bisbiguanide)[123] that is similar to chlorhexidine, yet more rapid in its action and is therefore hypothesized to be effective at lower concentration. It was used in ReNu MoistureLoc (Bausch & Lomb), which was removed from the market because of its reported association with the development of Fusarium keratitis.[135;136]

Subsequent analysis has shown that alexidine could not be directly related to this outbreak, however.[136]

Surfactants

Surfactants (surface-active agent) are added to contact lens solutions as wetting agents. They also lower the surface tension of the solution.[113] The two important purposes of a surfactant in a contact lens solution are, first being a detergent or cleaner to remove loose debris and deposits, and second to enhance wettability of the hydrophobic substrates.[123;137;137] Two distinct groups of surfactants are commonly used in contact lens solutions. Poloxamers, known under the trade name Pluronic, are non-ionic block copolymers, whereas the poloxamines, branded under the trade name Tetronic, are symmetrical block copolymers. Examples of surfactants include: Pluronic F87, Pluronic F127, Pluronic 17R4, Tetronic 1107, Tetronic 1304 or Tetronic 1307.[123]

Chelating agents

Chelating agents are added to solutions to improve the disinfection efficiency. Their purpose is to help in removal of tear film components, typically protein. The commonly used chelating agents in current contact lens solutions are ethylenediamine tetraacetic acid (EDTA), citrate or hydroxyalkylphosphonate (Hydranate).[123]

Demulcents

Demulcents are agents that consist of water-soluble polymers. Originally, they were used topically on the eye to protect and lubricate the mucous membrane in order to relieve dryness and irritation. More recently, they have been added to contact lens solutions to modify the lens surface to improve comfort. Commonly used demulcents are hydroxypropylmethylcellulose (HPMC) or propylene glycol.[123]

Other agents

Contact lens solution manufactures add other agents to their solutions to improve comfort and/or increase the efficiency of the solution.

Buffering and tonicity agents are included to obtain a certain tonicity and pH.[123;124] Examples of these agents are inorganic salts, non-ionic polyols, sodium chloride, potassium chloride, propylene glycol, borate buffers, phosphate buffers, citrate buffers, glycerine and mannitol.[123]

Other agents that can be found in different contact lens solutions are:

- Taurine, to enhance buffer capacity and acts as an antioxidant in the tear film, retina and cornea[138;139]
- Tromethamine, used as an alkalising agent and to maintain pH[123]
- Dexpanthenol, to enhance wettability and lubrication[140]
- Sorbitol, to aid lens wetting[123]
- C9-ED3A (nonanoyl ethylenediaminetriacetic acid),[140] a surfactant that is used in Alcon's OptiFree RepleniSH in combination with Tetronic 1304[123]

Effects of contact lens wear on the cornea

Approximately, 130 million people worldwide primarily use contact lenses to correct their refractive errors.[2] The metabolic activity of the avascular cornea is dependent on nutrients and oxygen supply.[42] The majority of the nutrients are derived from the anterior chamber, which is directly behind the cornea, but the oxygen has to be sourced mainly from the atmosphere.[40] A contact lens placed onto the cornea acts as a barrier to this oxygen exchange and as the initial materials used for contact lenses lacked sufficient oxygen transmissibility, lens wear resulted in chronic and acute signs of hypoxia.[3;5;7;141-150] These effects on the anterior and posterior cornea have been intensively studied and include central corneal clouding,[149;151] corneal swelling, neovascularisation, endothelial blebs[152] and other morphological endothelial responses.[153-155] With the introduction of SiHy lenses, providing a notable increase in oxygen transmissibility, the reported hypoxic problems seem to have decreased.[7;20;22;142] There are still morphological and physiological complications in the cornea with contact lens wear.[40;86;116;155;156] The emphasis of this thesis is the corneal epithelium, therefore, only the effects of contact lens wear on the corneal epithelium are described in more detail.

Sodium fluorescein

Corneal epithelial and anterior stromal insults can be visualized using a variety of vital dyes including sodium fluorescein, rose bengal, and lissamine green.[17;103] Sodium fluorescein, a yellow/orange substance, is the dye most commonly used to examine the ocular surface and to highlight CS.[17;103;157] It was first used by Paul Ehrlich in 1882 as an antibody labeling agent.[158] Shortly after this, Pflueger

instilled sodium fluorescein to examine the ocular surface and noted "CS".[159] In order to make sodium fluorescein more soluble in water, it is combined with sodium.[160] Topical application of sodium fluorescein is not toxic and should not cause any adverse reaction or stinging. It is likely though that multiple instillations of sodium fluorescein can cause a toxic epithelial response. Other side effects have also been observed, including nausea in 8% of patients, when sodium fluorescein was intravenously used during angiography.[161]

Corneal epithelium responses to contact lens wear

Typically, the factors that cause a physiologic, anatomic or metabolic response of the corneal epithelium to contact lens wear are of mechanical, hypoxic, inflammatory, infective or toxic/allergic origin.[17;19;103;155]

Corneal staining (CS)

From a clinical point of view, superficial punctate CS in various forms is probably the most commonly observed side effect of rigid and soft contact lens wear. Generally, the aetiology of CS can be divided into different categories: mechanical, hypoxic, dehydration, metabolic, allergic, toxic or infectious (Table 4).

Different types and varying levels and appearances of CS occur during contact lens wear and approximately 60% of the contact lens wearing population has CS to some degree.[162] Low grade CS can also be observed in non-contact lens wearers.[163;164] "CS" is not a condition itself but much rather it is used as a term to refer to an area of the epithelium that is disrupted or shows other pathophysiological changes, because they are fluorescent. There is controversy about what the dye is actually highlighting. It is suggested that sodium fluorescein stains and penetrates only dead or damaged cells,[94] but on the other hand there is also a report that fluorescing cells could still be alive.[165] Another theory is that this hyperfluorescence is an indication of gaps between neighbouring cells or localized loss of a group of cells causing sodium fluorescein to pool.[165]

Table 4: Categories of epithelial CS with soft contact lenses

Aetiology	Source	Appearance	Location
Mechanical	- High modulus	- dense, arcuate CS (SEAL)	- superior periphery, close to limbus
	- Lens binding	- dense arc or patch	- location of binding
	- Foreign bodies beneath lens	- zigzag track	- across corneal surface
	- Lens defects	- punctuate to patch CS depending on defect	- location of defect
	- Lens insertion or removal (abrasion)	- dense CS	- typically periphery (8 & 4 o'clock)
Dehydration	- Epithelial disruption, e.g. due to drying of corneal surface	- coarse punctate CS (smile-form)	- inferior, 4-5mm from limbus
	- Desiccation	- stipple staining	- central
Metabolic	- hypoxia	- dense CS	- generalized
Allergic	- Contents of contact lens Solutions, e.g. Thimerosal, benzalkonium chloride and chlorhexidine	- diffuse superficial punctuate CS	- generalized
Infectious	- Variety of pathogens	- superficial CS combined with stromal perfusion	- confined to area of ulceration or insult
Toxic	- unknown, probably certain components in contact lens solution	- diffuse superficial punctuate CS	- typically annular presentation, though can be all over cornea
	- non-neutralized peroxide	- diffuse superficial punctuate CS	- generally all over cornea

Grading of CS and clinical significance

The most commonly used grading scale to record the extent and severity of CS represents some version of a global five step graded (0-4) scale.[166-172] The increments of these five step scales usually not only represent the severity of CS but can also be used as guidelines for necessary intervention.[166;171;172] Therefore, no CS (grade 0) and a trace CS (grade 1), both of which not considered to be clinically significant, would not require any treatment. Mild CS (grade 2), not

considered clinically significant, but worth noting and should be monitored over time. However, moderate CS (grade 3) probably needs therapeutic intervention and severe CS (grade 4) requires treatment. [166;171;172] Sometimes, increments of 0.5 are added to the global five step scale, as they are hypothesized to improve precision of the assessments.[169;172] Illustrative photographs or pictures of each level are also often available to guide grading judgement[171] and descriptors of type and pattern of CS, e.g. punctuate, coalesced, etc. may also be used.[173] Grid schemes, more commonly used in research than in clinical practice and dividing the cornea in central, superior, inferior, nasal and temporal zones, are also used to evaluate CS.[174;175] Additionally, the type, depth and area of CS can be reported (Table 5).[166]

Table 5: Grading scale for CS characteristics (Adapted from: Terry et al.)[166]

Grade	Type	Extent: Surface Area	Depth
Grade 1	Micropunctate	1 – 15%	Superficial epithelium
Grade 2	Macropunctate	16 – 30%	Deep epithelium, delayed stromal glow
Grade 3	Coalescent	31 – 45%	Immediate localized stromal glow
Grade 4	Patch	> 45%	Immediate diffuse stromal glow

Table 6 shows the standards published by Terry et al.[166] An absolute change of grade by 1 or more over time, is additionally considered to be clinically significant.[166;171]

Table 6: Unacceptable CS (Adapted from Terry et al.[166])

Unacceptable CS – any of the following:
> Grade 2 type CS (macropunctate)
> Grade 1 type depth of CS (superficial epithelial involvement)
> Grade 1 extent of CS (1 – 15% surface involvement)

Jones et al. applied a cumulative scoring method, where they graded the degree of CS in each zone on a 0 (negligible) to 100 (severe) scale as well as they graded the percentage of each zone that exhibited CS also on a 0 (none) to 100% (total) score.[29] The "sector CS score" was then calculated by multiplying the degree of CS by the percentage of CS of the particular zone. The CS can either be recorded for each zone individual or considered as the sum of all 5 zones. This particular grading scale has been further modified and is now published under the term "CCLR Grading Scale"[176]. CS observed in the different experiments of this thesis was graded using the "CCLR Grading Scale" and a more detailed description of this scale will follow in the "General Methods" chapter. Peterson et al.[177] conducted an investigation to compare the level of significance of the CCLR grading scale to a traditional 0-4 scale. They suggested that CCLR global CS scores (GSS) can be categorized into the following sub groups of the 0-4 scale values. Where: Grade 0 = 0 -70 GSS, Grade 0.5= up to 399, Grade 1 = up to 728, Grade 1.5 = up to 1057, Grade 2.0 = up to 1387 and Grade 2.5 = 1716.

Solution induced corneal staining (SICS)

SICS observed with certain combinations of contact lens solutions and SiHy lenses has recently been reported.[29;31;178] It is referred to as solution sensitivity and has been hypothesized to be a result of a toxic reaction.[29;30] Also, a similar kind of CS had been observed with some conventional hydrogel lenses in combination with certain contact lens solutions. This SICS is typically of fine punctuate nature and usually most prominent in the peripheral cornea with only marginal central involvement (annular-shaped).[29;179] SICS has been reported to be usually asymptomatic[29;30] and to be most evident during the first 2-4 hours of contact lens wear with residual SICS after approximately 6 hours of contact lens wear.[29-31] Figure 7 shows an example of the appearance of SICS.

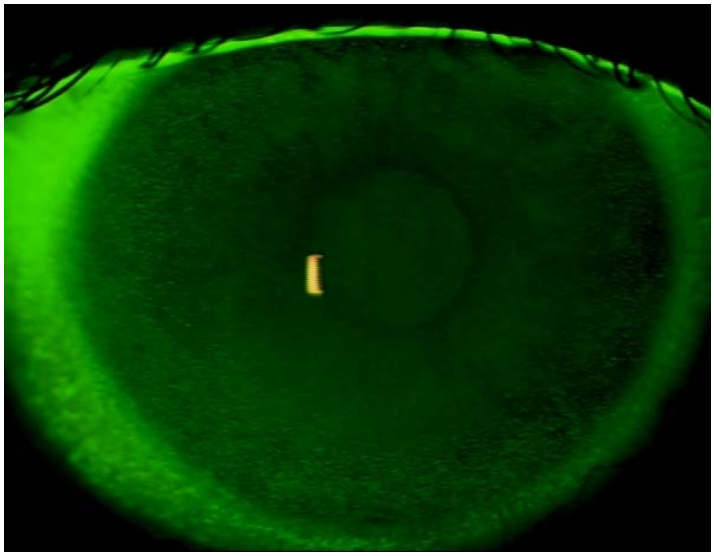


Figure 7: Appearance of SICS (Courtesy of CCLR)

The initial study about SICS with SiHy lenses was published by Jones et al.[29] They reported that significantly more asymptomatic SICS was observed when PureVision lenses (Bausch & Lomb) were used in combination with the PHMB-based solution ReNu Multiplus (Bausch & Lomb) than with the polyquad-based solution OptiFree Express (Alcon). Since then, other similar findings have been reported.[21;30;31;125;178-183] These studies seem to confirm that certain lens/solution combinations, in particular PureVision lenses in combination with PHMB-based solutions, especially ReNu MultiPlus, are associated with the highest amounts of SICS.

There are also various reports of this kind of SICS with conventional hydrogel lenses. PolyHEMA based contact lenses in combination with hydrogen peroxide systems have shown to result in less SICS than the same lens material in combination with either PHMB-based or polyquad-based systems.[174;184-189] Jones et al.,[184] conducted a study using FDA group II and IV lenses. Their conclusion was that increased levels of SICS occurred with regimes containing the highest levels of preservatives. This was particularly true with FDA group II lenses. Increased SICS with PHMB-based solutions in combination with FDA Group II lenses, compared to when the same lenses where used with hydrogen peroxide systems, was reported by Begley et al.[189] Cho et al.[185] stated that in their study the prevalence of SICS with polyHEMA lenses in combination with a PHMB-based solution was 71% compared to 32% with hydrogen peroxide based systems. A significant increase in SICS

with PHMB-based products after 28 days of lens wear (FDA Group II and IV) when compared to baseline was reported by Lebow et al.[187] and Stiegemeier et al.[188]

The mechanism behind this type of SICS is still unknown. It is suggested that certain components within the contact lens solution, mainly the preservative (disinfectant) are being adsorbed onto the lens surface and then released after lens insertion, causing a toxic reaction, resulting in the SICS.[29] Amos[125] suggested that the disinfectant alone was not responsible for SICS, but other components and the specific combination of components within a solution were important. It has also been proposed that the levels of SICS seen with these SiHy lenses are comparable to those seen with FDA Group II lenses (contain NVP) in combination with PHMB-based solutions. As it has been shown that the levels of lipid uptake for NVP-based lenses are higher than those for neutral or ionic lenses,[190;191] Jones et al. suggested that the lipid adsorption onto NVP based contact lens materials was a major cause for PHMB binding.[184] The result would be a subsequent release of toxic doses of PHMB onto the ocular surface. Dassanayake et al.[192;193] studied the uptake and release of eight soft lens materials and five MPS. They showed that FDA Group II lenses have increased uptake and release values when compared to FDA Group IV lenses which showed decreased uptake and release values. They did not detect a preservative uptake with polyquad-based materials. They proposed that the higher water content of FDA Group II lenses affects the uptake of chemicals, such as preservatives, resulting in a high release of those chemicals. PureVision also contains NVP and in combination with the special surface treatment (plasma oxidation process, creating silicate islands) the lipid uptake could be higher than with other ionic lenses or other SiHy materials, resulting in binding of certain components of the solution to the surface.[30] Schlitzer[194] showed that for FDA Group I and II lenses soaked in chlorhexidine (PHMB) or DYMED (polyquad) interacted with the antimicrobial agents. However, lenses cycled in DYMED, showed minimal uptake and therefore lower potential for ocular surface irritation. Karlgard et al.[195] investigated the in vitro uptake and release of ocular pharmaceutical agents by silicone-containing (lotrafilcon and balafilcon) and polyHEMA containing (etafilcon, alphafilcon, polymacon, vilfilcon, omofilcon) hydrogel contact lens materials. They showed that a rapid uptake and release was observed within 50 minutes, suggesting that the drug uptake and release appeared to be a function of lens material ionicity, water content and silicone component. Garofalo et al. studied FDA Group II and IV lenses, as well as two SiHy lenses (PureVision, Bausch & Lomb, and Focus Night and Day, CIBA Vision) in combination with PHMB and polyquad-based solutions over time. They found that SICS significantly increased after 1 – 2 hours for FDA Group II and SiHy

lenses in combination with PHMB-based products, followed by a SICS decrease after 6 hours of lens wear. FDA Group IV lenses, in combination with all solutions, produced only low amounts of SICS over the entire 6 hours. These observations seem to support the uptake and release theory of certain lens/solution combinations. The relatively quick recovery of SICS is probably an indication that the SICS is only superficial. The generally absence of increased symptoms of dryness or discomfort could also support the hypothesis that SICS is a manifestation of a relatively superficial phenomena.[29]

The reported general annular[29] appearance of this type of SICS is another interesting feature. SICS has been previously reported to be greater peripherally and inferiorly than centrally.[170;184;196] Jones et al.[29] suggested in their study that since all participants were myopic and the lenses were thicker in the periphery, more preservatives could have been absorbed in the thicker peripheral part. Another explanation is that as epithelial cells are generated in the periphery (limbus) and then move centripetal[40], peripheral cells are younger than those centrally, and may react differently when exposed to different environments.

Common to all the studies seems to be the great intersubject variability in amount and severity of SICS. Not everyone who is exposed to the same lens/solution combinations develops SICS. Jalbert et. al.[196] reported that even though participants were wearing the same lens/solution combination in both eyes, for some participants only one eye showed SICS. The reason for this is unclear.

Another common outcome of all these clinical studies is that it seems that the PHMB-based ReNu MultiPlus is the solution that in combination with certain lenses is associated with staining. The toxicity of contact lens solutions *in vitro* does not support this. Paradoxically, the majority of studies using corneal and other standard cell lines, show the opposite; polyquad-based and Aldox-based solutions induce more change to epithelial cells than ReNu Multiplus.[126;127;197-201] Mowrey-McKee et al.[197] using an immortalized human cell corneal epithelial cell line (HCE-T) showed that the solutions in order of increasing cytotoxicity potential were: SoloCare = COMPLETE Comfort Plus < ReNu MultiPlus < < OptiFree Express with Aldox. Labbe et al.[202] compared, benzalkonium chloride (BAK), previously shown to be toxic,[199;203] to polyquaternium-1 (PQ-1) using a rat model. This study indicated that high doses of PQ-1 were much less toxic than BAK. Dutot et al.[200] investigated the cytotoxicity of MPS used for contact lens disinfection on incubated conjunctival cell lines. They evaluated the capacity of one polyquad-based solution and three PHMB-based solutions to induce

necrosis. Their results were that all MPS induced necrosis with additional oxidative stress. They also showed that the polyquad-based MPS did induce oxidative stress and an increase in mitochondrial mass. Differences were found between the PHMB-based MPS solutions: They either induced a decrease in reactive oxygen species production with mitochondrial alterations, or an increase in reactive oxygen species production. Each solution stimulated specific cell death receptor activation. These differences found between the PHMB-based MPS (at the same concentrations) may have been due to their buffers or the EDTA concentration that differed slightly amongst solutions. Also, they suggest, a difference in PHMB polymer size would lead to differences in cytotoxicity. Bantseev et al.[204] have indicated that OptiFree Express (polyquad-based) has the potential for greater mitochondrial effects when compared to ReNu MultiPlus (PHMB-based) and Hanks' balanced salt control solution. Horwath-Winter et al.[198] demonstrated that four soft contact lens solutions (different preservatives) induced changes in mitochondria of human conjunctival cells only at higher doses. This damage to the mitochondria however, did not lead to cell death.

Few reports[205;206] however, indicate that the *in vivo* SICS results do support the *in vitro* toxicity observations. Santodomingo-Rubido et al.[205] tested six different MPS in various concentrations on one cell lines of Chinese hamster fibroblasts. Their results indicated, that OptiFree Express (polyquad-based) did induce less cytotoxicity than ReNu MultiPlus (PHMB-based). This has also been found by Pham et al.[206] Santodomingo-Rubido et al.[205] also showed that MPS with identical concentrations of PHMB can behave differently depending on solution formulation.

Imayasu et al.[207] examined the effects of MPS on corneal epithelial tight junctions on cultured human corneal epithelial cells. They tested four MPS with different preservatives, surfactants and buffers. Their results showed that even after frequent use, only one solution (non-buffered, PHMB-based with macrogolglycerol hydroystearate as the surfanctant) had no effect on the epithelial tight junctions. The other three solutions did cause a breakage of the epithelial tight junctions, therefore affecting the epithelial barrier function. Interestingly, these three MPS use different preservatives, namely PHMB, alexidine and polyquad. The common components of these three solutions are polaxamine as the surfactant and boric acid as the buffer. Testing these two components by themselves showed that polaxamine had almost no effect on the tight junctions, but boric acid-treated cells showed discontinuations and partially damaged line structures.[207]

Visualizing the corneal epithelium

Biomicroscopy of the eye

Microscopy enables the study of the morphology of the normal and pathological cornea. Instruments to do this include the ophthalmoscope, slit lamp, specular microscope, and CM.[208] Each of these non-invasive optical technologies have their origin in using reflected light to image the eye.[209]

A slit lamp typically uses oblique illumination and microscopic observation. It enables the viewing of oblique sectioned views of the living cornea, ocular lens or retina, by projecting a slit of light from a lamp onto the observed areas. The slit lamp is a versatile instrument due to its range of magnifications and possible slit adjustments and rotations. However, limitations are the shallow depth of field and that the weak reflectivity of the interior cornea being overwhelmed by the much larger reflections from the anterior and posterior surfaces. Another essential constraint is that the highest practical magnification that can be achieved is approximately 40x, with a lateral resolution of 30 μm.[86] Detailed information on the slit lamp principles and applications can be found in the literature.[17;210-212]

The specular microscope is also a reflected light microscope. Imaging is based typically on the specular reflection at the interface between the endothelium and aqueous humor, due to the difference in refractive indices. Specular reflection is achieved, when the angles of incidence and reflection are equal. Vogt[213] was the first to use this method to image the corneal endothelium *in vivo* and observed endothelial cell borders. After this, further adjustments and improvements have been made to minimize the effects of strong reflections, improve the narrow field of view and stabilize movements.[214-224] For more detailed principles and applications, the reader is referred to the literature.[87;216;225]

These instruments are attempts to overcome the problem of how to image *in vivo* an approximately 500 micron thick, transparent and moving object, the human cornea. The three-dimensional CM of the living eye was a major development in the instrumentation for biomicroscopy of the eye and has led to new diagnostic techniques and cellular descriptions of ocular disorders and pathologies.[225-229] As the CM was the instrument of choice for the experiments in this thesis, a more detailed discussion of its principles and applications is outlined in the following paragraphs.

Confocal microscopy (CM)

Optical principles of CM

Tissue analysis is commonly performed by histological examination of excised tissue. CM, a relatively new technique, provides a non-invasive option to study the cornea *in vivo* on a cellular level, at a magnification of about 500x to 700x. Inter- and intracellular appearances can be imaged using the light that is reflected within the tissue, as a consequence of changes of the index of refraction. In contrast to a slit lamp that has a large field of view with limited resolution, the optical principle of a CM is that "field of view is sacrificed for resolution".[86] The CM is a type of microscope that uses a small focused spot of light to illuminate and image a thick object, such as the cornea. Figure 8 shows a schematic diagram of the basic optical principle of a confocal arrangement.

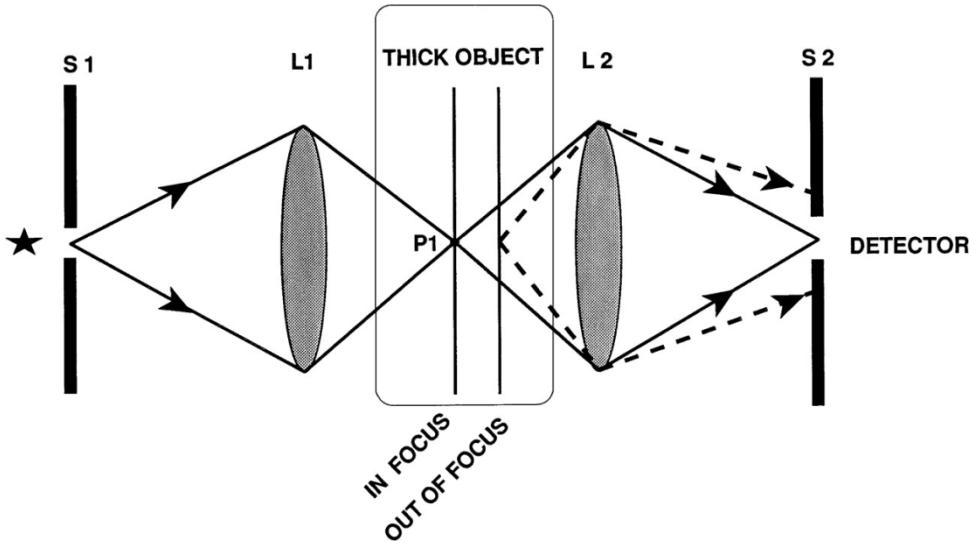


Figure 8: Basic optical arrangement of a clinical CM (with permission from Springer Science + Business Media)

The figure above illustrates a CM with two microscope objectives. For clinical use however, only a single objective lens is used, that serves for both illuminating and detecting the light. As can be seen, a point light source is focused to a point (P1) within a thin specimen (cornea) by lens L1. The second lens (L2) collects the light reflected from the small illuminated spot (P1) on the cornea and focuses the light through a slit (S2) onto a light detector. The term "confocal" has its origin in that both apertures, S1 and S2, are co-focused on the same point in the focal plane (e.g. cornea) which is

simultaneously illuminated and detected. The purpose of these apertures is to be a physical barrier to out of focus light. Due to this out of focus light elimination, images of higher contrast and axial and lateral resolution are possible.

When using the same wavelength of light and the same microscopic objective, the advantage of a CM over a non-CM is the enhanced transverse and axial resolution. Due to the improved axial resolution and focusing in and out of the cornea, the CM is an instrument that is capable of "optically sectioning" the cornea in approximately 4 – 10 µm thick slices, depending on the type of CM. The sectioning allows viewing structures of the cornea that scatter or reflect light, such as epithelial cells, keratocytes in the stroma, nerves and endothelial cells.[86] The transverse resolution of a CM is proportional to the numerical aperture (NA) of the microscope objective; the axial resolution is even more dependent on the NA. A high NA is necessary to obtain the maximal axial resolution and therefore to attain the best optical sectioning.[87;216] In addition to this, a higher NA is more efficient to collect light from weakly reflecting objects and also provides an increased light throughput which results in brighter images. On the other hand decreased field of view results from the increasing NA.[87]

Images taken with the CM are different from those obtained in histopathology where the tissue is typically vertically sectioned (along the thickness of the cornea). In CM, the plane of the images is orthogonal to the ocular surface, which provides "en face" corneal images.[87] Another difference between the two microscopy techniques is that in conventional light microscopy all points in the specimen are imaged simultaneously. CM illuminates and detects only a single spot on the specimen. Therefore, to form a two-dimensional image it is essential to either sequentially scan the illuminating spot over the area of the specimen or move the specimen.[226]

Further general formation on CM can be found in various reports.[87;227-230]

Types of CM

The confocal arrangement can be technically achieved in various ways and these arrangements will be discussed in the following paragraphs..

Tandem-scanning CM (TSCM)

Petran et al. developed a real time, direct view CM based on a spinning Nipkow disk.[230] The principle of a TSCM-based on a Nipkow disk is that the illumination light passes through a set of conjugate pinholes (diameter of 40 – 60 μm) that are arranged in several sets of Archimedes spirals. Each of these pinholes has a conjugate and equivalent pinhole on the other side of the disk where reflected light from the specimen passes through. Both illumination and reflected light are scanned parallel over the specimen to create a two-dimensional image.

The advantages of the real-time Nipkow-based TSCM include: (1) real-time operation, where images are acquired at video rates, (2) true colour confocal imaging, (3) specimen can be viewed with the eye, and (4) high transverse and axial resolution.[87] However, the big disadvantage of the TSCM-system based on the Nipkow disk is that only a small fraction of the illumination reaches the sample, due to the area/hole ratio of the disk (1-2%). Because of this very low light throughput, the illumination must be very high. As a result, the clinical use of a TSCM based on the Nipkow-disk for weakly reflecting specimens, such as living cells, may not be satisfactory.[225]

Slit-scanning CM (SSCM)

A SSCM scans the image of a slit, instead of a point, over the back focal plane of the microscope objective and synchronously descans the reflected light from the specimen. Varying the slit width balances the optimization of optical section thickness and image brightness. This is an important feature when imaging transparent samples. The main advantage of a SSCM compared to a point scanning Nipkow disk system is the increased light throughput. This allows the use of a lower intensity light source, enabling longer exposure for patients. Additionally, it is possible to image less reflective layers, such as the wing cells. The major disadvantages of a SSCM are that the microscope is strictly only confocal in the axis perpendicular to the slit height and that it provides a lower transverse and axial resolution compared to pin-hole based systems.[87;225] Figure 9 shows the schematic diagram illustrating the optical system of a clinical real-time SSCM. A double-sided mirror can be used for scanning and descanning and a halogen lamp (non-coherent “white” light) illuminates the slit. The detector has typically been a charged coupled device (CCD) camera. The cornea is coupled to the tip of the microscope objective using a methylcellulose gel or something optically uniform.

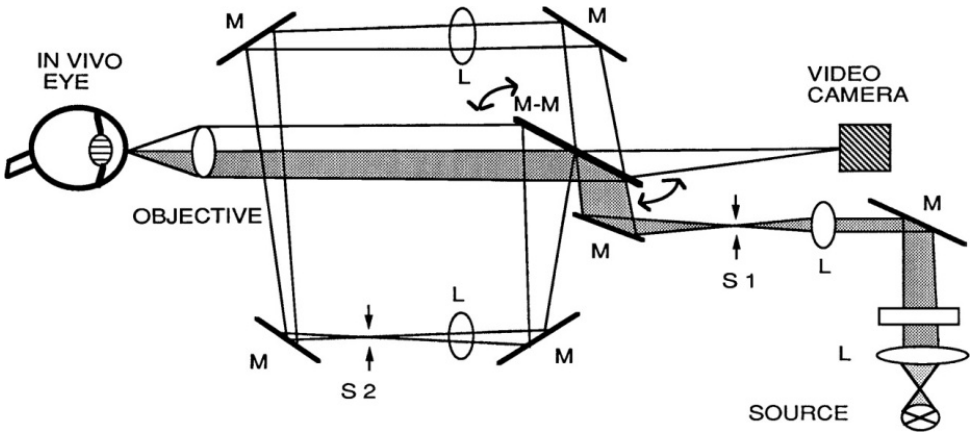


Figure 9: Schematic of the optical principles of a SSCM (with permission from Springer Science + Business Media)

At time of writing, SSCMs are commercially available from Tomey Corporation (Cambrigde, MA, USA), Nidek Technologies (Gamagori, Japan) and Helmut Hund (Wetzlar, Germany).[231] For the experiments in this thesis, a SSCM was used. The specific technical data for this instrument, ConfoScan 3 (Nidek Technologies, Gamagori, Japan), are outlined in Chapter 2 (General methods).

Laser-scanning confocal microscopy (LSCM)

The development of the LSCM is based on Minsky's patent[232] followed by Webb's[229] next step towards a clinical CM, the scanning laser ophthalmoscope. The LSCM is a CM that scans a laser beam spot of less than 1 µm in diameter sequentially over each point of the examined area by a set of galvanometer scanning mirrors. The reflected light is refocused by the microscope objective and imaged on a pinhole aperture in front of the photomultiplier.[48;86;87;216;231]

The Heidelberg retina tomography (HRT) (Heidelberg Engineering, Heidelberg, Germany) is an *in vivo* CM system that is established in the field of ophthalmology.[233-237] It is designed to image and assess the retinal optic nerve head using a 670nm laser.[233-237] For visualizing the anterior segment, Stave et al.[238] modified the HRT with a detachable objective system, the "Rostock cornea module" (RCM).

Clinical applications

In vivo CM of the cornea is quite widely used and a medical literature search resulted in summaries of over 900 published papers (source: Pubmed April 2009). This includes reports on examinations and observations of the cornea, its different structure, in health, disease, during contact lens wear, of cell density measurements or after refractive surgery. Summary information can be found in the review literature, examples being Masters et al.,[87;208;216] Efron,[86] Patel et al.,[48;156;239;240] McLaren et al.[241], Visser et al.[242], Zhihov et al.[243], Guthoff et al.[231], and Tervo et al.[244]

The emphasis of this thesis is the epithelium, especially during contact lens wear. Therefore, the following sections cover reports of the epithelium and alterations to the epithelium observed with CM.

The normal corneal epithelium

The normal corneal epithelium has been observed in various studies, specifically designed for either gaining information on the normal epithelium or as control for studies with broader experimental questions. The majority of the work has been conducted using the SSCM. Patel and McGhee propose that the slight increased image brightness and contrast when using the LSCM make certain features of the cornea appear qualitatively different.[48] Figure 10 shows CM images of the different layers of the normal corneal epithelium, including the sub-basal nerve layer.

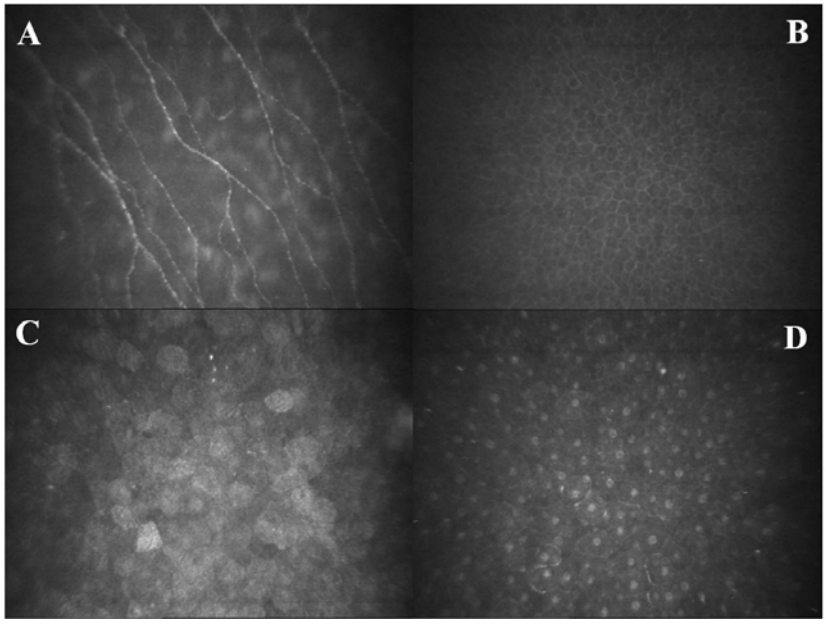


Figure 10: CM images of the different layers of the corneal epithelium, including the sub-basal nerve fibre layer. A: sub-basal nerve fibre layer, B: basal cell layer, C: wing cell layer, D: superficial cell layer

The superficial cells, as seen in Figure 10 D are characterized by a polygonal pattern. Large variations in brightness and granularity from cell to cell and also within cells can be seen. Also, some large, dark and featureless areas are apparent. In general, the cytoplasm ranges from light grey to dark grey with a reflecting nucleus and perinuclear dark halo.[243] The differences in reflectivity between cells of the cellular cytoplasm are thought to represent various stages of progression towards cell death. Wilson et al.[245] proposed that the darker cells are those that are about to desquamate. It is also reported that darker cells have less surface features and are less rough.[40] The cells are approximately 50 μm in diameter, but with wide individual variations.[86]

Epithelial wing cells (Figure 10 C) are typically more uniform in size and shape and form a mosaic-like pattern. A very thin reflective border, brighter then the cytoplasm can be seen in some cells. The cell bodies have the same reflectivity as superficial cells, and are smaller in size, approximately 20 μm. As the wing cells layers represent a morphological transition between basal cells and superficial

cells, bigger wing cells can be observed anteriorly, more towards the superficial cell layer and smaller wing cells are present towards the posterior side, close to the basal cell layer.[246;247]

An image of epithelial basal cells can be seen in Figure 10 B. These cells are tightly packed and appear as sections of cylindrical cells with bright borders and dark cytoplasm. As these cells are columnar in shape, with their major axis in the anterior-posterior directions,[39] the confocal view is normal to this axis which makes these cells smaller in diameter (approximately 10 μm) when compared to the other epithelial cells.[48;86;231;243]

The thin nerve fibrils of the sub-basal nerve plexus are shown in Figure 10 A and are located between the basal epithelium and Bowman's layer. The nerve bundles appear as straight and beaded reflective fibres that are distinct from the background.[86;231;243] Matsuda[248] has described the beads as axonal efferent and sensory terminals and consist of accumulations of glycogen and mitochondria.[249]. Patel and McGhee[250] have shown that the nerves radiate to a point inferior to the central cornea in a whorl-pattern across the cornea. Later, the same authors[240] also showed that the nerve plexus is a dynamic structure that moved centripetally.

The corneal epithelium during contact lens wear

Contact lens wear can cause distinct changes in corneal structure, morphology and thickness.[48;86;156;216;231;241;243]

Changes to the corneal epithelium during contact lens wear revealed with CM can be understood as compression of cells, redistribution of tissue, cell migration and proliferation. These alterations are generally the result of mechanical, metabolic or toxic disturbances. Other observations with the CM as a result of contact lens wear could also be interpreted as compression of cells and redistribution of tissue volume, cell migration and proliferation. Proliferation of basal cells, as suggested by Ladage et al.[251;252] is stimulated by contact lens wear. Lens wear slows desquamation of the superficial cells and restrains corneal epithelial turnover. A delayed desquamation response is thought to occur with contact lens wear, as the contact lenses protect the corneal surface from the normal shedding forces of the eyelids and therefore inhibit cell shedding.[77] Bansal et al.,[253] using SSCM showed that bright superficial epithelial cells were lower in number for contact lens wearers (446 ± 310 cells/mm^2) when compared to a non-contact lens wearing control group (620 ± 210 cells/mm^2). Tsubota and

Yamada,[254] using specular microscopy, found an increase in superficial cell size after 3 months of soft lens wear from 639 ± 84 μm^2 to 820 ± 99 μm^2. Their explanation for their observation was delayed epithelial desquamation due to contact lens wear. This appeared to be confirmed by O'Leary et al.[255] who showed that desquamated surface epithelial cells, harvested using an irrigation technique, were larger in contact lens wearers (1436 μm^2) compared to non-contact lens wearer (1225 μm^2). These findings were in agreement with observations made by Jalbert et al.[256] indicating that the hyper-mature state of the desquamated cells is the reason for their increased size. Various other authors[79;257] have reported that surface epithelial cell size has increased with all forms of contact lenses, including 24 hours wear of rigid orthokeratology lenses.[251;258] On the other hand, Eckard et al.[259] observed that the cell bodies of superficial cells were in general smaller in contact lens wearers (approximately 20 μm) compared to non-contact lens wearers (up to 50 μm). They also showed that an increase in superficial cell density occurred, centrally as well as peripherally.

An increase in density of Langerhans cells as a reaction of the ocular surface to contact lens wear has been reported.[260] The increase occurred mainly at the level of wing and basal cells. Continuous mechanical stimulation, exposure to different types of contact lens solutions and various stages of oxygen transmissibility are hypothesized to change the immune status of the cornea and possibly increase the risk of infections.[231]

In various studies,[72;79;261] CM has been used to assess corneal epithelial thinning generated from contact lens wear. Extended wear of a high Dk/t rigid lens appears to induce more epithelial thinning than a regular hydrogel lens. A hydrogel lens however, produces more epithelial thinning than a SiHy lens.[72]

Mucin balls are tear film debris observed in some patients wearing SiHy lenses and conventional hydrogel on an extended wear basis.[262-265] Various reports on mucin balls observed with the CM exist.[265-267] Miller et al.[265] reported the size of mucin balls to range in diameter between 20 and 50 μm and to sometimes deeply indent the corneal epithelium. They also showed that there was no inflammatory response and that no epithelial cells were seen beneath the mucin balls.

Effects of contact lens solutions on the corneal epithelium have also been examined using CM. Both, Chang et al.[268] and Imayasu et al.[269] have indicated that various levels of corneal irritation can occur as a response to different concentrations of preservatives used in contact lens solutions. The duration

of contact lens wear does also seem to influence the severity of disturbance. BAK has been shown to be the most toxic preservative used in ophthalmic preparations.[270] The extent of toxicity may depend on dosage and duration of exposure. It has been shown that an exposure for 3 hours to a moderate percentage of BAK (0.01%) results in loss of microvilli, degenerative membrane changes and desquamation of the superficial cell layer.[271] A concentration of 0.005% BAK has been demonstrated to induce desquamation of the surface layer within 30 minutes.[203] Harvey[1] investigated the effect of contact lens and contact lens solution interaction on the corneal epithelium using CM and observed brightly reflecting superficial cells in some participants exposed to a specific lens/solution combination. The suggestion she made was that this interaction of lens and solution probably resulted in a release of certain components of the solution that had been adsorbed onto the lens surface. However, she also hypothesized that not only could certain components have been adsorbed, they could have also entered the lens matrix, especially at the thicker lens edges of the, as the majority of the participants wore minus lenses. The release of certain components in both cases could have resulted in a toxic reaction on the ocular surface, visualized by these hyper-reflective superficial cells.

Literature review of hyper-reflective superficial epithelial cells

The serendipity of apparently whole (at least in terms of diameter) but hyper-reflective superficial cells observed by Harvey[1] in subjects who were exposed to a specific contact lens/solution combination led the conclusion that their presence is a result of this contact lens/solution interaction. These hyper-reflective superficial cells in combination with SICS are not mentioned in the literature.
Bright or hyper-reflective superficial cells have been rarely reported in the literature. They have been stated as being a result of normal cellular mitosis. The brighter superficial cells are thought to be either desquamating or damaged cells.

Various structures influence the interaction of the light beam and its transmission and absorptions. These structures include: cellular organelles, microvilli and microplicae, and glycocalyx.[40;259;272] Borchert et al.[272] also suggested that the presence of microdesmosomes in epithelial layers could explain brighter cell membranes of epithelial cells when compared with endothelial cells. Eckhard et al.[259] proposed that the same finding, the presence of microdesmosomes, could be applied to the

single superficial cell floating in the tear film even though it does not have a connection to the corneal surface. Wilson et al.[245] also suggested that the differences in cell cytoplasm reflectivity represent various stages of progression towards cell death. However, they proposed, that the darker cells are those that are about to desquamate. Jester et al.[273;274] suggested that expression of transketolase and aldehyde dehydrogenase (corneal crystallins), typically only found in normal transparent corneal cells, is reduced and that this would result in an opaque cornea with increased backscatter. The hypothesis of the presence of corneal crystallins in various corneal cell types and their importance in maintaining corneal transparency has been also been suggested by others.[275-277]

Benitez del Castillo et al.[278] investigated subjects with dry eyes using CM. They observed, in some subjects, the presence of superficial epithelial cells with increased reflectivity, but did not offer an explanation for their observation.

A study was conducted by Mocan and Irtek[279] to assess whether topical sodium fluorescein application prior to the CM procedure had any effect on the imaging characteristics of the corneal epithelium. They suggested that hyper-reflective superficial cells were more common in post-sodium fluorescein images, and specifically in patients having keratoconus.

A recent paper (2009) by Martone et al.[280] reported examining the long-term effects of preservative-free and preservative-containing antiglaucoma eye drops on the ocular surface. They found that the superficial epithelial cell layer showed hyper-reflective cell bodies with less prominent cellular outlines in patients using preservative containing antiglaucoma eye drops in comparison to patients using preservative-free antiglaucoma eye drops. These findings are in agreement with observations made by Ichijima et al.[203] and Labbe et al.[202] Ichjijima et al.[203] suggested that application of as little as 0.005% BAK causes a toxic reaction that leads to swelling and desquamating of superficial epithelial cells. These cells had a brighter hyper-reflective appearance. Labbe et al.[202] compared the toxicity of BAK and polyquaternium-1 (PQ-1) on rats using CM. They showed that rats who were exposed to BAK had more hyper-reflective superficial cells without visible nuclei when compared to rats who were exposed to PQ-1.

Research aims

The experiments designed for this thesis were intended to provide insight into what predictor variables are associated with hyper-reflective superficial cells. This thesis is organized into seven chapters. Chapter 2 describes the instruments and general methods used in each experiment. Chapter 3 reports results in normal corneal epithelium and the effect of age and dry eye symptoms on the appearance of hyper-reflective cells. Chapter 4 reports results on the effects of lens wear and contact lens solution usage. Chapter 5 is a report of an examination of the effect of diagnostic agents on the appearance of hyper-reflective cells. Chapter 6 and Chapter 7 discuss and summarize the results, conclude the findings and offer suggestions for future work in this area.

The specific research aims for this thesis are:

- To define the appearance of normal superficial epithelial cells.
- To investigate the variables that could possibly influence the appearance of hyper-reflective superficial cells. These were: age, dry eye symptoms, contact lenses, wear, contact lens solutions, contact lenses /solution combinations, prolonged wear of certain lenses and prolonged use of certain contact lens solutions, application of anaesthetic and use of sodium fluorescein (Table 7).

Table 7: Investigated variables that may cause the appearance of hyper-reflective cells

Variables that may cause appearance of hyper-reflective cells	Conclusion
Contact lens solution	**?**
Certain lens/solution combinations that induce SICS	**?**
Contact lens wear	**?**
Age	**?**
Dry eye symptom	**?**
Sodium fluorescein	**?**
Anaesthetics	**?**
Long-term use of same type of contact lens and solution	**?**

Hypotheses for this Thesis

The following hypotheses will be tested in this thesis:

- $h_0$1: Hyper-reflective cells will not be a typical observation in the normal superficial epithelium.
- $h_0$2: Dry eye symptom will not be a predictor for the appearance of hyper-reflective cells.
- $h_0$3: Age will not be a predictor for the appearance of hyper-reflective cells.
- $h_0$4: Contact lens/solution combinations symptom will not be a predictor for the appearance of hyper-reflective cells.
- $h_0$5: Certain contact lens/solution combinations that induce SICS will not be a predictor for the appearance of hyper-reflective cells.
- $h_0$6: Long-term use of the same type of lens and solution will not be a predictor for the appearance of hyper-reflective cells.
- $h_0$7: Contact lens wear will not be a predictor for the appearance of hyper-reflective cells.
- $h_0$8: Sodium fluorescein will not be a predictor for the appearance of hyper-reflective cells.
- $h_0$9: Topical anaesthetics will not be a predictor for the appearance of hyper-reflective cells.

The different experiments conducted for this thesis are organized in three chapters. The first experimental chapter (Non-Cl Wearers) will contain the experiments in which the normal superficial epithelium was defined and observed and in which the superficial epithelium of symptomatic dry eye and asymptomatic post-menopausal women was examined.

The experiments that aimed to investigate the effect of various contact lens/solution combinations, the effect long-term use of the same type of contact lenses and solutions and the effect of solution by itself on the superficial epithelium will be reported in the second experimental chapter (Effect of Contact Lenses and Solution).

The third experimental chapter (Effect of Diagnostic Agents) will include the experiments intended to study the effects of fluorescein and topical anaesthetic on the superficial epithelium.

Chapter 2

General Methods

This chapter describes methods, procedures, diagnostic agents and instruments used consistently during the different experiments in this thesis. Also, grading and image analysis that have been performed in the experiments are detailed. Any modifications that occurred to the procedures described in this chapter are described in the method sections of the respective experiments. If not stated otherwise the following were performed.

Confocal microscopy (CM)

Instrumentation

CM was performed using the ConfoScan 3 (Figure 11) which provides *in vivo* images of the human cornea using slit scanning CM. A small illuminated slit is projected into the cornea through one half of the objective lens. The reflected light passes through the other half of the lens and a second slit, having the same size and in the same focal plane as the illumination slit. The image of this second slit is then detected by a CCD-camera and displayed on a PC monitor. The slits are scanned across the specimen, in order to view a larger corneal area.

The light that illuminates the first slit is from an Osram Xenophot HLX 64625 FCR 12 V, 100W halogen lamp. The slits are in vertical arrangement measuring 15 mm in height and 0.28 mm in width. An immersion lens (Achroplan 40x/0.75W, Zeiss, Germany) with a working distance of 1.98 mm, numerical aperture of 0.75 and a front area of 16.61 mm^2, is the objective. The images are of an area of 450 x 340 μm, and have a nominal lateral resolution of 1 μm and a nominal depth resolution of approximately 10 μm.[281] A mean magnification of 500x is obtained on a 15" display (1024 x 768 pixels). A maximum of 350 images was taken at a rate of 25 images per second.[281]

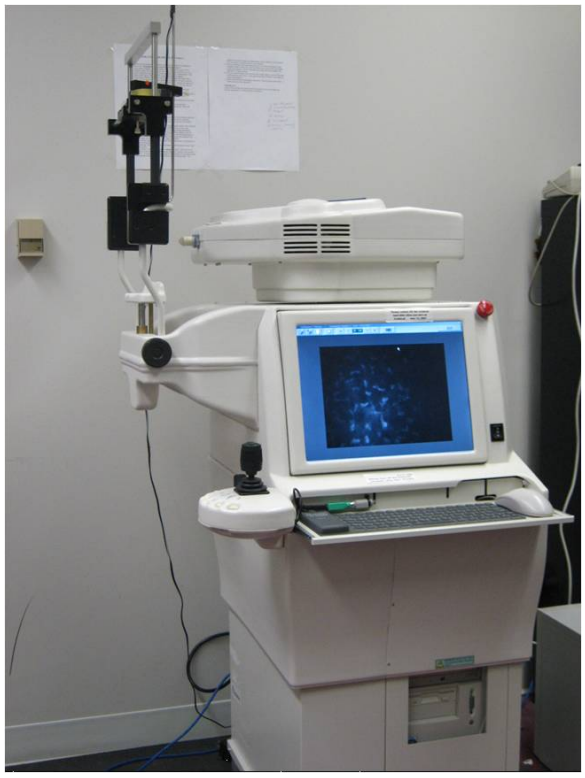


Figure 11: ConfoScan 3

Procedures

Prior to image acquisition, corneal integrity was determined for all participants. For this after instillation of sodium fluorescein into the lower fornix of participants, corneas of participants were examined using a slit lamp biomicroscope. Participants were then briefed on what to expect during the confocal microscopy procedure. The chin and forehead rests and the tip of the objective lens were wiped with 70% isopropyl alcohol swabs (Alcohol Swab, Becton Dickinson and Company, Oakville, ON, Canada). One drop of anaesthetic (Alcaine, proparacaine hydrochloride 0.5%, Alcon Canada Inc., Mississauga, Canada) was instilled into the lower fornix of each eye and after approximately 1 minute, participants typically indicated that their eyes felt numb. A drop of an ophthalmic gel (GenTeal, hypromellose lubricant 0.3% (w/v), Novartis Ophthalmics, Mississauga, ON, Canada) was placed on the tip of the objective lens and was used as a coupling medium between the cornea and objective lens. The chin and forehead of each participant was placed on the head rest of the CM

(Figure 12), and the device was advanced until the gel connected to the cornea. Measurements were taken on each eye at the corneal apex and on the temporal side close to the limbus. For the central measurements, participants were asked to look straight ahead. A height adjustable fixation target with a small, red light emitting diode (LED) was used on the non-imaged eye to control fixation. After imaging was completed, the objective lens was withdrawn, the fixation target was moved to 30 degrees temporally and the procedure was repeated to obtain images of the temporal cornea close to the limbus. The same process was then used for the other eye. The CM examination lasted between 5 – 15 minutes, with approximately 30 – 60 seconds per scan.

After completing the procedure, participants were asked to wait in the reception area until the anaesthetic had worn off completely. The cornea was then checked again with the slit lamp biomicroscope with and without sodium fluorescein.

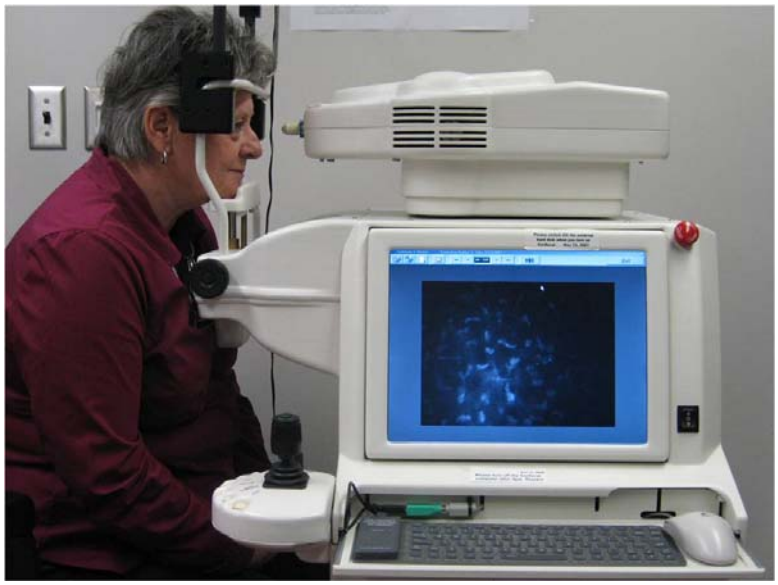


Figure 12: Positioning of the participants at the CM

Inclusion and exclusion criteria

For each of the experiments reported in this thesis, participants' eligibility was determined based on the following inclusion and exclusion criteria.

Inclusion criteria

A person was eligible if she or he:

1. Was correctable to a visual acuity of 6/9 (in each eye) or better with his or her habitual correction.
2. Was willing and able to maintain the visit schedule.
3. Was at least 17 years of age, falls within an age-matching range to the lens wearing group and had full legal capacity to volunteer.
4. Had read, understood and signed an information and consent letter.
5. Had astigmatism of ≤ 1.00 D cyl.
6. Had no active ocular disease.
7. Had normal binocular vision, i.e. no strabismus, no amblyopia.
8. Had no systemic disease affecting ocular health.
9. Was not using any systemic or topical medications that may affect ocular health, accommodative function, or the ocular physiological response to the contact lenses.
10. Had no known ocular or systemic allergies that could interfere with contact lens wear.
11. Had had an oculo-visual examination in the last two years.

Any additional inclusion or exclusion criteria specific to a certain experiment will be in the methods section of each respective experiment.

Exclusion criteria

A person was ineligible if she or he:

1. Had any active ocular disease.
2. Had any lid or conjunctival abnormalities or CS.
3. Was using a topical ocular prescription or any ocular topical over-the-counter medication.
4. Was a participant in any other clinical or research study.
5. Had had corneal refractive surgery and other ocular surgery.
6. Was aphakic.
7. Had known sensitivity to the diagnostic pharmaceuticals to be used in the study.
8. Had blepharitis.
9. Was pregnant, lactating or planning a pregnancy at the time of enrolment.
10. Had pinguecula/pterygium that, in the investigator's judgement, made contact lens wear inadvisable.

Diagnostic agents

The following diagnostic agents were used:

- Fluorets (sodium fluorescein sodium ophthalmic strips USP 0.25mg, Laboratoire Chauvin, France)
- Alcaine (proparacaine hydrochloride 0.5%, Alcon Canada Inc., Mississauga, Canada
- GenTeal (hypromellose lubricant 0.3% (w/v), Novartis Ophthalmics, Mississauga, ON, Canada)
- SoftWear Saline (sterile, isotonic saline solution containing an antimicrobial buffer system, consisting of sodium borate, boric acid and sodium perborate, generating up to 0.006%

hydrogen peroxide stabilized with phosphoric acid; CIBA Vision Canada Inc., Mississauga, ON, Canada)

Sodium fluorescein instillation

In order to be consistent throughout the experiments, the following procedure was used for sodium fluorescein instillation.

Two drops of saline were added to the tip of a fluoret. The fluoret was then vigorously shaken over a garbage bin in order to remove the excess. The participants were instructed to look up and away and that their lower eye lid would be gently touched. For insertion, the lower lid was gently pulled down and the flat side of the fluoret was placed onto the lower fornix just inside the lid margin. After this, the participants were asked to blink.

Grading of superficial cellular appearance

CM images of the superficial epithelium have a variety of different morphological appearances. The following grading scale (Figure 13) was developed to characterize the appearance of superficial cells.[282;283]

0 = indistictive cellular appearance

1 = Presence of cells with more prominent margins

2 = Presence of cells with prominent margins and contents

3 = Presence of hyper-reflective cells

The number of hyper-reflective cells in a given image was counted each time a grade of 3 (presence of hyper-reflective cells) was assigned. The number of hyper-reflective cells in each image were converted to number of cells per image area (cells/mm$^{2)}$. This cell density was calculated by dividing

the number of hyper-reflective cells in a given image by 0.1562, as the area of a CM image was measured to be 440x355 µm.

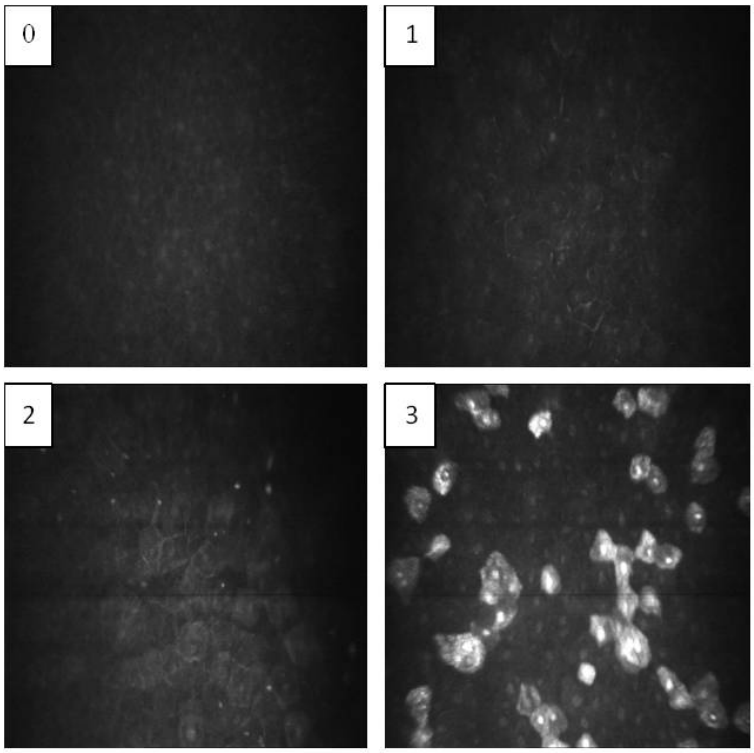


Figure 13: Examples for the different grades for superficial cellular appearance[282;283]

Grading of corneal staining (CS)

Any CS, reported during the different experiments for this thesis, was graded using the CCLR GSS (global CS score).[176;284] CS was recorded in the temporal, superior, nasal, inferior and central zones of both eyes after instillation of sodium fluorescein dye (Figure 14). The type of CS was then graded for each zone on a scale of 0 (none) to 100 (patch), and the extent was recorded as the percentage of the zones showing CS, also on a scale from 0 (no CS) to 100 (entire zone covered by CS. The ZSS (zone CS score) of each zone was then calculated (type x extent), resulting in a value in the range of 0 to 10,000. The sum of all five ZSS was divided by five to provide the GSS.

For statistical analysis in this thesis, if not stated otherwise, the GSS and the ZSS for central and temporal cornea were used.

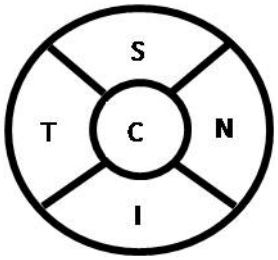


Figure 14: Corneal zones for Global Staining Score (GSS): C=central, I=inferior, S=superior, N=nasal and T=temporal

Image analysis

The previously described grading scale was developed to compare the different appearances of the superficial epithelium between various studies. However, a criticism of this descriptive grading scale is that it has not been validated. Another weak point of this grading scale is that it is subjective as it involves an experienced person to decide on a grade. Therefore, image analysis was used to objectively identify hyper-reflective superficial cells in CM images.

The content of a digital image is represented by an arranged assay of pixels and each of these pixels is associated with particular image information, such as brightness, grey tone levels, colour, etc. A histogram graphs the summary of the total pixel count of every possible value of brightness in an image. The tonal range of, for example, an 8 bit gray scale, ranges from 0 (black) to 255 (white). Image processing and especially analysis are commonly used to extract this numerical or graphical information from the characteristics of an image. [285] Hence, image analysis enables an objective quantification of elements or attributes of interest in an image, such as hyper-reflective cells, due to differences in brightness.

Image processing and analysis software

Image J 1.42p,[286] the public domain image processing and analysis software was used to analyze CM images obtained during the different experiments of this thesis.

Method for image analysis

If possible, for each participant, each time point/visit, and each corneal location one representative CM image of the superficial epithelium was chosen. Each image was then imported into and opened in Image J[286]. A median filter (low-pass filter) with a radius of 20 pixels was applied to each image to achieve a blurring effect and to remove cell edges and some noise in the image. Of particular interest was to be able to virtually remove bright cell nuclei and small reflection artefacts within an image. Images were then binarised (image is turned into an image that contains only black (0) and white (255) pixels) using the "Auto Local Threshold v1.0" plug-in. The "Bernsen"-method[287] with a radius of 15. Figure 15 illustrates the steps of image processing, for both images without and with hyper-reflective cells (1A-C and 2A-C, respectively).

For image analysis a region of interest (ROI) was chosen by manually placing and adjusting an elliptical measuring tool over the desired area. The desired area was determined by manually identifying the location and area of visible superficial epithelium in the original, unaltered image and choosing the same location and area in the processed imaged. Image analysis was then performed within the ROI using the following measures: area, mean gray value, and standard deviation.

Using linear transformation, the number (#) of white pixels (255) in the ROI (area) was then calculated from the measured mean gray value by applying the following formula:

$$\# = \frac{mean\ gray\ value * area}{255}$$

The coverage (%) of white pixels in the chosen ROI of each image was calculated and used to objectively quantify the number of hyper-reflective cells and will be referred to as "hyper-reflective cell area" in this thesis. Statistical analyses will be performed using this percentage value.

Statistical analyses were also performed and reported on the standard deviations of the untransformed gray values that were obtained by Image J.

Only data-sets of ideal images of the superficial epithelium were included in the image analyses, and partly because of this the image analyses sample sizes for the different experiments may vary. The sample sizes used for image analyses will therefore be explicitly reported in the result section of each experiment.

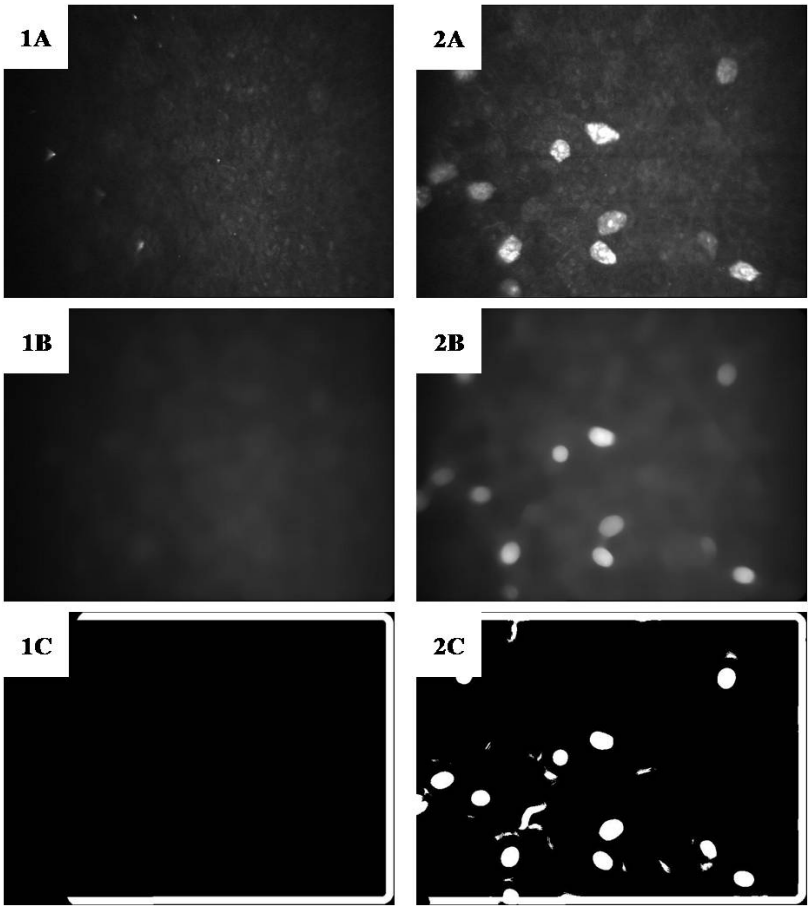


Figure 15: Examples of different steps of the image processing: 1A=original image of superficial epithelium without hyper-reflective cells, 1B=image after applying median filter, 1C=image after applying "Local AutoThreshold". 2A=original image of superficial epithelium with hyper-reflective cells, 2B=image after applying median filter, 2C=image after applying "Local Auto Threshold"

Contact lenses

Following is a list (Table 8) of the properties and specifications of all the various contact lenses that were used during various experiments in this thesis. It will be stated in the methods section of the respective experiment that involved contact lenses, which of these lenses were used. All lenses were fitted according to manufacturers' guidelines.

Table 8: Contact lens properties and specifications

Proprietary Name	**ACUVUE® OASYS™**	**ACUVUE® Advance™**	**O_2Optix™**	**PureVision™**
Referred to in thesis	Oasys	Advance	O_2Optix	PureVision
Manufacturer	Vistakon® Johnson & Johnson Vision Care Inc. Jacksonville, FL, USA	Vistakon® Johnson & Johnson Vision Care Inc. Jacksonville, FL, USA	CIBA Vision Corp., Duluth, GA, USA	Bausch & Lomb Inc., Rochester, NY, USA
Material (USAN)	senofilcon A	galyfilcon A	lotrafilcon B	balafilcon A
FDA classification	I	I	I	III
Health Canada licence #	67836	63133	35518	64120
EWC (%)	38%	47%	33%	36%
Dk/t (-3.00D)	147	86	138	101
BOZR (mm)	8.40	8.3, 8.7	8.4	8.6
Diameter (mm)	14.0	14.0	14.2	14.0
Sphere powers (D)	+8.00D to -12.00	+8.00D to -12.00	-1.00D to -6.00	+6.00D to -12.00

Contact lens solutions

Following is a list (Table 9) of the specifications of all the contact lens solutions that were used during various experiments in this thesis. It will be stated in the methods section of the respective experiment that involved contact lens solutions, which of these solutions were used.

Table 9: Contact lens solution specifications

Proprietary Name	**ReNu Multiplus® MPS**	**OPTI-FREE® RepleniSH® MPS**	**SoloCare Aqua™**	**Clear Care™**
Name referred to	ReNu MultiPlus	RepleniSH	SoloCare Aqua	Clear Care
Manufacturer	Bausch & Lomb Inc., Rochester, NY, USA	Alcon® Laboraties , Fort Worth, GA, USA	CIBA Vision Corp., Duluth, GA, USA	CIBA Vision Corp., Duluth, GA, USA
Health Canada #	02230538	0229767	02247116	02245661
Preservative and Disinfectant/ Cleaning agent	0.0001% DYMED (PHMB)	0.001% polyquaternium-1 (Polyquad®) 0.0005% MAPD (Aldox®)	0.0001% PHMB	3% Hydrogen Peroxide
Buffer	Boric acid, sodium borate	Boric acid	Tromethamine	Phosphate
Chelating agent	Hydroxyalkyl-phosphonate (Hydranate)	Citrate (citric acid)	0.025% EDTA	--
Surfactant / wetting agents	Poloxamine (Tetronic 1107)	TearGlyde™ (Poloxamine [Tetronic® 1304] + nonanoyl ethylene-diaminetriacetic acid [C-9 ED3A])	Dexpanthenol, Sorbitol, Pluronic F127	Pluronic 17R4
other component(s)		--	--	Sodium Chloride 0.79% (stabilized with phosphonic acid)

Statistical analysis

Statistical analyses were performed using Statistica 8 (StatSoft Inc., Tulsa, OK, USA) and InStat3 (GraphPad Software Inc., La Jolla, CA, USA). Graphs were plotted with Statistica 8 (StatSoft Inc., Tulsa, OK, USA), Excel (Microsoft Office XP and 2007, Microsoft Corp., Redmond, WA, USA) and R[288] (Department of Statistics and Mathematics, WU Wirtschaftsuniversitaet Wien, Vienna, Austria).

CS data and image analyses data were analyzed using parametric tests. Grading data were analyzed using non-parametric tests and counts of hyper-reflective cells were analyzed using chi square and non-parametric tests. The specific statistical tests used for the different experiments will be stated in the methods section of each experiment. The level of statistical significance was set to $p \leq 0.050$.
Unless reported differently, all error-bars shown in the graphs are 95% confidence intervals.

To put the obtained study results into perspective, sample size calculations for hyper-reflective cell count were performed using a web-based power calculator[289] and verified by GPower.[290] Therefore, for sample size calculations, the ratio of observations of hyper-reflective cells within the sample of each experiment was kept constant and a significance level of 0.05 and a power of 80% was aimed for.

Chapter 3

Non-CL Wearers

General Introduction

The superficial epithelium is the outermost layer of the corneal epithelium. Its cells are approximately 40 - 50 µm wide and 4 µm in depth and are mostly of hexagonal shape and firmly attached to each other.[40] In general, superficial cells do not show keratinisation and their flattened nuclei are smooth. A high amount of glycogen in larger and smaller granules can be found in wing cells and in the superficial epithelium. During hypoxia and wound healing the amount of glycogen drops.[53] Superficial cells of the corneal epithelium have finger-like projections of microvilli and also microplicae that are thought, particularly microvilli, to stabilize the precorneal tear film.[76;84] The cornea looses approximately 14% of its surface cells each day. Before this so called exfoliation, the nuclei of these surface cells condense and shrink and the connection to the underlying squamous layer loosens and the cells disintegrate or are swept away during the blinking process. With scanning microscopy,[85] "light" and "dark" cells with varying density have been observed and it has been suggested, that the light cells are the youngest of the superficial cells and have just arrived at the surface, whereas the dark cells are older and about to desquamate. It is also reported that darker cells have less surface features and are less rough.[40] Increased levels of hyper-reflectivity of superficial cells possibly indicating damaged or desquamating cells, have been reported.[243]

It has been proposed[40;291] that reflectivity of cells is based on the light scattering phenomenon and that several structures influence the interaction of the light beam and its transmission and absorption. These factors are cellular organelles and membranes, microplicae and microvillae as well as glycocalyx. It was hypothesized by Borchert et al.[272] that the presence of microdesmosomes in epithelial layers explains brighter illumination in cell membranes of epithelial cells.

Numerous CM studies have examined and described the corneal epithelial layers in normal and abnormal conditions.[48;86;87;156;208;216;243;292-294] Morphologic changes of the corneal epithelium, such as decreased cell density in basal and superficial cell layers and few bright reflective cells in the superficial layer have been shown with *in vivo* CM in subjects with symptoms of dry eyes.[243;278;295]

The experiments described in this chapter are attempts to monitor quantitatively the superficial epithelium of normal and younger people and of older (post-menopausal) people who have symptoms of dry eyes. The specific aim was to observe if hyper-reflective cells occur under normal physiological circumstances.

Superficial epithelium of younger non-contact lens wearers

In this experiment it was intended to investigate and characterize the appearance of the superficial epithelium over time of a non-contact lens wearing control group. Also of interest was if hyper-reflective cells would be present as a normal superficial epithelial feature.

Relevant data for this thesis section were collected during a study conducted at the CCLR and sponsored by CIBA Vision (CIBA Vision, Corp., Duluth, GA, USA).[296;297] Study participants consisted of 26 contact lens wearers and a control group of 12 age and gender matched non-contact lens wearers. As the focus of this experiment was the superficial epithelium of a non-contact lens wearing control group, only data from the control group of the initial study, will be discussed in this experiment.

Objectives

The specific objectives of this experiment were:

- To characterize the appearance of the superficial epithelium of normals/non-contact lens wearers over time.
- To observe if hyper-reflective superficial epithelial cells occur.

Methods

Participants

12 non-contact lens wearing participants enrolled in this experiment and informed consent was obtained prior to enrolment.

Study design and study visits

Ethics clearance was obtained from the Office of Research Ethics at the University of Waterloo, and informed consent obtained before enrolment. The study was a prospective nine-month clinical trial.

For this study, two separate visits were scheduled. An initial screening and baseline visit, followed by another visit nine months after the baseline visit.

At the screening and baseline visit, history and ocular health (anterior eye) assessment (including slit lamp biomicroscopy with sodium fluorescein) determined eligibility. CM was then performed using the procedures described in Chapter 2. At the nine month visit, ocular health was checked and CM images taken.

Procedures

At the baseline visit, CM was performed on both eyes at the corneal apex only, whereas at the nine month visit it was performed at the corneal apex and on the temporal side on both eyes.

CS was assessed using sodium fluorescein.

Grading and Analysis

Images of the superficial epithelium were identified and the appearance of the cells subjectively graded using the grading scale described in Chapter 2.

CS was graded according to the CCLR GSS (Chapter 2). All observed CS during this experiment was not graded by the thesis author, but by a different investigator.

Image analysis was performed as described in Chapter 2.

Statistical analysis

Image data were analyzed repeated measures ANOVA.

Results

Study participants

Eight female and four male participants were enrolled. The average age of the female participants was 33.25 ± 6.90 (range 24 to 47 years) and the average age of the male participants was 30.75 ± 11.9 (range 20 to 45 years). One participant was discontinued after baseline measurements as he moved away. Therefore, the results of 11 participants are reported. Table 10 shows some of the participants' characteristics.

Table 10: Participants' dioptric characteristics (mean ± SD)

		OD	OS
K-readings	Flat K	43.0 ± 1.4	42.9 ± 1.4
	Steep K	43.5 ± 1.5	43.5 ± 1.6
Corneal cylinder		-0.7 ± 0.3	-0.8 ± 0.3
Refractive error	Sphere	0.0 ± 0.6	0.1 ± 0.5
	Cylinder	-0.4 ± 0.4	-0.4 ± 0.4

Superficial cellular appearance and presence of hyper-reflective cells

Table 11 shows the superficial cellular appearance grade given for each participant at baseline (OD and OS at the corneal centre) and at the 9 month visit (OD and OS, central and peripheral cornea). Figure 16 shows the differences in superficial appearance grades for the central cornea between the base line and 9 month visit for each eye. As can be seen in both (Table 11 and Figure 16), even though grade 3 (presence of hyper-reflective cells) was given a few times at the 9 month visit, the appearance of superficial cells seems to not change over time in the normal superficial epithelium. This is confirmed by statistical analysis for the differences (9 months – baseline) in superficial appearances where no difference was found between right and left eye (Wilcoxon matched pairs $p>0.050$). For participants whose epithelium was graded as 3, the numbers of hyper-reflective cells (cells/mm^2) are listed in Table 12. Due to the small number of participants exhibiting hyper-reflective cells, statistical analysis between baseline and the 9 month visit was not performed.

Table 11: Superficial cellular appearance grade for each eye at baseline and the 9 month visit

	Baseline		9 Month			
	OD central	OS central	OD central	OD temporal	OS central	OS temporal
ID # 31	1	0	1	1	1	1
ID # 32	1	1	1	1	0	2
ID # 33	1	1	2	2	1	1
ID # 34	1	--*	1	0	1	1
ID # 35	1	0	2	3	2	3
ID # 36	2	1	1	3	2	1
ID # 37	1	1	2	1	3	2
ID # 38	0	0	2	1	1	2
ID # 39	1	1	1	1	1	2
ID # 40	1	1	1	1	1	2
ID # 42	--*	--*	1	0	0	3
Median	**1**	**1**	**1**	**1**	**1**	**2**
Min	**0**	**0**	**1**	**0**	**0**	**1**
Max	**2**	**1**	**2**	**3**	**3**	**3**

*no superficial appearance grade was given, as it was impossible to obtain images of the superficial epithelium

Table 12: Numbers of hyper-reflective cells (cells/mm^2) for each eye at baseline and the 9 month visit, when hyper-reflective cells were present (i.e. participants assigned an appearance grade 3 in Table 2)

	Baseline		9 Month			
	OD central	OS Central	OD central	OD temporal	OS central	OS temporal
ID # 31	0	0	0	0	0	0
ID # 32	0	0	0	0	0	0
ID # 33	0	0	0	0	0	0
ID # 34	0	--	0	0	0	0
ID # 35	0	0	0	26	0	19
ID # 36	0	0	0	6	0	0
ID # 37	0	0	0	0	26	0
ID # 38	0	0	0	0	0	0
ID # 39	0	0	0	0	0	0
ID # 40	0	0	0	0	0	0
ID # 42	--	--	0	0	0	6
Sum	**0**	**0**	**0**	**32**	**26**	**23**
Median	**0**	**0**	**0**	**0**	**0**	**0**
Min	**0**	**0**	**0**	**0**	**0**	**0**
Max	**0**	**0**	**0**	**26**	**26**	**19**

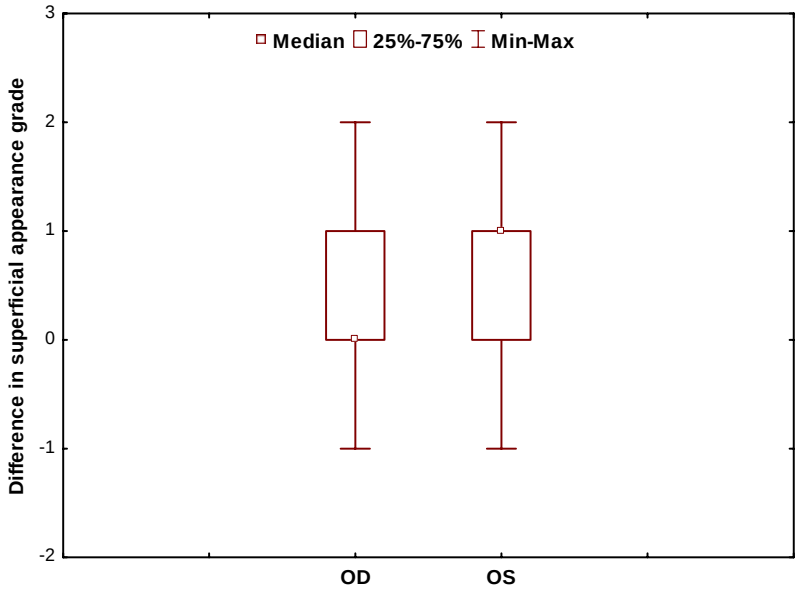


Figure 16: Differences in superficial appearance grade for the central cornea between the baseline and 9 month visit, separately for OD and OS

Figure 17 is an example of the superficial epithelium (OD) of a study participant. Image A was obtained at the baseline visit and image B at the nine months visit. As can be seen the cells look similar in both images and were graded as 1 (presence of cells with more prominent margins).

Corneal staining (CS)

Table 13 lists the CS scores acquired for baseline and after nine months. For each eye and each visit (averaged over participants respectively) the GSS (mean ± SD) and the ranges of CS as well as the CS score (mean ± SD) and ranges of CS for the different corneal quadrants are shown. As can be seen no CS was observed for the temporal quadrants for both eyes and for both time points. The CS that was present in the other corneal quadrants was clinically insignificant and statistical analyses were not performed.

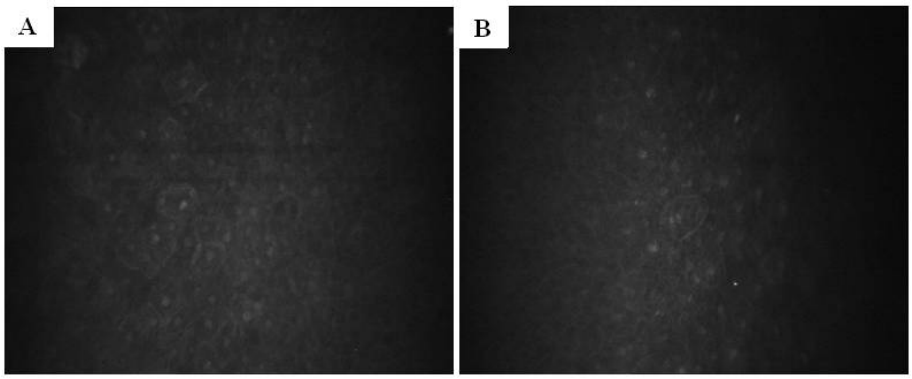


Figure 17: Example of images of the central superficial epithelium (OD) of one participant (ID#32) at the baseline visit (A) and after 9 month (B)

Table 13: Corneal staining (mean ± SD, range) for each eye at baseline and the 9 month visit

	Baseline (mean ± SD) (range)		9 months (mean ± SD) (range)	
	OD	**OS**	**OD**	**OS**
GSS (sum of 5 zones/5)	11 ± 18 0 - 60	10 ± 14 0 - 40	5 ± 8 0 - 24	14 ± 28 0 - 90
Temporal	0 ± 0 0 - 0	0 ± 0 0 - 0	0 ± 0 0 - 0	0 ± 0 0 – 0
Superior	0 ± 0 0 - 0	7 ± 23 0 - 75	6 ± 14 0 - 40	0 ± 0 0 – 0
Nasal	4 ± 12 0 - 40	1 ± 5 0 - 15	3 ± 9 0 - 30	26 ± 75 0 – 250
Inferior	12 ± 33 0 - 100	18 ± 38 0 - 120	36 ± 63 0 - 200	40 ± 80 0 – 200
Central	27 ± 90 0 - 300	0 ± 0 0 - 0	3 ± 9 0 - 30	3 ± 9 0 - 30

Image analysis

Complete data sets were available for seven participants and therefore statistical analysis for the image analysis part was performed on a sample size of 7.

No statistical significance (RmANOVA p=0.312) in hyper-reflective cell areas was found between OD and OS. Also no statistical significant difference (RmANOVA p=0.542) in hyper-reflective cell areas of the images was noticed between baseline and 9 months (OD and OS combined). The interaction between eyes and visits in hyper-reflective cell areas was also not significant (RmANOVA p=0.786, Figure 18).

Figure 19 shows that the standard deviations of images of the superficial epithelium were not significantly different (RmANOVA p=0.190) between eyes at baseline and 9 months.

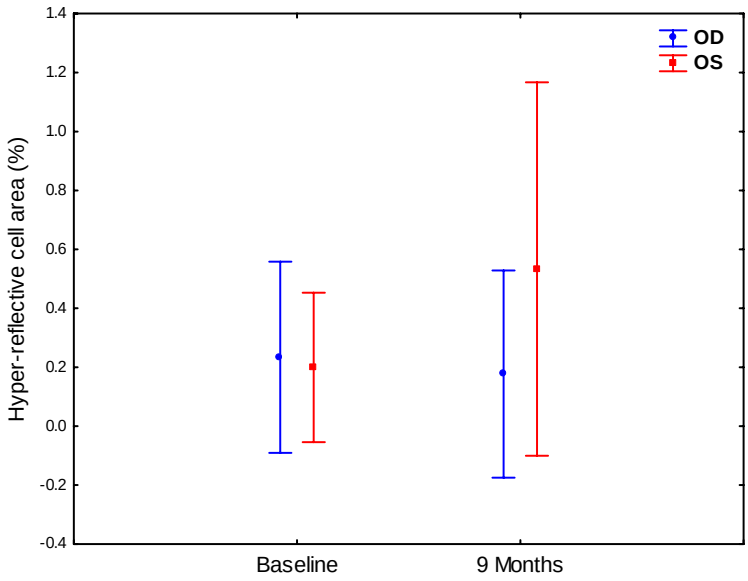


Figure 18: Hyper-reflective cell areas (%) of images of the central superficial epithelium (OD and OS separately) of normal, non-contact lens wearing participants at baseline and 9 months

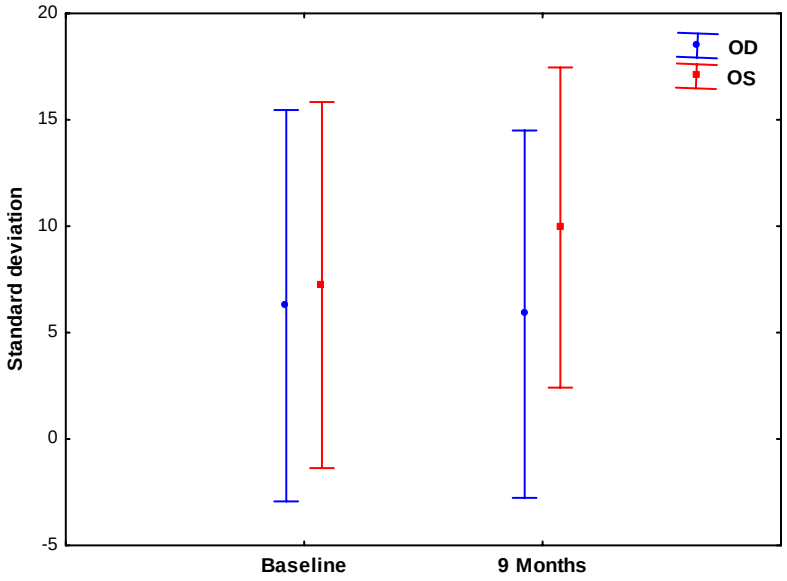


Figure 19: Mean standard deviations of images of the superficical epithelium of normal, non-contact lens wearing participants at baseline and 9 months

Conclusion

Superficial cellular appearance was similar at baseline and after nine months, suggesting that superficial cellular appearance does not change over time.

Very few random hyper-reflective or brighter cells were observed. For these participants who had hyper-reflective cells, there was no temporal CS and clinically insignificant central CS.

No differences in objective measures of superficial epithelial images over time were found.

Superficial epithelium of dry-eyed postmenopausal non-contact lens wearing women

The previous experiment showed that the appearance of the superficial epithelium of a non-contact lens wearing control group does not change over time and that hyper-reflective cells are not a habitual observation. The participants who were enrolled in the previous experiment were in general younger. Therefore the present experiment aimed to examine if age had an effect on the appearance of the superficial epithelium of non-contact lens wearers. Additionally to age, dry-eye symptom was also investigated to see if they are grouping for hyper-reflective cells. Dry-eye symptoms were specifically chosen as a predicting variable as it is mentioned in the literature to be associated with hyper-reflective cells.[278]

Relevant data for this experiment were obtained from a study which aimed to correlate various clinical and morphological changes found in participants symptomatic of dry eye with tear film and ocular surface biomarkers in post-menopausal women.

Objectives

The specific objectives of this experiment were:

- To investigate the appearance of the superficial epithelium of dry-eye symptomatic and asymptomatic postmenopausal women.
- To investigate if age has an effect on the appearance of the superficial epithelium.
- To observe if hyper-reflective cells occur.

Methods

Study participants

18 female participants were enrolled into the dry eye symptomatic (test) group (moderate to severe symptoms) determined using OSDI (ocular surface disease index) score[298] and 18 female participants

were enrolled into the asymptomatic (control) group. All participants were Caucasian and non-contact lens wearer. Informed consent was obtained prior to enrolment.

Study specific inclusion & exclusion criteria

A person was eligible for inclusion in the study if she:

1. Had moderate or severe dry eye symptoms based on the dry eye questionnaire and half of the time felt they needed to use eye drops for dry eye symptoms (dry eye group)
2. Was post-menopausal.

A person was excluded from the study if she:

1. Was on hormone replacement therapy (HRT).
2. Had ceased menses due to autoimmune disorders, mumps, chemotherapy, pelvic irradiation or smoking.
3. Had rheumatoid arthritis, diabetes, Sjogren's syndrome or any other systemic disease affecting ocular health.
4. Was using any systemic or topical medications (other than eye drops for dry eye symptoms) that may affect ocular health and neuro-endocrine system function.

Study design and study visits

Ethics clearance was obtained from the Office of Research Ethics at the University of Waterloo.

Data and observations were collected at one scheduled appointment (screening combined with the study visit).

Participants' eligibility was based on ocular history and symptoms of dry eye and tear film evaluation. Case history, dry eye questionnaire and analogue scales, ocular surface assessment and tear film evaluation (non-invasive tear break up time and phenol red thread test) were also carried out to determine participant eligibility. Before the study appointment, participants were asked to cease any use of rewetting drops for at least 24 hours.

Procedures

Ocular surface CS was evaluated using sodium fluorescein dye and was graded using the CCLR GSS, as described in Chapter 2.

CM was performed according to the procedures illustrated in Chapter 2, on the corneal apex of one randomly selected eye.

Grading and Analysis

Images of the superficial epithelium were identified and the appearance of the cells subjectively graded using the grading scale described in Chapter 2.

CS was graded according to the CCLR GSS (Chapter 2). Approximately 40% of the observed CS during this experiment was graded by the thesis author; the remainders were graded by two other investigators as the images were being acquired. For this reason, the grading was not masked.

Image analysis was performed as described in Chapter 2.

Statistical analysis

CS and image analysis data were analyzed using a t-test for independent groups (Student t-test).

Results

Participants

The mean age of the 18 participants in the test group was 63.4 years (median 61.5 years, range 48 to 78 years). The mean age of the 18 participants in the control group was 63.2 years (median 62.5 years, range 55 to 75 years).

Superficial cellular appearance and presence of hyper-reflective cells

Table 14 lists the mean, minimum and maximum grades given for the dry eye symptomatic and the asymptomatic group. As can be seen grade 3 (presence of hyper-reflective cells) was only given in the dry eye group. Only three participants manifest hyper-reflective or bright cells. The distributions of the grades given for cellular appearance for each group (test and control) are shown in Figure 20.

Table 14: Superficial cellular appearance grades for the dry eye and the non dry eye group

	Dry eye symptomatic group	Asymptomatic group
Median	1	1
Min	0	0
Max	3	2

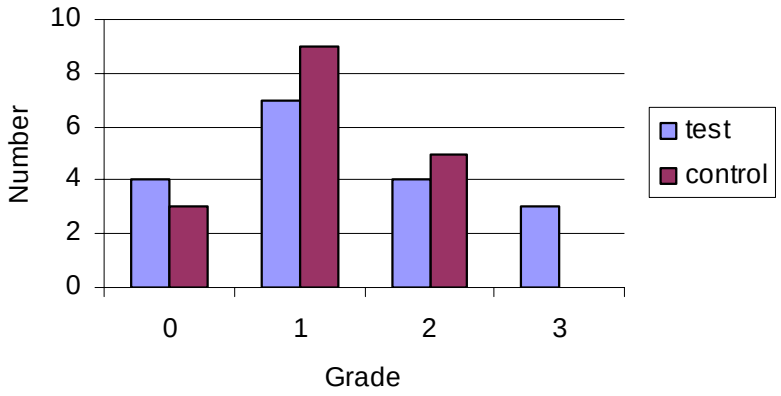


Figure 20: Superficial cellular appearance grade

For the three participants who were given a grade 3, the numbers of hyper-reflective cells (cells/mm^2) was calculated and the numbers listed in Table 15. As seen in this table, the number of hyper-reflective cells was small.

Table 15: Numbers of hyper-reflective cells (cells/mm^2) in participants receiving grade 3 for superficial cellular appearance

Participant ID	Numbers of hyper-reflective cells (cells/mm^2
7	13
12	19
18	13

Figure 21 gives an example of the superficial epithelium of (A) a dry eye symptomatic participant (ID#4) and (B) an asymptomatic participant (ID#33). The appearance of the superficial cells was graded as grade 1 (presence of cells with more prominent margins) for in both images.

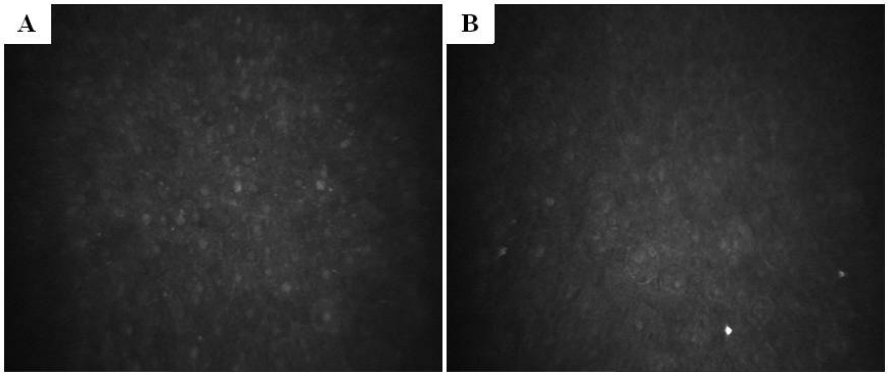


Figure 21: Examples of the superficial epithelium for a symptomatic dry eye participant (A) and an asymptomatic participant (B)

Corneal staining (CS)

The CS results are based on the sum of the CS scores (two eyes) for each study participant. The GSS and the ZSS (mean ± SD and range) found with sodium fluorescein are listed in Table 16. The total CS score was significantly higher (Student t-test p=0.041) in participants in the test group than those in the control group.

Table 16: Corneal staining (mean ± SD, range) for the test (dry eye symptomatic) and the control (asymptomatic) groups

	Test (n=18) (mean ± SD) (Range)	Control (n=18) (mean ± SD) (Range)
Total CS score (sum of 5 zones, 2 eyes)	1840 ± 2567 0 - 10250	569 ± 832 0 – 3175
Temporal	285 ± 506 0 - 2000	68 ± 125 0 – 425
Superior	321 ± 522 0 - 2000	86 ± 180 0 – 625
Nasal	306 ± 526 0 - 2000	132 ± 378 0 – 1300
Inferior	747 ± 785 0 - 2500	214 ± 261 0 – 750
Central	182 ± 480 0 - 2000	69 ± 247 0 - 1050

The CS scores for the central quadrant of the three participants who had hyper-reflective cells are listed in the following table (Table 17). It can be seen, that for ID#12 and 18 no central CS was recorded and for ID#7 the score was very low (25 out of 10000).

Table 17: Corneal staining scores for the central cornea and number of hyper-reflective cells, of the three participants exhibiting hyper-reflective cells

ID	CS score (centre)	Number of hyper-reflective cells
7	25	2
12	0	3
18	0	2

Image analysis

There was no statistically significant difference (Student t-test p=0.072) in hyper-reflective cell areas between the two groups (Figure 22). However, it can be seen that the range in hyper-reflective cell areas was larger in the dry eye symptomatic group than the range n the asymptomatic group, suggesting that some images in the dry eye group contained a higher number of white objects (cells or artefacts) or that the images were brighter on average in some. The standard deviations of the dry eye symptomatic and asymptomatic group are shown in Figure 23. No statistically significant difference

(Student t-test p=0.238) was found, but again the range is bigger in the symptomatic dry eye group compared to the asymptomatic group.

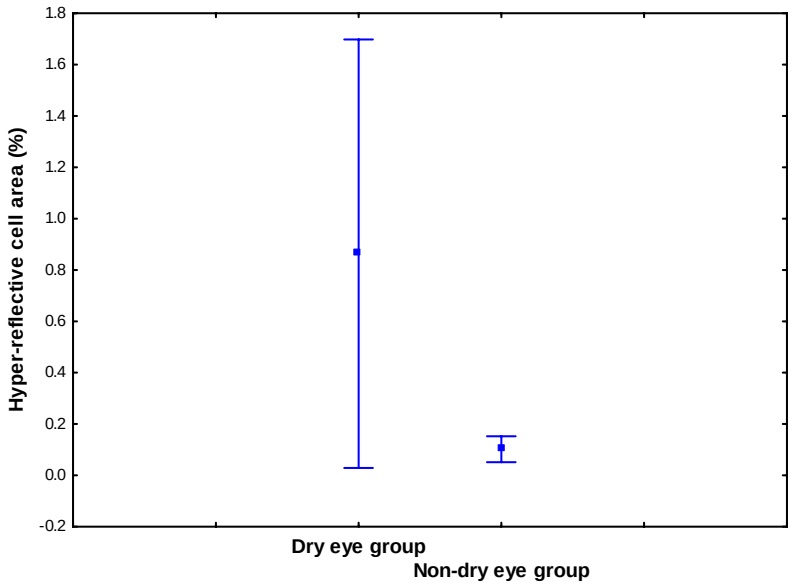


Figure 22: Hyper-reflective cell areas (%) for symptomatic dry eye and asymptomatic group

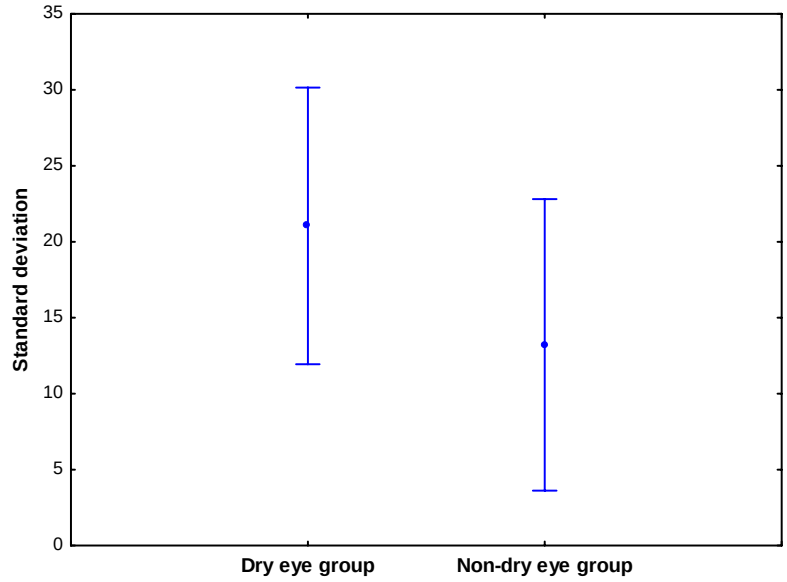


Figure 23: Standard deviation for symptomatic dry eye and asymptomatic group

Conclusion

No difference was found in superficial cellular appearance between the dry eye symptomatic and the asymptomatic group (median grade was 1). However, in three participants of the dry eye symptomatic group, small numbers of hyper-reflective or bright cells were present. No or clinically insignificant CS was reported for these three participants, suggesting, that the occurrence of these hyper-reflective cells may be associated with dry eye or with normal cell turnover, e.g. desquamated cells[243], and not linked to CS. Generally, statistically significant higher amounts of CS were observed in the dry eye symptomatic group when compared to the asymptomatic group.

Image analysis showed that that there was no difference between images of dry eye symptomatic and asymptomatic participants.

General Discussion

The two experiments in this chapter intended to define quantitatively the superficial epithelium of normal and postmenopausal dry eye symptomatic and asymptomatic participants.

Superficial epithelium of younger, non-contact lens wearers

The superficial epithelium of young, non-contact lens wearers was observed over time. CM images obtained of the superficial cells in this present experiment appear to be similar to the descriptions and images from previous CM studies of the normal superficial epithelium.[48;86;87;208;216] The central superficial cellular appearance at baseline was similar to the appearance of the cells nine months later, suggesting that the superficial cells to not change in appearance over time. The superficial cellular appearance of central and temporal cornea at the nine month visit was similar. No statistical analysis was performed, as the eleven participants enrolled into this study received similar grades for the superficial cellular appearance and therefore there was no or very small variance.

Small numbers of hyper-reflective or bright cells were seen in a few participants at the nine month visit only. Interestingly, one participant had hyper-reflective cells in both eyes at the temporal position, whereas for the others hyper-reflective cells only occurred in one eye and also in one position. These rare observations of hyper-reflective cells in this sample (n=11) suggest that hyper-

reflective cells are not normally observed in the normal superficial epithelium. For the difference in proportions (0/11 and 2/11) of observed hyper-reflective cells to be significant at 80% power and with a 0.05 significant level an overall sample size of 38 participants would be required.

No CS was observed in the temporal cornea and the reported central CS was clinically insignificant. This finding supports other reports that the prevalence of CS in normal, non-contact lens wearing people is low.[56;299;300] Therefore, the presence of these hyper-reflective cells that were observed in these participants does not appear to be associated with CS. Their appearance could be due to be a normal process of the turnover of the epithelium and the reported differences in brightness of superficial cells that are about to be desquamated.[85;243]

A critique of the grading scale used to compare the cellular appearance is that the differences between grade 0 (indistinctive cellular appearance), grade 1 (presence of cells with more prominent margins) and a grade 2 (presence of cells with prominent margins and contents) is just an attempt to describe the appearance of the cells. If this was actually a worsening or change of cellular appearance towards hyper-reflective cells is not confirmed. However, the step to grade 3 (presence of hyper-reflective cells) may be more meaningful. Another weak point of this grading scale is that it is subjective. The objective quantification of images of superficial epithelium was developed and it was anticipated that the presence of hyper-reflective cells in an image could be measured as an increase in image brightness and variance. In order to obtain representative results with image processing and analysis, ideal images of the superficial epithelium had to be selected. A representative image was determined by the clear presence of superficial epithelial cells in the majority of the image. However, this was not always possible for each participant, time point and, corneal location. Only seven complete data sets (n=7) with ideal superficial epithelial images were used for image analysis for the experiment quantifying the superficial epithelium of normal, non-contact lens wearing participants. Image analysis revealed that no difference in image brightness occurred over time (9 months). Also image brightness of the right and left eye was not different. This finding is in agreement with the subjective grading of the superficial epithelium of normal, non-contact lens wearers, where no difference over time and between eyes was noted. The measured hyper-reflective cell areas were close to 0 for all the visits, as well as for right and left eyes. This indicates that the measured areas contained mainly the acellular appearing black background. However, the standard deviations suggest that some small,

white areas may have been present after the image processing procedure or that some images were brighter on average.

Superficial epithelium of dry-eyed postmenopausal women

The second report in this chapter was of superficial cellular appearance in a group of symptomatic dry eye post-menopausal women and a group of asymptomatic post-menopausal women.

CM was performed on the central cornea of one randomly assigned eye. No difference in superficial cellular appearance was found between the two groups and their appearance is similar to previous descriptions of superficial cells imaged using CM.[48;87;208;216] Small numbers of hyper-reflective cells were observed in three participants in the dry eye group. This reinforces statements made in previous reports of highly reflective superficial cells in dry eyed people.[243;278;295] Benitez-del-Castillo et al.[278] also made the qualitative observation that basal epithelial reflectivity was observed only in dry eyes. This present experiment consisted of 18 symptomatic dry eye participants and 18 asymptomatic participants and there was no difference in observations of hyper-reflective cells between the two groups. For the difference in proportions (3/18 and 0/18) of observed hyper-reflective cells to be significant at 80% power and with a 0.05 significant level a sample size of 44 participants in each group would be required.

Significantly more CS was observed in the symptomatic group compared to the asymptomatic group. This finding would support the suggestion that staining should be one of the methods to diagnose dry eye disease.[298] However, no or clinically insignificant CS was observed when looking specifically at the three participants who had hyper-reflective cells. This would suggest CS is not associated with their appearance. Again, the presence of these few cells could be explained by the normal shedding mechanism of the cornea.

Image processing and analysis did not show differences between the hyper-reflective cell areas of the groups. This supports the grading of the appearance of the superficial epithelium where no statistical significant difference between the two groups was found. The higher standard deviations measured in the dry eye symptomatic group, implies that some images in this group contained variability in image brightness. If some hyper-reflective cells were present in some images there would be increased image brightness (Figure 22). This suggestion is supported by the observation that for each of the

three participants in the dry eye symptomatic group a small number of hyper-reflective cells were present (Table 15 and Figure 20).

Miscellaneous

A limitation of CM is that it is not known if the same corneal position is re-inspected, especially when comparing conditions over time. This difficulty of finding the same corneal position is due to the normal corneal epithelium not having detectable landmarks and the small field of view of the CM. An attempt was made to minimize discrepancy of positioning of the confocal objective lens on the cornea between measurements, by using an adjustable fixation target. Participants were asked to fixate onto this fixation target with the eye that did not undergo CM.

It may also be argued that the use of sodium fluorescein prior to CM and the application of any ophthalmic solution onto the cornea, such as the anaesthetics used during the CM procedure, may have had an effect on the appearance of the superficial cells. The effect of this was part of another experiment and will be addressed and discussed in Chapter 5.

In conclusion, the results obtained during the experiments of this chapter indicated that hyper-reflective cells are not usually observed in the normal superficial epithelium of non-contact lens wearer as well as not in post-menopausal dry eye symptomatic and asymptomatic women.

The focus of the following chapter is to investigate if contact lenses, contact lens solutions or combinations of lenses and solutions will have an effect on the presence of hyper-reflective cells.

Chapter 4

Effect of Contact Lenses and Solutions

General Introduction

The hyper-reflective cells reported in the Master's thesis by Harvey[1] suggested that the presence of these cells may be associated with the presence of SICS caused by specific lens/solution combinations.

The interaction of certain lens/solution combinations is linked to SICS.[29-31;125;178;179;182;184;187;189] PureVision lenses (Bausch & Lomb) when used with ReNu MultiPlus (Bausch & Lomb) have been stated as the combination to produce the highest amounts of SICS.[29;31;178] The manifestation of SICS is generally asymptomatic,[29;30] suggesting that the increased amount of SICS is so superficial that it is clinically irrelevant. *In vivo* CM has revealed that contact lens wear does affect the cornea at a cellular level.[86;156] Harvey's[1] findings seem to add to this observations, but hyper-reflective superficial cells in association with SICS have not been mentioned in the literature.

The purpose of the experiments described in this chapter was to see if Harvey's[1] observation of hyper-reflective cells is reproducible and to investigate what contact lens/solution combinations are associated with hyper-reflective cells. Another aim was to examine if applying solutions by themselves onto the cornea and if exposing the cornea to the prolonged use of the same contact lens/solution combination are associated with hyper-reflective cells.

Effect of lens/solution combinations on the superficial corneal epithelium 1

Having quantified and characterized the normal appearance of the superficial epithelium of non-contact lens wearers and having established that age and dry-eye symptom are not predicting variables for hyper-reflective cells; this experiment was designed to investigate the effect of different lens/solution combinations on the superficial cellular appearance. In particular, would the lens/solution combination be a predicting variables for hyper-reflective cell appearance was of

interest for this thesis, as Harvey[1] had suggested that certain lens/solution combinations may be associated with the presence of hyper-reflective cells.

Relevant data for this thesis were collected as part of a study conducted at the CCLR. The purpose of the experiment was to compare the effect of an investigational PHMB-based MPS (test) to the polyquad-based RepleniSH (Alcon, control). The two solutions were used in combinations with four marketed SiHy lenses: PureVision (Bausch & Lomb), O2Optix (CIBA Vision) Acuvue Advance (Advance) and Acuvue Oasys (Oasys, Johnson & Johnson, respectively).

Objectives

The specific objectives of this experiment were:

- To observe if hyper-reflective superficial epithelial cells occur with the different lens/solution combinations.
- To compare the appearance of central and temporal superficial epithelium.

Methods

Participants

The aim was to recruit up to 120 adapted soft contact lens wearers. Participants had be adapted soft lens wearers who currently wore two-week or monthly replacement lenses. Daily disposable lens wearers were not eligible. There were no specific requirements regarding previous lens material or care products. Any participant who habitually used rewetting drops was asked to discontinue use during the study.

Study specific inclusion & exclusion criteria

A person was eligible if he/she:

1. Was an adapted soft contact lens wearer.

2. Had normal binocular vision (no strabismus, no amblyopia, and anisometropia less than or equal to 1.00 D).

3. Had a distance contact lens prescription between +6.00D to -8.00 DS.

4. Agreed to wear the study lenses on a daily wear basis and not use any lens rewetting drops.

A person was ineligible if he/she:

1. Had a difference in biomicroscopy grading between the two eyes of greater than 1.0 on a 0.0 to 4.0 scale at the baseline visit.

2. Currently wore daily disposable contact lenses.

3. Currently wore lenses on a continuous or extended wear basis.

4. Had any ocular pathology or severe insufficiency of lacrimal secretion (dry eyes) that would have affected the wearing of contact lenses.

5. Had a pinguecula/pterygium that, in the investigator's judgment, made contact lens wear inadvisable.

6. Had corneal distortion resulting from rigid lens wear

Study solutions

Participants were instructed to clean and disinfect their lenses using either the test solution (PHMB-based) or the polyquad-based control solution (RepleniSH). For details of the control solution, the reader is referred to Table 9 in Chapter 2 (General Methods). Details of the test solution are shown in Table 18. Each participant used each study solution in the right or left eye, according to the randomization table. The investigators were masked as to the type of solution being used in each eye (single-masked). Participant compliance with the lens care instructions was checked at the final appointment. To avoid lenses being switched and to prevent potential incompatibility of the care regimen with the lens case polymer each participant used a Microblock™ (CIBA Vision) case for the test solution and an Alcon OptiFree case for the RepleniSH solution (i.e. the participant used two separate cases, one for each eye).

Table 18: Test solution details

Identifier	Test solution
Drug identification #	--
Preservative and disinfectant/ cleaning agent	0.0001 % PHMB
Buffer	No disclosure
Chelating agent	No disclosure
Surfactant/wetting agent	No disclosure

Study lenses

Four marketed SiHy lenses were used in this study and participants were randomly assigned to one of the four lens designs according to a randomization table. The four lens designs were: Advance, Oasys, PureVision and O2Optix. For lens details and specifications, the reader is referred to Table 8 in Chapter 2 (General Methods).

Study design

Ethics clearance was obtained from the Office of Research Ethics at the University of Waterloo prior to commencement of the study. Health Canada provided approval for the use of the investigational test solution and for RepleniSH as this solution, at the time of the study, had not been launched in Canada. Informed consent was obtained from all participants prior to enrolment in the study. Figure 24 outlines the study design. This study was conducted as a two-week prospective contralateral eye clinical trial, using a randomized design. The study consisted of four groups of at least 25 participants, (up to a total maximum of 120), each group wearing one of four SiHy lens brands bilaterally: PureVision, O_2Optix, Advance or Oasys (assigned randomly).

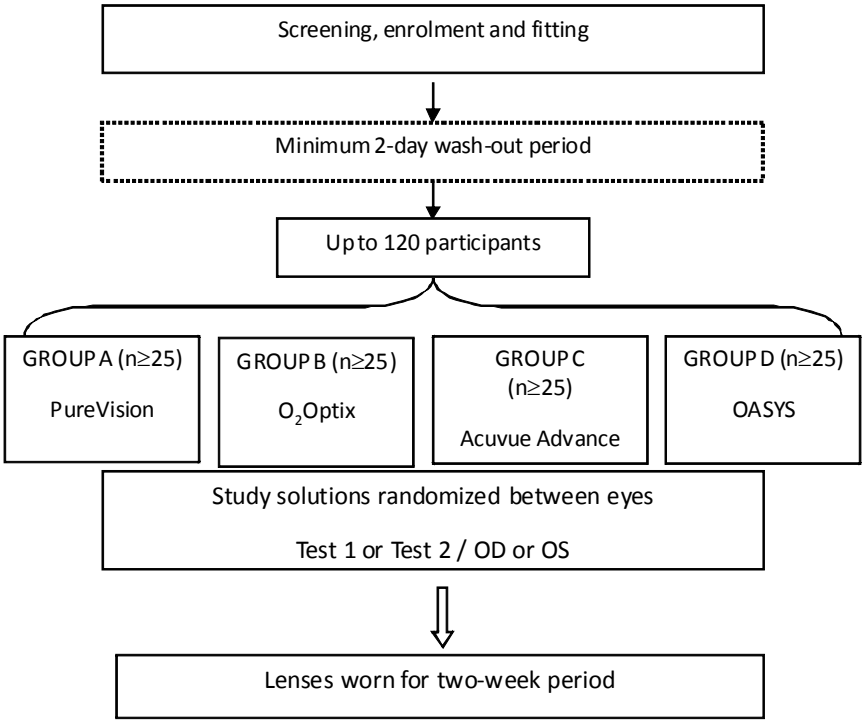


Figure 24: Study design

Procedure for assigning participants to treatment groups and randomization

As participants were recruited, they were randomly assigned to lens type, solution to be used for each eye, and order in which CM and the other clinical procedures were conducted at the study visits. Once randomized, the procedure to be conducted first at each clinical visit for each participant remained fixed (i.e. if CM was conducted on the day before dispensing and the other procedure was conducted at the dispensing visit, then CM was conducted on day 13 and the other procedure on day 14).

Study visits

Following an initial one-hour screening and fitting visit, four appointments were scheduled for each participant, including (1) pre-dispensing visit, (2) dispensing visit, (3) follow-up visit Day 13, and (4) follow-up visit Day 14 (a total of 5 study visits per participant).

Informed consent was obtained prior to the screening visit. At the initial screening and fitting appointment participants' eligibility was confirmed. At this visit, the study lenses that had been

randomly assigned to the participant were examined to ensure that an appropriate fit could be achieved.

On the day before the dispensing visit (pre-dispensing visit, conducted after a minimum two-day washout period during which participants wore their habitual spectacles), either CM or the other procedures were performed (as determined by the randomization schedule), followed on the next day (dispensing visit) by the remaining assessment. At the 13-day (± 3) and 14-day (± 3) visits, CM or the other procedures were performed again, in the sequence specified by the randomization table. At the final 14-day visit, all lenses were collected and the participants returned all study products.

CS using sodium fluorescein was assessed and recorded prior to CM for all study visits.

Procedures

Ocular surface staining was evaluated using sodium fluorescein. CM was performed according to the procedures illustrated in Chapter 2.

Grading and Analysis

Images of the superficial epithelium were identified and the appearance of the cells subjectively graded using the grading scale described in Chapter 2.

CS was graded according to the CCLR GSS (Chapter 2). Approximately 30% of the observed CS during this experiment was graded by the thesis author; the rest was graded by two different investigators.

Image analysis was performed as described in Chapter 2.

Statistical analysis

Hyper-reflective cell counts were analyzed using a Fisher's exact test (chi-square 2x2 contingency table). Superficial cellular appearance was analyzed using Wilcoxon-matched pairs test. CS and image analysis data were analyzed using repeated measures ANOVA.

Results

Participants

One hundred participants completed the study (87 female, 13 male). The mean age of the participants was 24 years (median 23 years, range 18 to 51 years).

Superficial cellular appearance and presence of hyper-reflective cells

Table 19 lists the median and range of superficial cellular appearance grades given for the different lens/solution combinations over time and for the central and temporal cornea. It can be seen that the appearance of the superficial cells for the test solution (in combination with all the lenses) at Day 14 was graded higher when compared to baseline. The grades given for the control solution (RepleniSH) were similar for both baseline and Day 14.

Differences in superficial appearance grade (grade for test solution minus grade for RepleniSH) at baseline and at Day 14 were calculated for each lens and position. Statistical analyses were then performed using Wilcoxon-matched pair test for the differences in grades found for each lens. The differences in superficial appearance (central) between test solution and RepleniSH were statistically significant higher at Day 14 when compared to baseline for PureVision ($p=0.006$, Figure 25), Oasys ($p<0.001$, Figure 27) and Advance ($p<0.001$, Figure 28). Only O_2Optix did not show statistical significant differences in superficial appearance ($p=0.828$, Figure 26). For the temporal cornea the differences in superficial appearance were statistically significant higher at Day 14 compared to baseline for Oasys, PureVision and Advance ($p<0.001$, $p=0.010$ and $p<0.001$, respectively). There was no statistical significant difference ($p=0.955$) in superficial appearance between baseline and Day 14 for O_2Optix.

Table 19: Superficial cellular appearance grades over time for the different lens/solution combinations and corneal positions* [α]

	Baseline				Day 14			
	Test		RepleniSH		Test		RepleniSH	
	C	T	C	T	C	T	C	T
Advance [α]								
Median	1	2	1	2	3	3	1	2
Min	0	0	0	0	1	1	0	1
Max	3	3	3	3	3	3	3	3
O2Optix								
Median	1	1	1	1	2	2	1	2
Min	0	1	0	0	0	0	0	0
Max	2	3	3	3	3	3	3	3
Oasys [α]								
Median	1	2	1	2	3	3	1	2
Min	0	1	0	0	1	1	0	0
Max	3	3	3	3	3	3	3	3
PureVision [α]								
Median	1	1	1	1	2	3	1	2
Min	0	0	0	0	1	1	0	1
Max	3	3	2	2	3	3	3	3

* C = central, T = temporal

[α] Statistically significant ($p<0.050$) difference (test-RepleniSH) in superficial cellular appearance Baseline and Day 14 for central and temporal

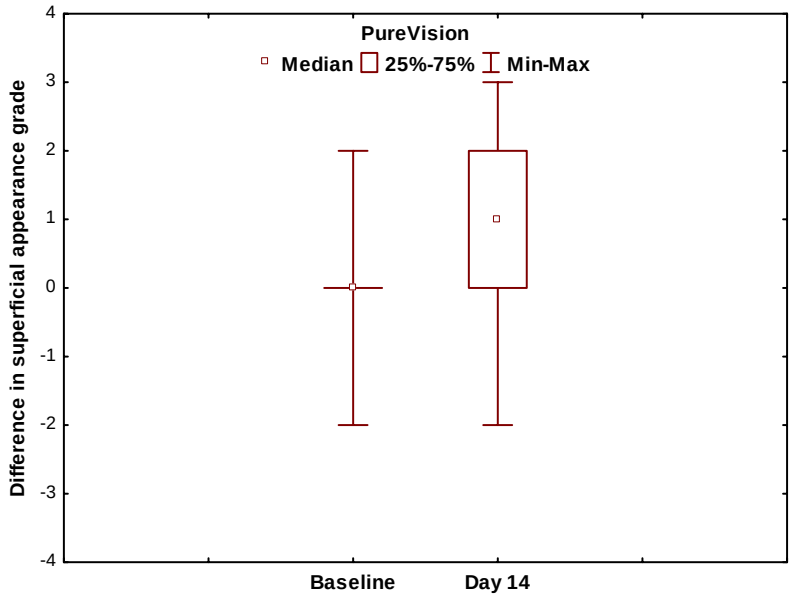


Figure 25: Differences in superficial appearance grade (central) between test solution and RepleniSH at baseline and Day 14 (PureVision)

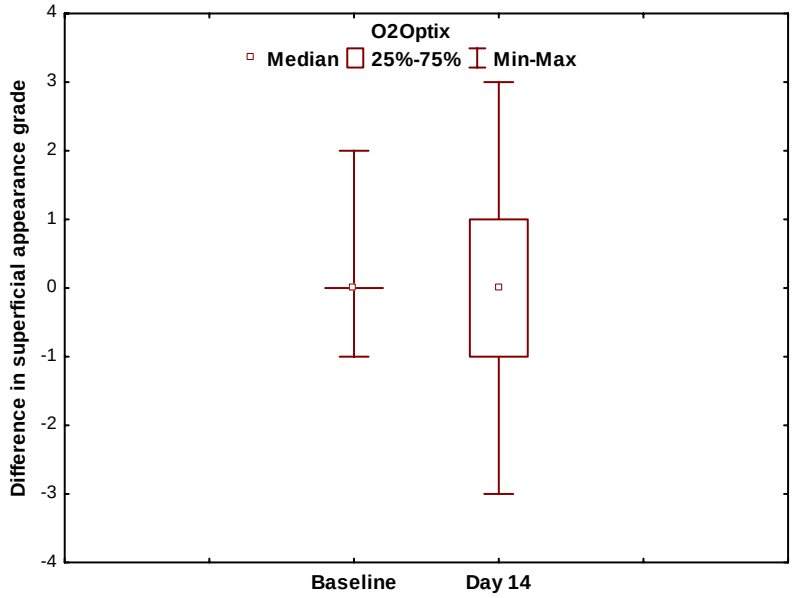


Figure 26: Differences in superficial appearance grade (central) between test solution and RepleniSH at baseline and Day 14 (O_2Optix)

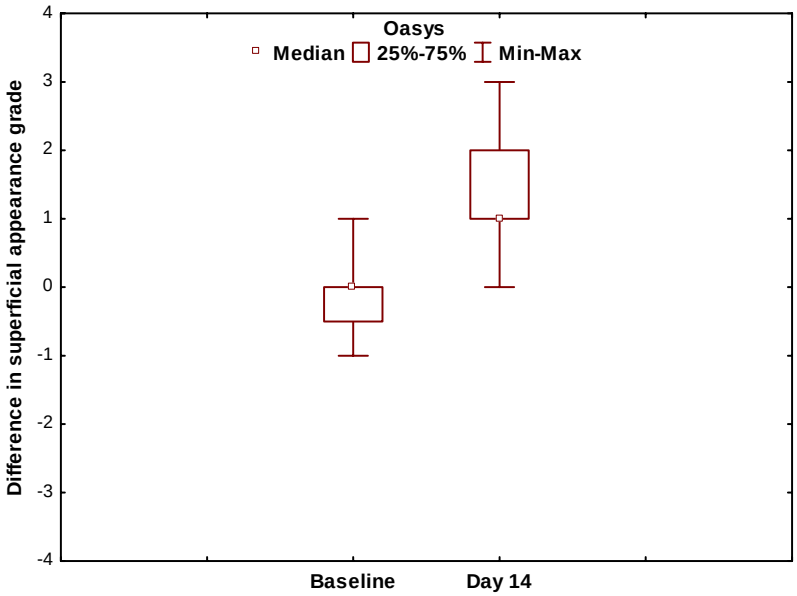


Figure 27: Differences in superficial appearance grade (central) between test solution and RepleniSH at baseline and Day 14 (Oasys)

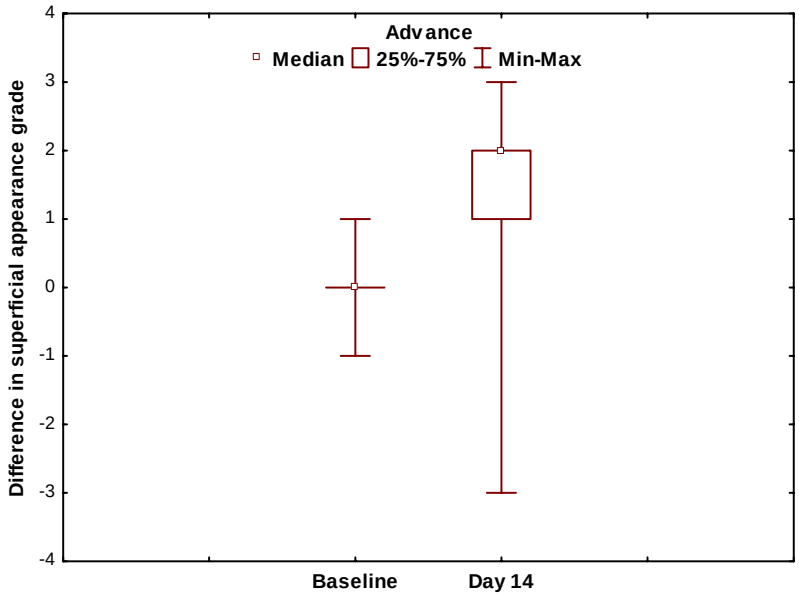


Figure 28: Differences in superficial appearance grade (central) between test solution and RepleniSH at baseline and Day 14 (Advance)

Hyper-reflective superficial epithelial cells were identified in some corneas both at the dispensing visit and the Day 14 visit. Table 20 lists the numbers of hyper-reflective cells (cells/mm^2) when grade 3 (presence of hyper-reflective cells) was given. The majority of hyper-reflective cells were counted at Day 14 with the test solution in combination with either lens. O_2Optix in combination with the test solution showed the least amount of hyper-reflective cells.

Table 20: Numbers of hyper-reflective cells (cells/mm^2) over time, when grade 3 (presence of hyper-reflective cells) was given for the different lens/solution combinations corneal positions*

	Baseline				Day 14			
	Test		RepleniSH		Test		RepleniSH	
	C	T	C	T	C	T	C	T
Advance $^{\alpha}$								
Sum	173	64	45	13	1216	1191	38	45
AVE	6.9	2.6	1.8	0.5	48.7	47.6	1.5	1.8
SD	89.6	38.4	38.4	12.8	128.0	160.1	38.4	32.0
Median	0	0	0	0	38	45	0	0
Min	0	0	0	0	0	0	0	0
Max	90	38	38	13	128	160	38	32
O2Optix								
Sum	0	32	26	45	147	250	102	198
AVE	0.0	1.3	1.0	1.8	5.9	10.0	4.1	7.9
SD	0.0	6.4	5.1	6.3	15.6	34.9	14.2	15.3
Median	0	0	0	0	0	0	0	0
Min	0	0	0	0	0	0	0	0
Max	0	32	26	26	70	173	70	58
Oasys $^{\alpha}$								
Sum	64	45	19	6	903	980	38	26
AVE	2.7	1.9	0.8	0.3	37.6	40.8	1.6	1.1
SD	9.8	7.9	3.9	1.3	32.7	44.4	5.7	4.1
Median	0	0	0	0	29	32	0	0
Min	0	0	0	0	0	0	0	0
Max	45	38	19	6	102	186	26	19
PureVision $^{\alpha}$								
Sum	19	70	0	0	749	877	58	128
AVE	0.8	2.8	0.0	0.0	30.0	35.1	2.3	5.1
SD	3.8	11.7	0.0	0.0	55.6	53.7	8.0	11.5
Median	0	0	0	0	0	19	0	0
Min	0	0	0	0	0	0	0	0
Max	19	58	0	0	256	230	38	51

* C = central, T = temporal

$^{\alpha}$ Statistically significant difference in observation of hyper-reflective cells between test solution and RepleniSH at Day 14

Figure 29 shows the numbers of hyper-reflective cells (cells/mm^2) at the central cornea observed for the different lens/solution combinations at Day 14. Figure 30 illustrates the same, but for the temporal cornea.

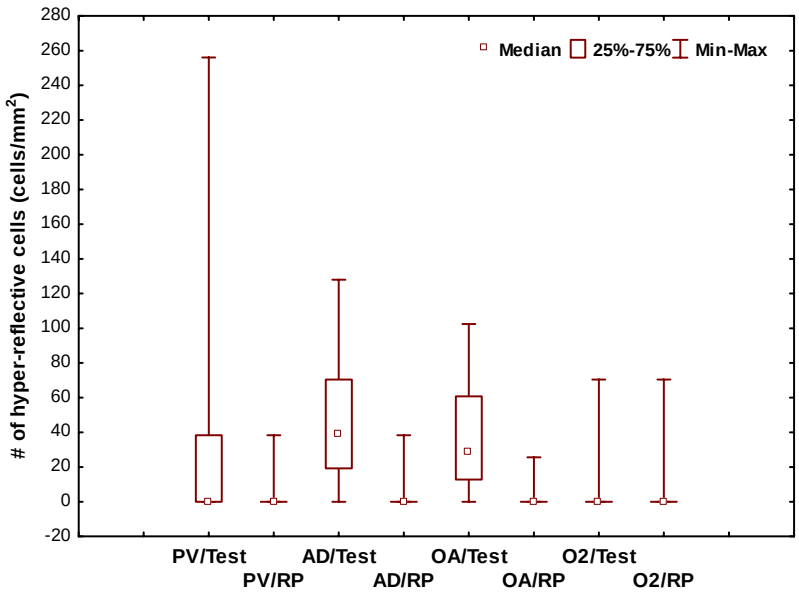


Figure 29: Numbers of hyper-reflective cells (cells/mm^2, central cornea) at Day 14 for the different lens/solution combinations (AD=Advance, OA=Oasys, O2=O_2Optix, PV=PureVision, Test=test solution, RP=RepleniSH)

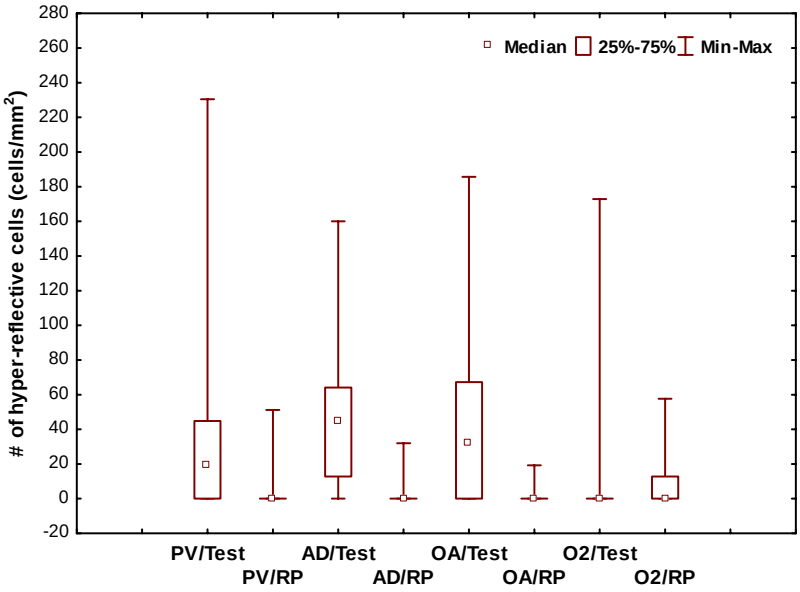


Figure 30: Numbers of hyper-reflective cells (cells/mm^2, temporal cornea) at Day 14 for the different lens/solution combinations (AD=Advance, OA=Oasys, O2=O_2Optix, PV=PureVision, Test=test solution, RP=RepleniSH)

Statistical analyses using a Fisher's exact test (chi-square test 2x2 contingency table) on hyper-reflective cell count revealed that the number of hyper-reflective cells was statistically significantly dependent ($p<0.001$) on which solution was used. The presence of hyper-reflective cells was statistically significantly associated with the use of the test solution, especially if used in combination with PureVision, Advance and Oasys (Fisher's exact test $p=0.005$, $p<0.001$ and $p<0.001$, respectively). There was no statistically significant association (Fisher's exact test $p=0.989$) with hyper-reflective cell number with solution for O_2Optix.

Figure 31 shows images of the superficial cells of one participant (randomly assigned to wear PureVision lenses) at the baseline visit for both solutions. Images of the superficial cells of the same participant at the two week visit (for both solutions) are shown in Figure 32. As can be seen, the cells at the baseline visit appear similar for both eyes. However at the two week, visit the eye that was exposed to the test solution had hyper-reflective cells and was graded a 3 (presence of hyper-reflective cells).

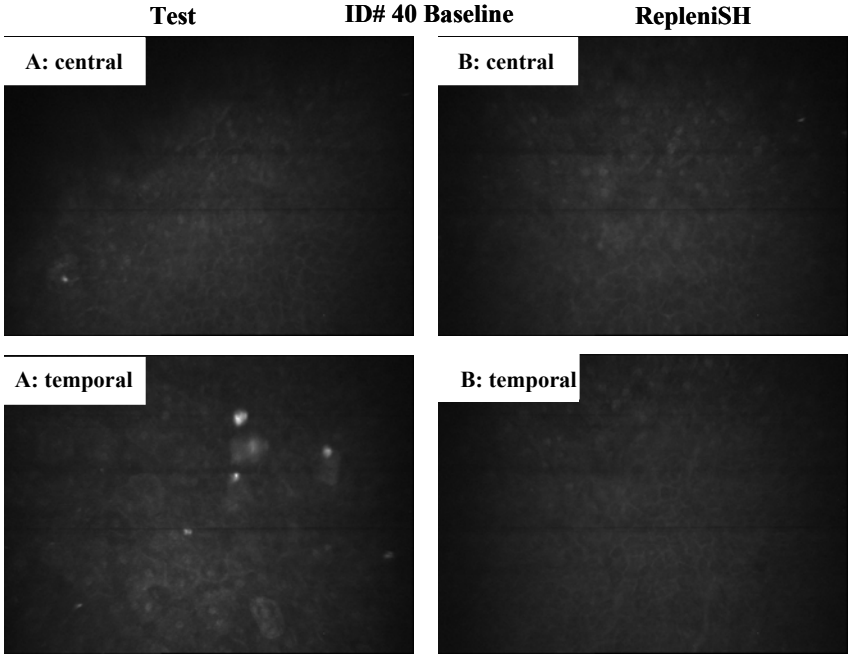


Figure 31: Example of the superficial cellular appearance of a participant, showing the central and temporal cornea at the Baseline visit with the test and the control (RepleniSH) solution

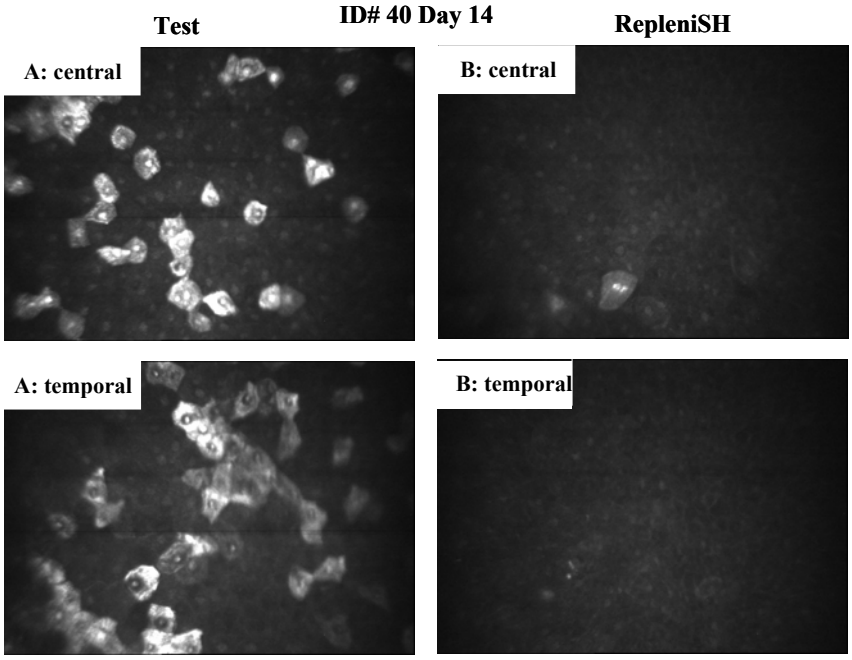


Figure 32: Example of the superficial cellular appearance for the same participant, showing the central and temporal cornea at Day 14 with the test and the control (RepleniSH) solution

Corneal staining (CS)

Table 21 lists the average CS scores over time for the different lens/solution combination. Figure 33 shows CS for the different lenses in combination with the test solution and RepleniSH for baseline and Day 14. A statistical significant difference (RmANOVA p=0.021) in CS was found. Tukey post hoc testing showed that there was significantly more CS at Day 14 with the test solution in combination with Advance (Tukey HSD, p<0.001), with Oasys (Tukey HSD, p=0.005) and with PureVision (Tukey HSD, p<0.001). No statistical significant difference (Tukey HSD, p=0.664) in CS was found for the test solution in combination with O_2Optix.

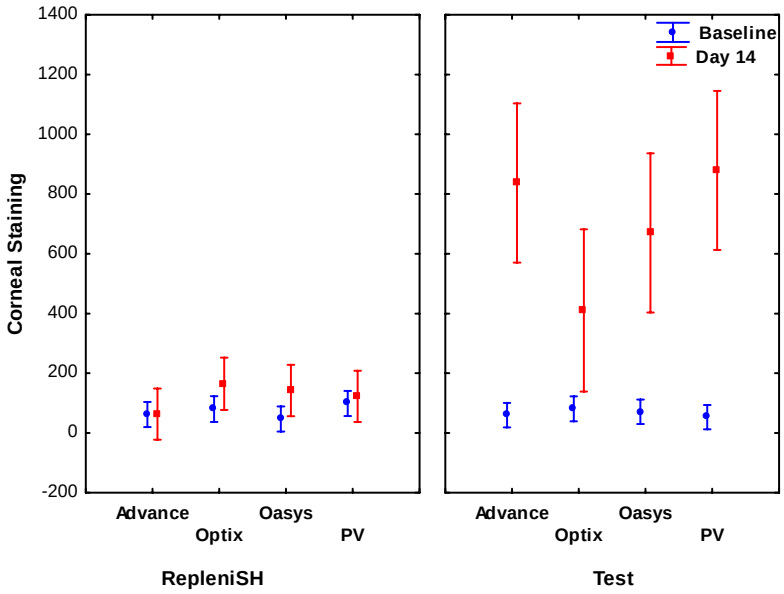


Figure 33: Corneal staining for the different lenses in combination with the test solution and RepleniSH for baseline and Day 14

Table 21: Corneal staining (mean ± SD, range) for the different lens/solution combinations at baseline and 2 weeks

	Baseline (mean ± SD (range)		2 weeks (mean ± SD (range)	
	RepleniSH	Test	RepleniSH	Test
Advance GSS	62 ± 90 0 - 395	60 ± 79 0 – 320	63 ± 62 0 - 232	837 ± 574 80 - 1920
Temporal	28 ± 140 0 – 700	24 ± 78 0 – 375	28 ± 64 0 – 200	837 ± 703 0 – 2250
Inferior	114 ± 174 0 – 700	170 ± 293 0 – 1400	181 ± 296 0 – 1000	1168 ± 856 0 – 4200
Nasal	44 ± 140 0 – 600	54 ± 136 0 – 525	8 ± 32 0 – 150	847 ± 624 0 – 2000
Superior	25 ± 59 0 – 250	46 ± 86 0 – 250	95 ± 171 0 – 750	870 ± 665 0 – 2100
Central	99 ± 358 0 - 1750	4 ± 20 0 – 100	4 ± 21 0 - 105	464 ± 597 0 - 1800
O2Optix GSS	77 ± 89 0 - 409	78 ± 112 0 – 442	158 ± 344 0 - 1610	397 ± 790 0 - 3140
Temporal	43 ± 129 0 - 625	69 ± 148 0 – 500	77 ± 233 0 – 1050	342 ± 827 0 – 3000
Inferior	227 ± 247 0 – 100	226 ± 344 0 – 1120	264 ± 414 0 – 1800	643 ± 930 0 – 3500
Nasal	71 ± 166 0 – 720	41 ± 127 0 – 612	160 ± 438 0 – 2000	398 ± 842 0 – 3000
Superior	47± 110 0 - 500	52 ± 129 0 – 500	151 ± 360 0 – 1600	420 ± 872 0 – 3500
Central	14 ± 45 0 - 200	17 ± 82 0 – 400	139 ± 365 0 - 1600	182 ± 604 0 - 2700
Oasys GSS	47 ± 96 0 - 380	71 ± 37 0 – 542	142 ± 228 0 - 1120	670 ± 518 60 - 1900
Temporal	45 ± 146 0 – 625	67 ± 172 0 – 750	59 ± 184 0 – 900	636 ± 610 0 – 2000
Inferior	73 ± 115 0 – 375	102 ± 172 0 – 500	166 ± 277 0 - 1200	288 ± 930 0 – 4000
Nasal	41 ± 111 0 – 400	90 ± 228 0 – 1000	145 ± 316 0 – 1200	507 ± 555 0 – 2000
Superior	49 ± 120 0 – 400	77 ± 181 0 – 750	283 ± 360 0 – 1400	633 ± 561 0 – 1750
Central	25 ± 125 0 - 625	18 ± 80 0 – 400	58 ± 202 0 - 900	586 ± 583 0 - 200
PureVision GSS	99 ± 140 0 - 428	53 ± 69 0 – 240	123 ± 112 0 - 375	879 ± 751 30 - 2920
Temporal	61 ± 140 0 – 500	17 ± 33 0 – 125	40 ± 111 0 – 500	927 ± 774 0 – 2500
Inferior	284 ± 400 0 – 1225	192 ± 273 0 – 900	271 ± 338 0 – 1250	1085 ± 842 0 – 4000
Nasal	54 ± 120 0 – 450	35 ± 78 0 – 300	114 ± 254 0 – 875	944 ± 902 0 – 3600
Superior	64 ± 162 0 – 700	19 ± 76 0 – 375	138 ± 166 0 – 500	1016 ± 1062 0 – 4900
Central	32 ± 126 0 - 625	3 ± 15 0 – 75	50 ± 185 0 - 900	422 ± 663 0 - 2250

On Day 14 statistically significant more (RmANOVA $p<0.001$) CS was observed with the test solution (Figure 34) for the temporal compared to the central cornea.

Figure 35 shows CS for the central and temporal cornea with the test solution in combination with the different lenses. Significantly more corneal staining was found for the temporal cornea (RmANOVA $p=0.023$). Post-hoc testing revealed that the Advance/test combination resulted in more corneal staining temporally than the Advance/test combination centrally as well as with the O2Optix/test combination centrally (Tukey HSD $p=0.027$ and $p=0.014$, respectively). Also, the PureVision/test combination produced temporally significantly higher amounts of CS than the O2Optix/test combination temporally and centrally, as well as the PureVision/test combination centrally (Tukey HSD $p=0.046$, $p=0.021$ and $p<0.001$, respectively).

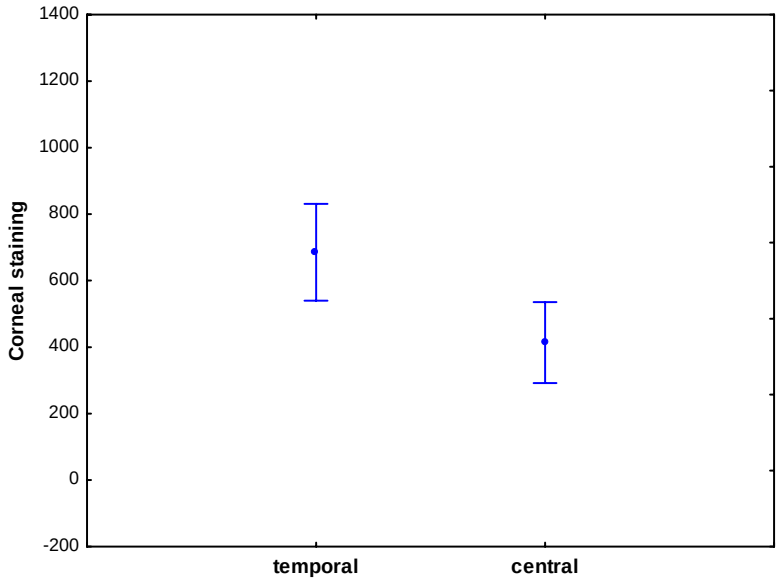


Figure 34: Corneal staining at Day 14 with the test solution for central and temporal cornea

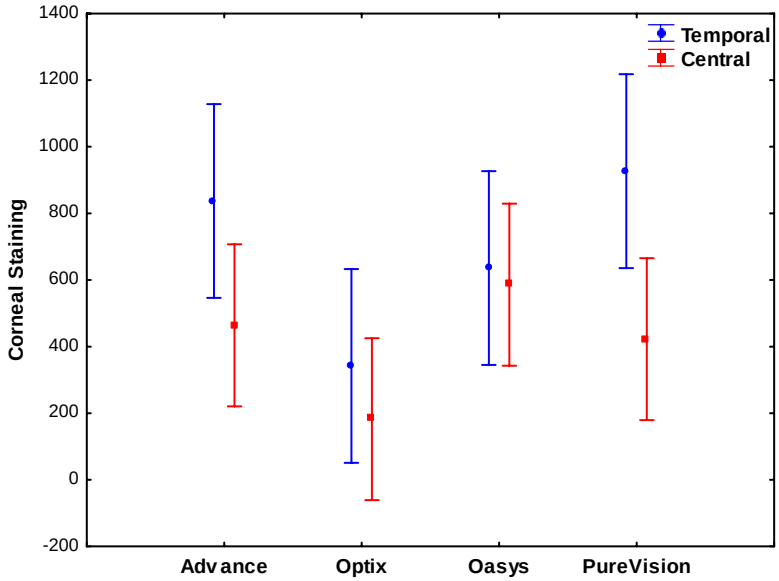


Figure 35: Corneal staining at Day 14 (temporal vs. central) for the test solution in combination with the different lenses

Comparing the CS scores between central and temporal cornea at Day 14 for the different lens solution combinations, Figure 36 shows that there was no statistical significant difference (RmANOVA p=0.264) in CS for RepleniSH in combination with either lens. Also the CS scores are so small, that they might be regarded as clinically insignificant.

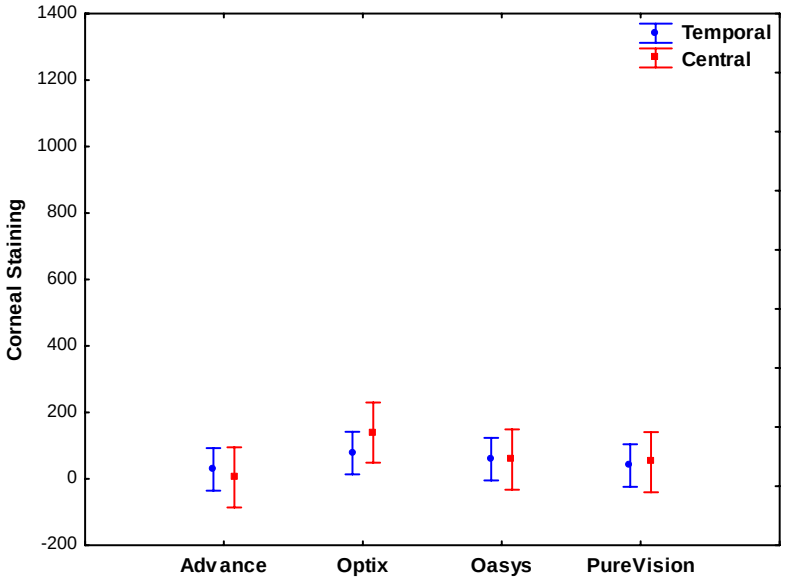


Figure 36: Corneal staining at Day 14 (temporal vs. central) for RepleniSH in combination with the different lenses

Image analysis

Image analysis was performed on superficial epithelial images obtained at the two week visit, as at this visit hyper-reflective cells were detected in some participants. Statistical analysis (RmANOVA) was performed separately for the central (n=51) and temporal (n=70) cornea because central and temporal images could not be obtained for all participants. Only complete data sets (images of the superficial epithelium for OD and OS) were included in statistical analysis.

Figure 37 shows the hyper-reflective cell areas of superficial epithelial images of the central cornea for the test and the control solution. Statistically significant differences (RmANOVA $p=0.037$) in hyper-reflective cell area were found between images of the test solution and images of the control solution. It can be seen that there were significantly greater hyper-reflective cell areas in images with the test solution compared to images with the control solution. A similar result (RmANOVA $p<0.001$) was observed for the temporal cornea (Figure 38).

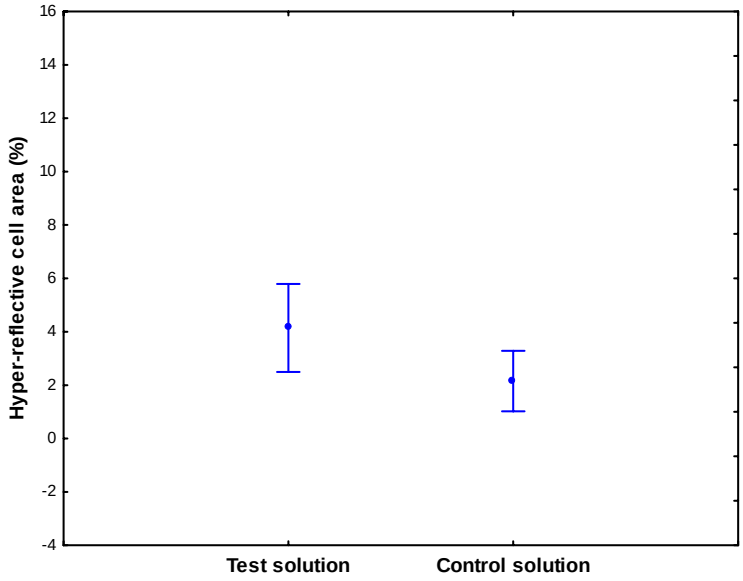


Figure 37: Hyper-reflective cell areas (%) of superficial epithelial images of the central cornea for the test and control solution

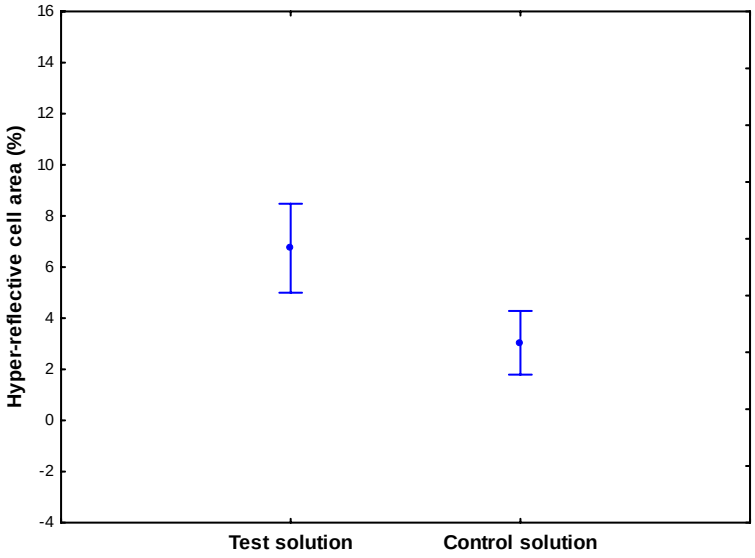


Figure 38: Hyper-reflective cell areas (%) of superficial epithelial images of the temporal cornea for the test and control solution

Figure 39 and Figure 40 illustrate the standard deviations of the images of the superficial epithelium (test and control solution) of the central and temporal cornea, respectively. For locations, statistically higher (RmANOVA $p=0.004$ and $p<0.001$, respectively) values and greater standard deviations were measured on images with the test solution compared to images from the control solution groups.

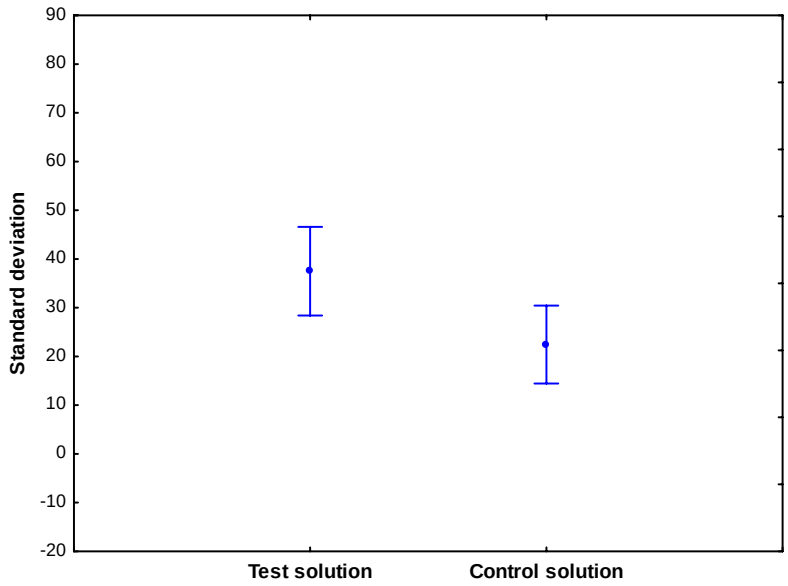


Figure 39: Standard deviations of superficial epithelial images of the central corneal for the test and control solution

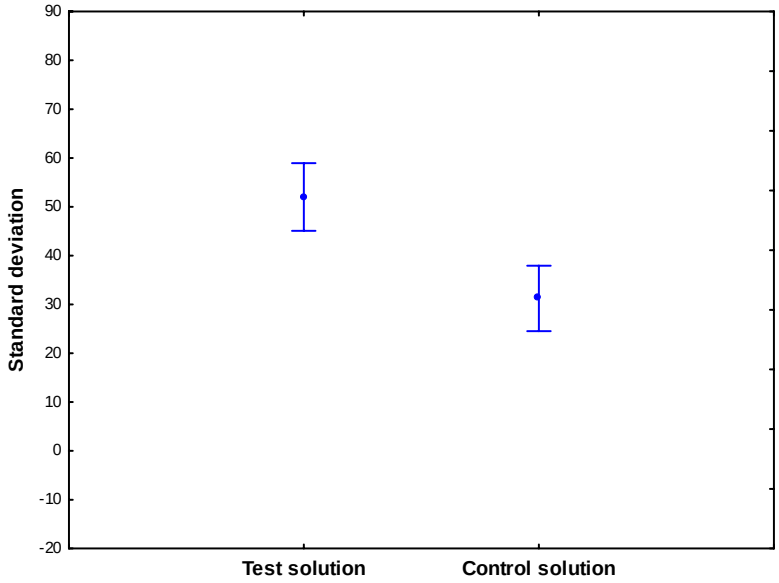


Figure 40: Standard deviations of superficial epithelial images of the temporal cornea for the test and control solution

Hyper-reflective cell areas of superficial epithelial images in the different lens/solution combinations were not statistically significantly different (RmANOVA p=0.615) in the central cornea (Figure 41). Figure 42 plots the standard deviations for the images of the central cornea and for the different lens solution combination. Statistical analysis revealed that differences in standard deviations for the different lens/solution combinations approached significance (RmANOVA p=0.073). Post-hoc testing (Tukey HSD) showed that statistically significantly higher (Tukey HSD p=0.009) standard deviations occurred with the Advance/test combinations than with the Advance/control combinations.

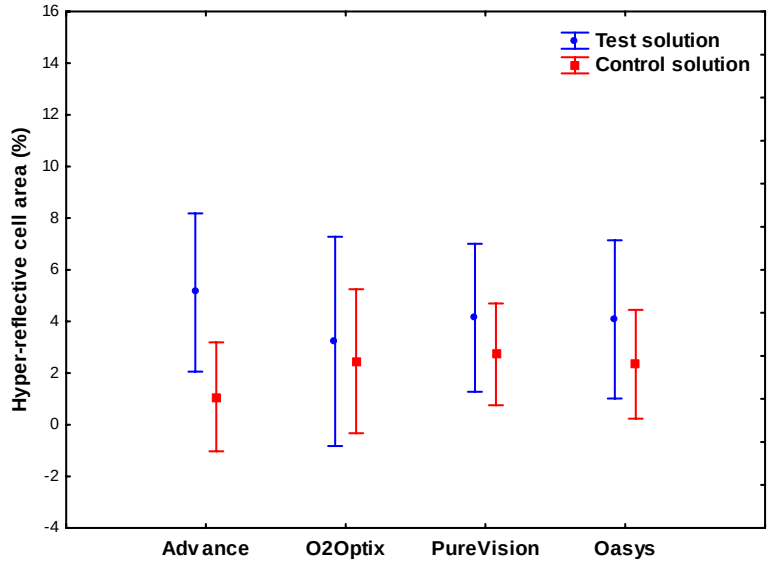


Figure 41: Hyper-reflective cell areas for superficial epithelial images (central) for the different lens/solution combinations

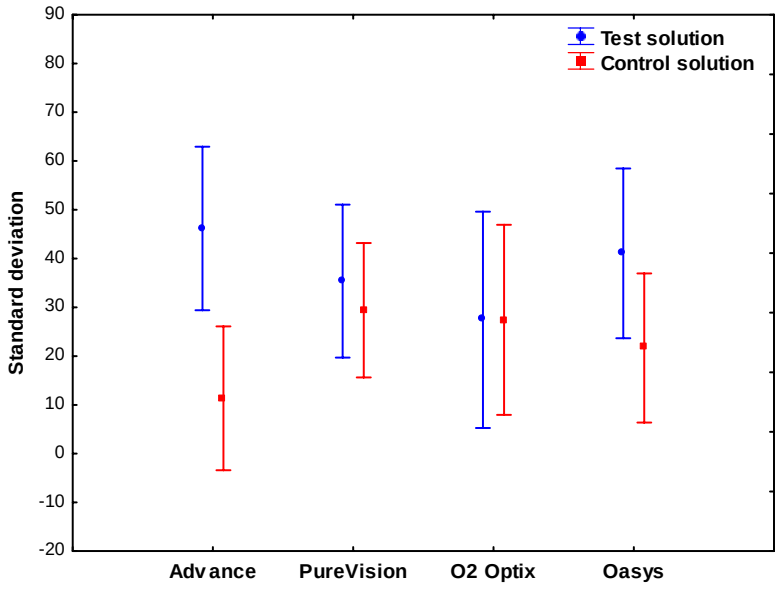


Figure 42: Standard deviations of superficial epithelial images (temporal) for the different lens/solution combinations

At the temporal cornea, a statistically significant difference (RmANOVA p=0.002) in hyper-reflective cell areas of superficial epithelial images was found for the different lens/solution combinations (Figure 43). Post-hoc testing (Tukey HSD) revealed that the Advance/test combination had significantly greater hyper-reflective cell areas than Advance/control, O_2Optix/test, PureVision/control, and Oasys/control combinations (Tukey HSD p<0.001, p=0.005, p=0.005, and p=0.073, respectively).

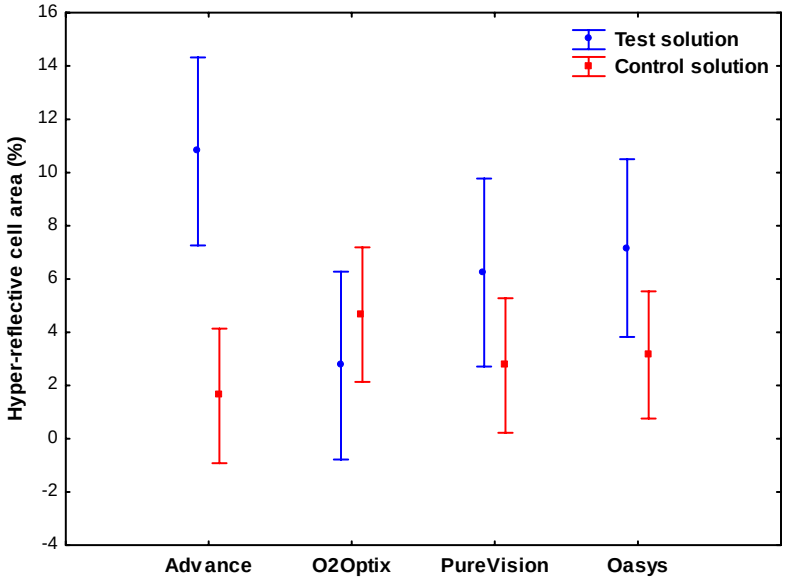


Figure 43: Hyper-reflective cell areas (%) for superficial epithelial images (temporal) for the different lens/solution combinations

The standard deviations (Figure 44) of superficial epithelial images (temporal) for the different lens/solution combinations also revealed statistically significant differences (RmANOVA p=0.001). Post-hoc testing (Tukey HSD) showed that the Advance/test combination resulted in statistically significantly higher standard deviations than the Advance/control, PureVision/control,O_2Optix/test, and the Oasys/control combinations (Tukey HSD p=0.006, p=0.010, p=0.002, and p=0.001, respectively). The standard deviations with the Oasys/test combination were also statistically significantly higher (Tukey HSD p=0.005) than with the Advance/control combination. The Oasys/test combination produced statistically significantly higher (Tukey HSD p=0.026) standard

deviations than the Oasys/control combination as well as the O_2Optix/test combination (Tukey HSD p=0.035).

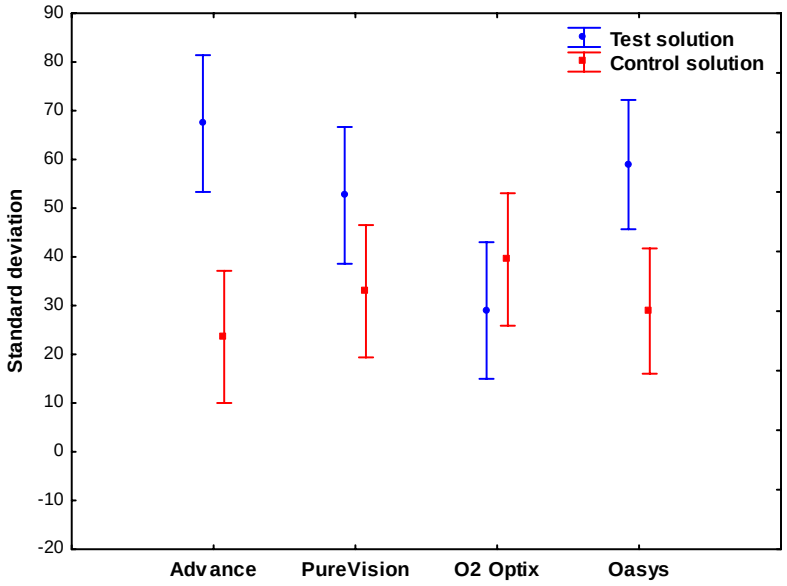


Figure 44: Standard deviations of superficial epithelial images (temporal) for the different lens/solution combinations

Conclusion

Increased numbers of hyper-reflective cells and higher levels of SICS were found with the PHMB-based test solution compared to the polyquad-based RepleniSH, suggesting that hyper-reflective cells and SICS co-occur.

Images of the superficial epithelium of eyes exposed to the test solution were on average brighter and had greater variance than images taken from the superficial epithelium of eyes exposed to RepleniSH, implying the presence of hyper-reflective cells in these brighter images.

Effect of lens/solution combinations on the superficial corneal epithelium 2

The previous experiment showed that an increased number of hyper-reflective cells occurred when eyes were exposed to certain lens/solution combinations, in particular when the PHMB-based solution was used with the lenses. The experiment also demonstrated that CS with the test solution was higher than with the polyquad-based control solution. The effect of further lens/solution combinations on the superficial epithelium was therefore investigated in the following experiment. Of special interest was the effect on the superficial epithelium when a hydrogen peroxide-based solution was used. Hydrogen peroxide is suggested to be the gold-standard solution and not to result in SICS.[30;31;178]

Relevant data for this experiment were collected as part of a study conducted at the CCLR.[301] The purpose of this study was to compare the effect of Clear Care (CIBA Vision) a hydrogen peroxide disinfecting system to the effect of the polyquad-based RepleniSH (RepleniSH, Alcon), both in combination with two SiHy contact lenses (O_2 Optix, CIBA Vision and Acuvue Oasys (Oasys), Johnson & Johnson) on the corneal epithelium.

Objective

The specific objective of this experiment was:

- To observe if hyper-reflective superficial epithelial cells occur with the specific lens/solution combinations.

Methods

Participants

Twenty-five adapted soft contact lens wearers were recruited for this study.

Study specific inclusion & exclusion criteria

A person was eligible if he/she:

1. Had normal binocular vision (no strabismus, no amblyopia, and anisometropia less than or equal to 1.00 D).

2. Was a current soft lens wearer replacing their lenses every 2 weeks to 1 month.
3. Had a distance contact lens prescription between +6.00D to -10.00 DS and could be successfully fitted with both study lens types.
4. Agreed to wear the study lenses on a daily wear basis.

A person was ineligible if he/she:

1. Had a known sensitivity to the contact lens care solutions or diagnostic pharmaceuticals used in the study.
2. Wore daily disposable lenses.
3. Wore lenses on a continuous or extended wear basis.
4. Was unable to wear contact lenses successfully without using rewetting drops.

Study solutions

The two care regimens used in this study were Clear Care and RepleniSH. Details regarding these care regimens can be found in Table 9 (Chapter 2 General Methods).

Participants were instructed to clean and disinfect their lenses after each wearing period, using either Clear Care (CIBA Vision) or RepleniSH (Alcon). Participant compliance with the lens care instructions was checked at the follow-up appointments and additional containers of solution were provided, if necessary. No rewetting drops, enzyme or surfactant cleaners were used during the study.

Study lenses

O_2Optix and Oasys were used in this study. Please refer to Table 8 in General Methods for lens details. The participants wore lenses on a daily wear basis for at least eight to ten hours per day and at least six days per week for the duration of the eight-week study.

Study design

The protocol was submitted to the Office of Research Ethics at the University of Waterloo. The study was conducted as an eight-week, prospective, contralateral eye, clinical trial with a partly double masked and randomized cross-over design (see Figure 45). The investigator was masked to the lens care system and the participant was masked to the lens type. Data were collected at seven scheduled

appointments (screening, two baseline and four additional study visits). Each baseline visit was preceded by a washout period of two days of no lens wear.

Study visits

Following an initial screening appointment to ensure participants' eligibility, they were randomly assigned to wear an O_2 Optix lens in one eye and an Oasys lens in the other eye. A washout period of at least two days followed this visit, during which participants were instructed to wear their spectacles only.

At the baseline visit for phase one, procedures that were part of the initial study were performed on both eyes before the first pair of contact lenses was dispensed. Participants were then randomly assigned to use either the Clear Care or RepleniSH care regimen for the first phase of the study. The other solution was dispensed in the second phase.

Follow-up visits were conducted after two and four weeks of lens wear.

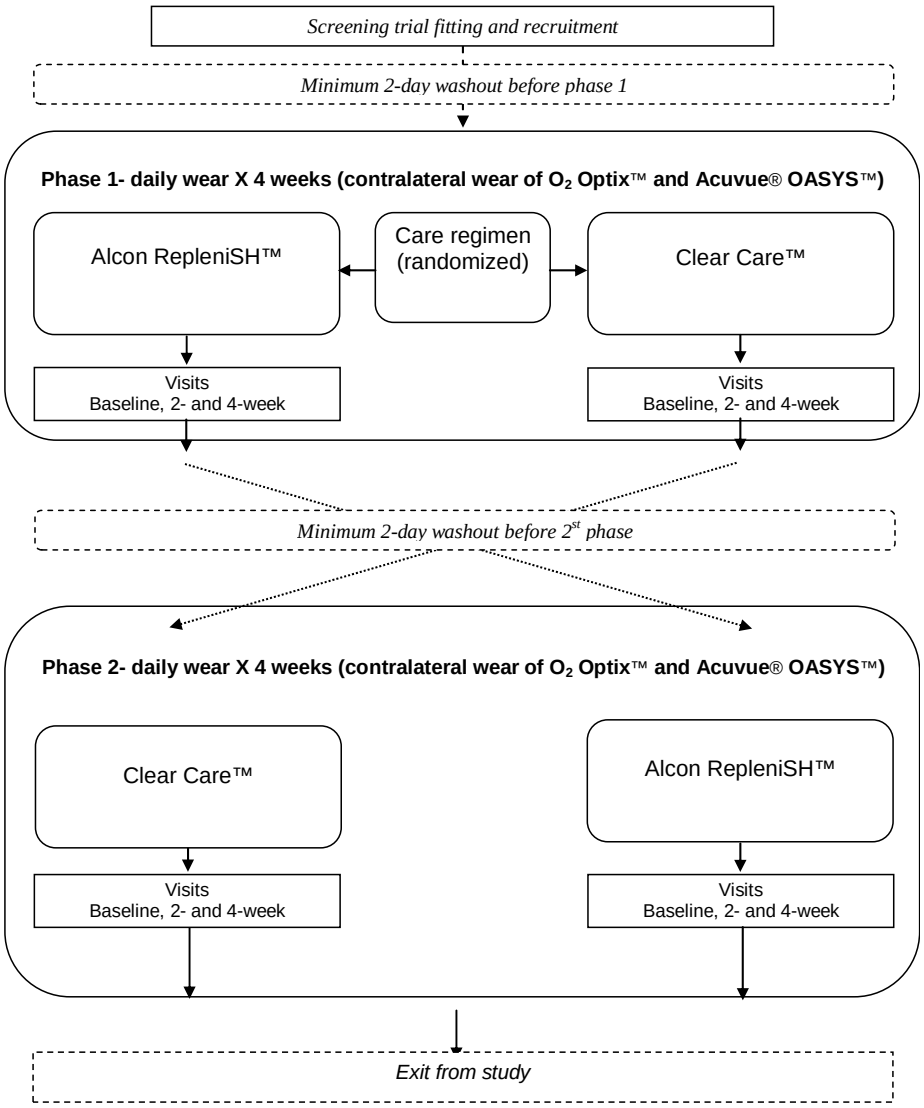


Figure 45: Study Design

Procedures

Ocular surface staining was evaluated using sodium fluorescein. CM was performed on both eyes at the 4 week visit of each phase according to the procedures illustrated in Chapter 2.

Grading and Analysis

Images of the superficial epithelium were identified and the appearance of the cells was subjectively graded using the grading scale described in Chapter 2.

CS was graded according to the CCLR GSS (Chapter 2). CS during this experiment was graded by a different investigator, not the author.

Image analysis was performed as described in Chapter 2.

Statistical analysis

Statistical analysis for CS data was performed using repeated measures ANOVA.

Results

Participants

Twenty-eight participants were enrolled into the study. Two out of the 28 were enrolled but not dispensed because they did not meet the inclusion criteria listed in the protocol. Their data are therefore not reported. Of the 26 participants dispensed study lenses and solutions (nine male; 17 female), the mean (± standard deviation) age was 31 ± 12 years (range 17 to 59 years).

Superficial cellular appearance and presence of hyper-reflective cells

Table 22 shows the appearance (median and range) of superficial cells for the four lens/solution combinations and for the two different corneal locations.

Table 22: Superficial cellular appearance*

	Clear Care				RepleniSH			
	O_2Optix		Oasys		O_2Optix		Oasys	
	C	P	C	P	C	P	C	P
Median	1	2	1	2	1	1	1	2
Min	0	0	0	0	0	1	0	0
Max	3	3	3	3	2	2	3	3

* (C = central and P = peripheral)

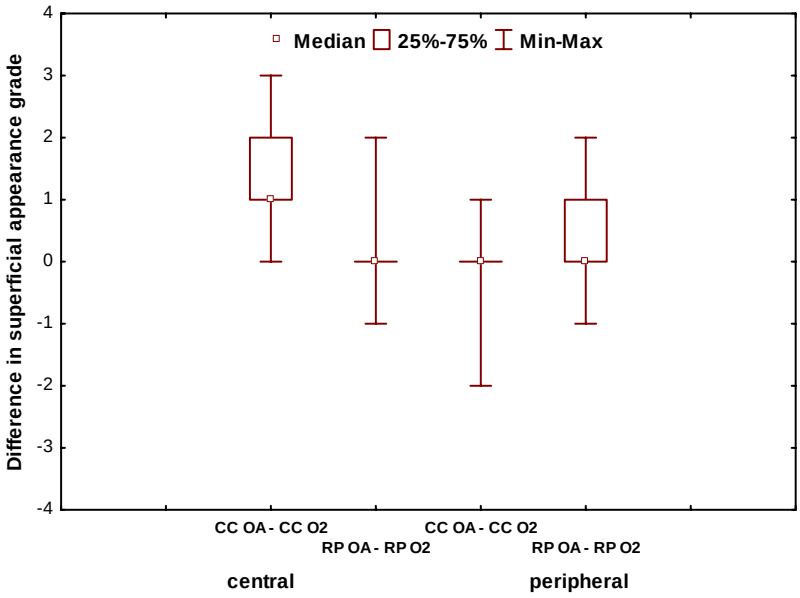


Figure 46: Differences in superficial appearance grade between Clear Care (OA – O_2) and RepleniSH (OA – O_2) for central and peripheral cornea

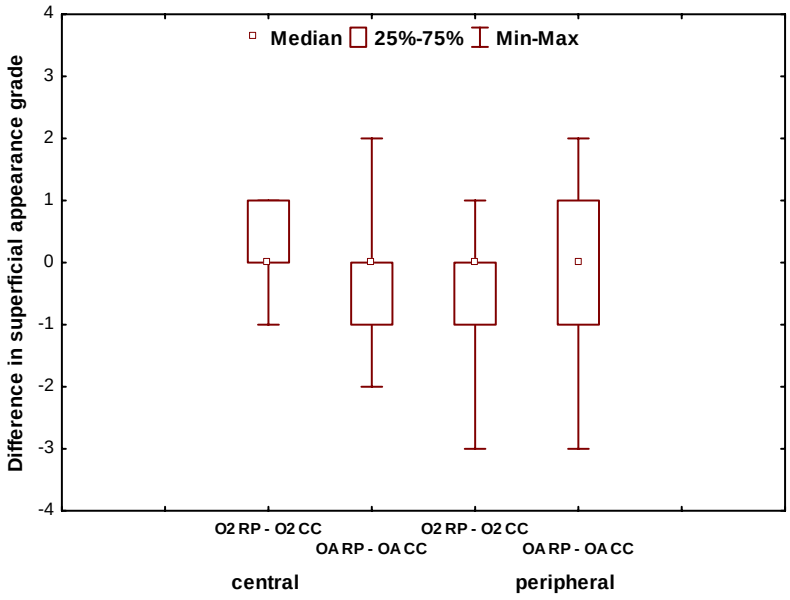


Figure 47: Differences in superficial appearance grade between O_2Optix (RepleniSH - Clear care) and Oasys (RepleniSH - Clear Care) for central and peripheral cornea

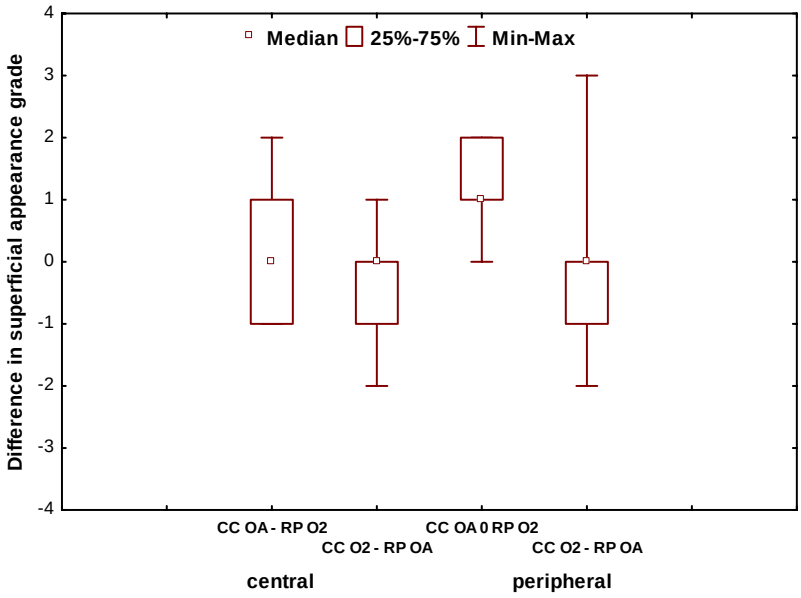


Figure 48: Differences in superficial appearance grade between Clear Care/Oasys – RepleniSH/O_2Optix and Clear Care/O_2Optix – RepleniSH/Oasys for central and peripheral cornea

Figure 46, Figure 47 and Figure show the differences in superficial appearance grade for central and temporal cornea when Clear Care was used with either Oasys or O_2Optix, or when RepleniSH was used with either Oasys or O_2Optix (Figure 46), when Clear Care/Oasys was compared to RepleniSH/O_2Optix (Figure 47) as well as when Clear Care/O_2Optix was compared to RepleniSH/Oasys (Figure 48). Statistical analyses showed that the differences in central superficial appearance grade were significantly higher (Wilcoxon matched pairs $p<0.001$) when Clear Care was used with Oasys and O_2Optix than when RepleniSH was used with these lenses. The differences in peripheral superficial appearance grade were also significantly higher (Wilcoxon matched pairs $p=0.006$) when the differences in grades between the Clear Care/Oasys combinations and the RepleniSH/O_2Optix combinations were compared to the differences between the Clear Care/O_2Optix combinations and the RepleniSH/Oasys combinations. All other comparisons were statistically insignificant (Wilcoxon matched pairs $p>0.050$).

A grade of three (presence of hyper-reflective cells was reported for a few participants. The number of observed hyper-reflective cells present in images varied and was small; this is represented in Table

23. As can be seen in these two tables, for the O_2 Optix /RepleniSH combination there were no hyper-reflective cells.

Table 23: Mean numbers of hyper-reflective cells (cells/mm^2) measured for the different lens/solution combinations and for the different positions

	O_2Optix	Oasys
Clear Care -central	1.3 ± 5.1 (0 to 26)	3.2 ± 10.2 (0 to 45)
Clear Care -peripheral	4.5 ± 10.9 (0 to 38)	3.2 ± 10.9 (0 to 45)
RepleniSH -central	0.0 ± 0.0 (0 to 0)	1.2 ± 5.1 (0 to 26)
RepleniSH–peripheral	0.0 ± 0.0 (0 to 0)	1.9 ± 5.1 (0 to 19)

Figure 49 shows the superficial epithelium (central) of both eyes for a study participant (ID#3) during phase one (A) and phase two (B). The grades for superficial cellular appearance were grade 1 (presence of cells with more prominent margins) for OD (phase one and phase two) and for OS (phase two). Grade 2 (presence of cells with prominent margins and contents) was given for OS (phase one).

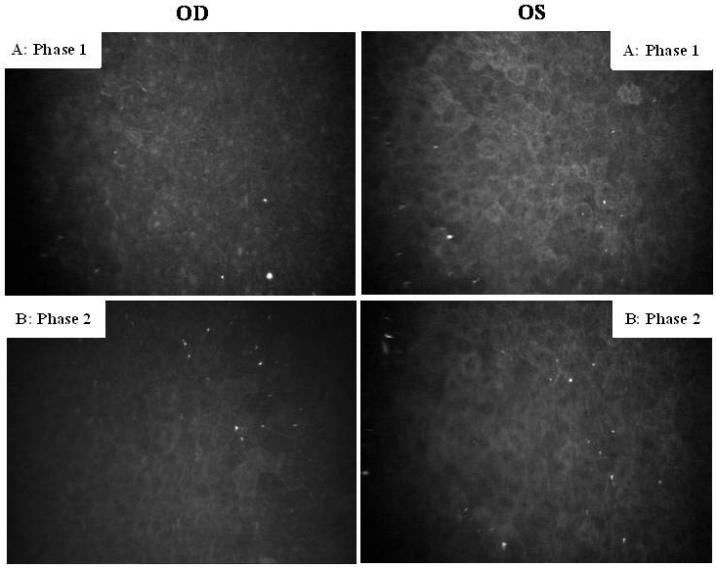


Figure 49: Examples of the central superficial epithelium (OD and OS) of one study participant (ID#3) for phase one (A) and phase two (B)

Corneal staining (CS)

Table 24 lists the CS scores for the different lens/solution combinations averaged for all participants. As can be seen, all observed CS scores were very small and perhaps clinically insignificant.

Table 24: Corneal staining (mean ± SD, range) for the different lens/solution combinations

	RepleniSH (mean ± SD) (range)		Clear Care (mean ± SD) (range)	
	Oasys	**O2Optix**	**Oasys**	**O2Optix**
GSS (0 – 10000)	91 ± 216 (0 – 1077)	79 ± 210 (0 – 990)	66 ± 169 (0 – 815)	26 ± 39 (0 – 150)
Temporal	33 ± 90 (90 – 375)	90 ± 314 (0 – 1500)	83 ± 375 (0 – 1875)	2 ± 10 (0 – 50)
Inferior	78 ± 148 (0 – 560)	135 ± 408 (0 – 2000)	97 ± 311 (0 – 1500)	83 ± 173 (0 – 750)
Nasal	152 ± 172 (0 – 2250)	114 ± 400 (0 – 2000)	70 ± 223 (0 – 1000)	14 ± 53 (0 – 250)
Superior	141 ± 258 (0 – 1200)	34 ± 74 (0 – 250)	44 ± 96 (0 – 375)	29 ± 103 (0 – 500)
Central	49 ± 201 (0 – 1000)	20 ± 50 (0 – 200)	36 ± 103 (0 – 400)	4 ± 13 (0 – 50)

Figure 50 shows that there was no significant difference (RmANOVA p=0.231) in CS (GSS) for the different lens/solution combinations. Also, there was no significant difference in CS between lenses (RmANOVA p=0.910) nor between solutions (RmANOVA p=0.392). There was also no statistically significant difference (RmANOVA p=0.272) in CS between central and temporal cornea for the different lens/solution combinations (Figure 51).

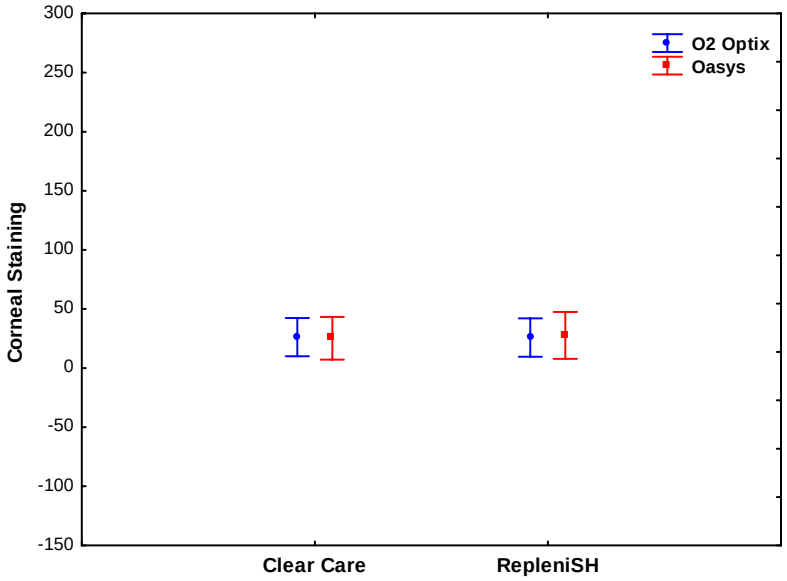


Figure 50: Corneal staining for the different lens/solution combinations

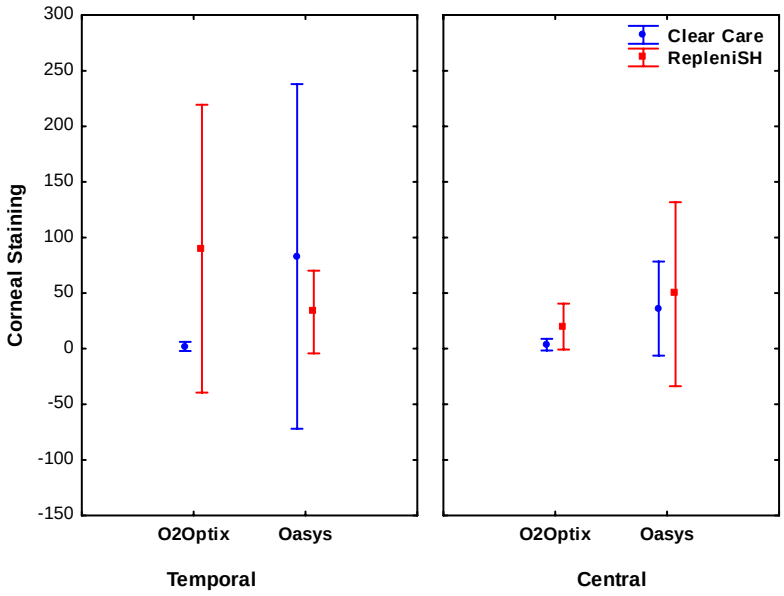


Figure 51: Corneal staining for central and temporal cornea and for the different lens/solution combinations

Corneal staining and presence of hyper-reflective cells

Table 25 lists those participants who exhibited hyper-reflective cells at the different corneal positions with the different lens/solution combinations. The CS score and the number of hyper-reflective cells are given. As can be seen, no participant exhibited any hyper-reflective cells with the O_2Optix/RepleniSH combination, as mentioned earlier. ID#11, ID#15 and ID#23 were the only participants who exhibited hyper-reflective cells for different lens/solution combinations. If hyper-reflective cells were observed, interestingly no CS occurred except for ID#11 using Oasys/Clear Care and Oasys/RepleniSH.

Table 25: Corneal staining (S) and numbers (cells/mm^2) of hyper-reflective cells (#) for those participants that received grade 3 for cellular appearance for the different lens solution combinations and corneal positions (C = central, T = temporal)

	O2Optix/ Clear Care				Oasys/ Clear Care				O2Optix/ RepleniSH				Oasys/ RepleniSH			
	C		T		C		T		C		T		C		T	
ID	S	#	S	#	S	#	S	#	S	#	S	#	S	#	S	#
9	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0	19
11	0	26	0	38	250	26	0	45	--	--	--	--	1000	26	--	--
15	--	--	0	32	--	--	0	6	--	--	--	--	--	--	0	19
18	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0	6
19	--	--	0	13	--	--	--	--	--	--	--	--	--	--	--	--
23	--	--	--	--	0	45	0	32	--	--	--	--	0	6	--	--
25	--	--	0	26	--	--	--	--	--	--	--	--	--	--	--	--

Image analysis

CM images of the superficial epithelium could not be identified for all lens/solution combinations and corneal positions for each participant. A complete image data set (all combinations for both corneal locations) was available only for one participant. Six complete data sets were available for the central cornea and four complete data sets for the temporal position. Due to the missing data, statistical analysis was not done and Figure 52 illustrates the hyper-reflective cell areas and Figure 53 the standard deviations. To show the data distributions extreme values and outliers are included in the figures. As can be seen there was a great deal of variance in the hyper-reflective cell areas and standard deviations between the different lens/solution combinations as well as within the different lens/solution combinations.

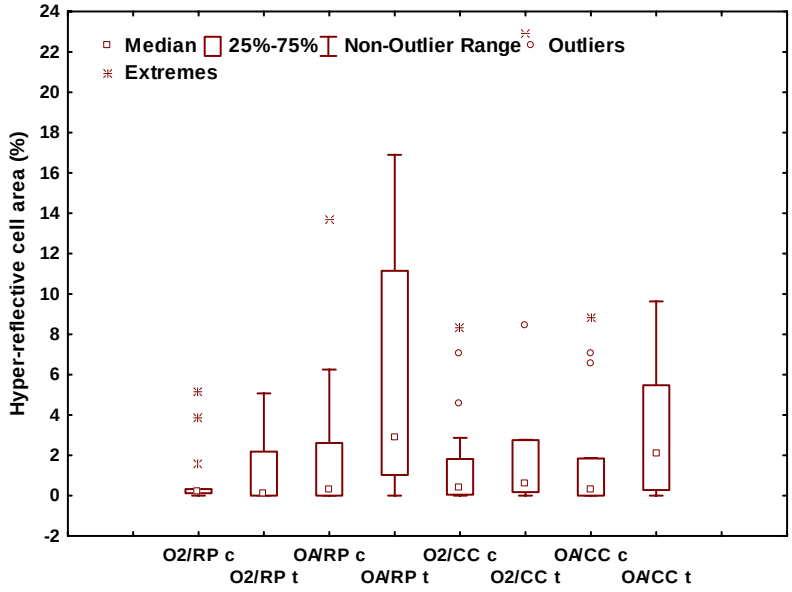


Figure 52: Hyper-reflective cell areas (%) for the different lens/solution combinations and corneal positions (O2=O_2Optix, OA=Oasys, RP=RepleniSH, CC=Clear Care)

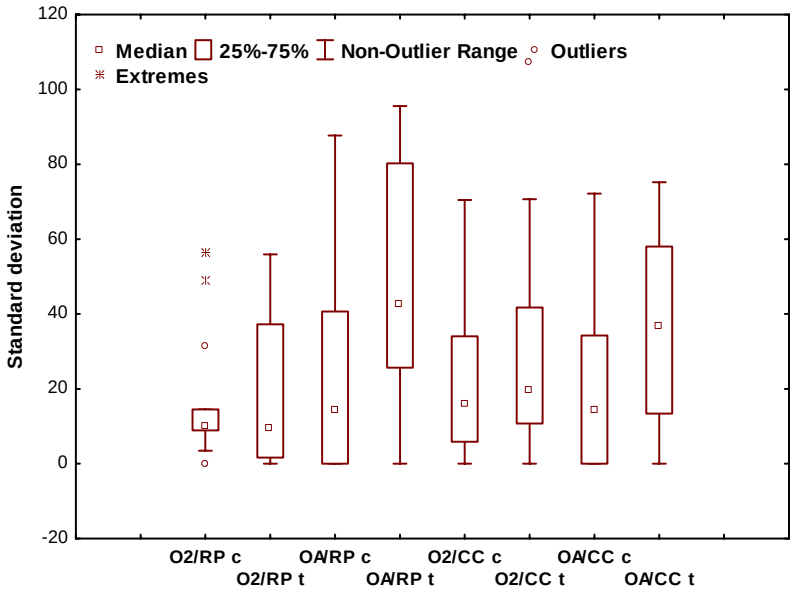


Figure 53: Standard deviations for the different lens/solution combinations and corneal positions (O2=O_2Optix, OA=Oasys, RP=RepleniSH, CC=Clear Care)

Conclusion

Small numbers of hyper-reflective cells were observed in this study. Clinically insignificant SICS was observed for these participants.

There appeared to be no obvious difference in hyper-reflective cell prevalence between the different lens/solution combinations.

Effect of long-term use of the same type of contact lens and contact lens solution on the superficial corneal epithelium

The conclusions drawn from the previous experiments in this chapter were that hyper-reflective cells predominantly seem to occur when a specific lens/PHMB-based solution combination was used. However, the lens/solution combinations in the previous experiments were only worn for a short period of time (two weeks and one month respectively for the two experiments). Therefore, it was of special interest if the long-term use of the same lens type and the same type of solution would have an effect on the superficial epithelium. This idea that long-term use of the same type of lens and same type of solution might be predicting variables for hyper-reflective cells was based on the results obtained during the first experiment of this chapter, where increased numbers of hyper-reflective cells with the PHMB-based solution were observed at Day 14.

The present experiment therefore aimed to investigate if the long-term use of a contact lens type (hydrogel or SiHy) in combination with a type of contact lens solution (PHMB-based or polyquad-based) would have any effects on the appearance of the superficial epithelium.

Objective

The specific objective of the experiment was:

- To determine whether the prolonged use of different combinations of contact lens solutions and contact lenses is a predicting variable for the appearance of hyper-reflective cells.

Methods

Participants

Eight adapted soft contact lens wearers were enrolled in this experiment.

Study specific inclusion & exclusion criteria

A person was eligible if he/she:

1. Was a current soft lens wearer and wore contact lenses six or more days/week.
2. Had had two years of ≥ 80% use of one of the specified care systems and 100% use of this system in the last year.
3. Had been a full time daily wear user of either i) SiHy or ii) FDA Group IV hydrogel soft contact lenses for the last 2 years with no breaks > 1 month and none in the last 3 months.

Study design

Ethics clearance was obtained from the Office of Research Ethics at the University of Waterloo and informed consent was obtained prior to enrolment.

This was a one-visit, single-masked study of existing soft contact lens wearers who have consistently used either PHMB-containing or non PHMB-containing soft lens soaking solutions. Four participants were in the PHMB group and four in the non-PHMB group.

Participants were pre-screened by a questionnaire to identify who had primarily used either: a) PHMB-containing care products or b) non PHMB-containing (polyquaternium-1) care products for two years. The investigator was masked to the participant's habitual contact lens care system at the

time of the experiment. This was accomplished by having an ophthalmic technician administer the questionnaire and the investigator was not shown the results until after the clinical assessment.

Data were collected at one scheduled appointment. This appointment was a combined screening and assessment visit. The screening part was to ensure the participant was eligible for the study and to determine the appropriate study group, according to Table 26. If a participant was eligible the study procedures were performed.

Table 26: Summary of numbers of participants

	PHMB-users	Polyquaternium-1 users	Total
FDA Group IV users	2 (A)	2 (C)	4
SiHy users	2 (B)	2 (D)	4
Total	4	4	8

Test groups (A & B)

A. FDA Group IV lens wearers, PHMB-solution users in the last two years

B. SiHy lens wearers, PHMB users in the last two years

Participants who were enrolled in the test groups must have had two years predominant use of selected PHMB-containing contact lens care products (see below). 'Predominant' is identified as ≥80% over the past 2 years plus 100% in the last year.

- Bausch & Lomb Sensitive Eyes
- Bausch & Lomb ReNu Multiplus
- Advanced Medical Optics Complete MoisturePlus*
- Advanced Medical Optics Equate

*Because this solution had been taken off the market, participants were eligible if they had previously used this product and had switched to a different PHMB-containing care product within the past year.

Control Groups (C & D)

C. FDA Group IV lens wearers, polyquaternium-1 solution users in the last two years

D. SiHy lens wearers, polyquaternium-1 solution users in the last two years.

Participants who were enrolled in the control groups must have had two years (see above) use of non PHMB-containing (Polyquaternium-1) contact lens products, e.g. Alcon OptiFree Express

Study visits

Participants were asked to not wear their contact lenses for two hours prior to the combined screening and assessment visit, but were asked to bring their lenses. An ophthalmic technician administered the screening questionnaire in order for the investigator to be masked as to the participant's habitual contact lens care system. This questionnaire also provided information on contact lens and care product usage. The participant's suitability was then assessed to ensure that the participant met all inclusion/exclusion criteria. Contact lens wear history was recorded and CS was assessed using sodium fluorescein. Participants were then asked to insert their lenses in order to assess lens fit. Lenses were removed and CM was performed on both eyes (central and temporal).

Procedures

CM was performed as explained in Chapter 2.

Grading and Analysis

Images of the superficial epithelium were identified and the appearance of the cells was subjectively graded using the scale described in Chapter 2.

CS was graded according to the CCLR GSS (Chapter 2). All CS during this experiment was graded by the thesis author.

Image analysis was performed as described in Chapter 2.

Results

Participants

Eight participants (two male, six female) were enrolled in the experiment, two in each group. The mean age of the participants was 28 ± 9.7 years, ranging from 21 to 49 years.

Lenses and solutions

The following table (Table 27) lists the lenses and solutions, as well as the number of years used, for each participant in the different groups.

Table 27: List of lenses and solutions used (time) for each participant

	Lenses	years	Solution	years
Group A (FDA IV, PHMB)				
ID#7	Focus Monthly	12	Complete	10
ID#5	Acuvue 2	3	ReNu Multiplus	3
Group B (SiHy, PHMB)				
ID#2	O2Optix	5	Sensitive Eyes	6
ID#4	Oasys	2	Complete	2
Group C (FDA IV, Polyquad)				
ID#8	Frequency 55	8	OptiFree Express	5
ID#6	Acuvue 2	10	OptiFree Express	5
Group D (SiHy, Polyquad)				
ID#1	Focus Night&Day	8	OptiFree Express	8
ID#3	O2Optix	2	OptiFree Express	9

Superficial cellular appearance and presence of hyper-reflective cells

Table 28 shows the superficial cellular appearance grade (both eyes and both corneal positions) for each group and participant. As can be seen, grade 3 (presence of hyper-reflective cells) was only seen once, for the temporal cornea of the left eye of ID#6 (group C, polyquad with FDA 4). The number (cells/mm^2) of hyper-reflective cells for this participant is shown in Table 29.

Table 28: Superficial cellular appearance grade for each participant (both eyes and corneal locations)*

	OD		OS	
	C	T	C	T
Group A (FDA IV, PHMB)				
ID#7	1	2	2	2
ID#5	2	2	1	2
Group B (SiHy, PHMB)				
ID#2	1	1	1	1
ID#4	1	2	2	2
Group C (FDA IV, Polyquad)				
ID#8	1	2	2	2
ID#6	1	2	2	3
Group D (SiHy, Polyquad)				
ID#1	1	1	2	2
ID#3	1	1	1	2

* C = central, T = temporal

Table 29: Numbers of hyper-reflective cells (cells/mm^2) for each participant (both eyes and corneal locations)*, when grade 3 (presence of hyper-reflective cells) was given

	OD		OS	
	C	T	C	T
Group A (FDA IV, PHMB)				
ID#7	0	0	0	0
ID#5	0	0	0	0
Group B (SiHy, PHMB)				
ID#2	0	0	0	0
ID#4	0	0	0	0
Group C (FDA IV, Polyquad)				
ID#8	0	0	0	0
ID#6	0	0	0	13
Group D (SiHy, Polyquad)				
ID#1	0	0	0	0
ID#3	0	0	0	0

* C = central, T = temporal

Figure 54 shows images of the central superficial cells in the right eyes for participants in Group A (FDA IV and PHMB) and Group B (SiHy and PHMB). The superficial cellular appearance is similar for the four participants. Grade 1 (presence of cells with more prominent margins) was given to ID#7, ID#2 and ID#4. Participant ID#5 received a grade 2 (presence of cells with prominent margins and contents). The bright lines seen in the images of ID#5 and ID#4 are artefacts resulting from the gel

used as coupling medium. Statistical analyses of superficial cellular appearance and for hyper-reflective cell count were not performed due to the small number of participants in each group as well as almost no variance in the data.

Images of the central superficial cells of the right eyes for all the participants in Group C (FDA IV and polyquad) and Group D (SiHy and polyquad) are shown in Figure 55. Again, the images look similar and they also look similar to the images in Figure 54. Grade 1 (presence of cells with more prominent margins) was given for all participants.

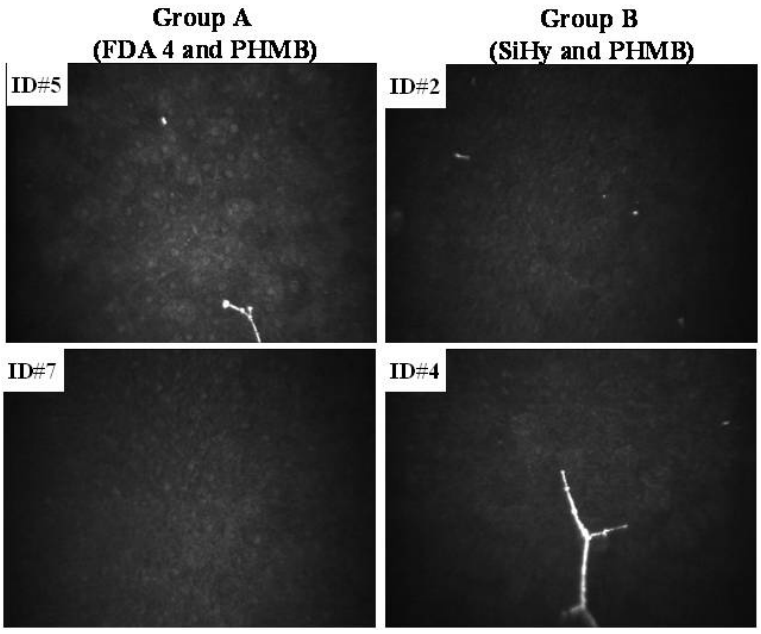


Figure 54: Examples of the superficial cellular appearance (OD only) for each of the participants in Group A and Group B

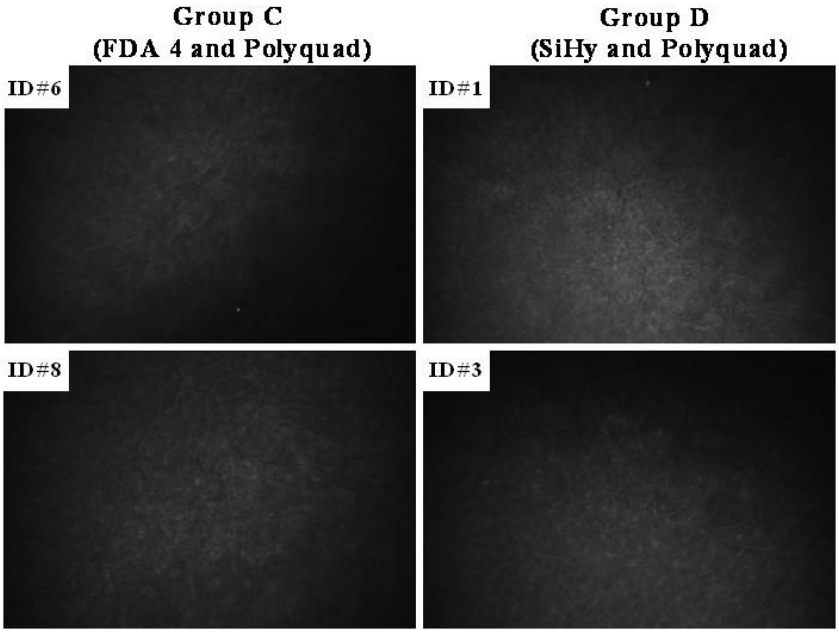


Figure 55: Examples of the superficial cellular epithelium (OD only) for each of the participants in Group C and Group D

Corneal staining (CS)

Table 30 shows the CS scores for the individual participants assigned to the test groups (use of PHMB based solutions). It can be seen that there are great variations in CS in each group as well as between the groups. The obtained CS scores are mainly clinically insignificant. Table 31 lists the CS scores for the individual participants assigned to the control groups (use of polyquad based solutions). Again, a great deal of variations was observed between the participants and between groups. All CS was considered clinically insignificant. The only participant who showed hyper-reflective cells was ID#6 (group C). The bright cells were observed in the temporal cornea of the left eye, however, as can be seen in Table 31 no CS was observed in this quadrant.

Table 30: Corneal staining for each participant in the two test groups (PHMB)

	Group A (PHMB with FDA IV lenses)				Group B (PHMB with SiHy lenses)			
	OD		OS		OD		OS	
	ID#5	ID#7	ID#5	ID#7	ID#2	ID#4	ID#2	ID#4
GSS (0 – 10000)	860	25	275	50	60	550	50	425
Temporal	875	0	375	0	125	500	0	500
Inferior	1000	125	250	250	50	500	250	250
Nasal	1500	0	250	0	125	750	0	875
Superior	875	0	375	0	0	625	0	250
Central	50	0	125	0	0	375	0	250

Table 31: Corneal staining for each participant in the two control groups (Polyquad)

	Group C (polyquad with FDA IV lenses)				Group D (polyquad with SiHy lenses)			
	OD		OS		OD		OS	
	ID#6	ID#8	ID#6	ID#8	ID#1	ID#3	ID#1	ID#3
GSS (0 – 10000)	60	20	75	55	85	135	25	0
Temporal	0	0	0	50	125	125	0	0
Inferior	250	0	375	125	250	50	0	0
Nasal	50	50	0	50	50	250	0	0
Superior	0	50	0	50	0	0	125	0
Central	0	0	0	0	0	250	0	0

Image analysis

Table 32 lists the hyper-reflective cell areas and standard deviations (SD) of central images of the superficial epithelium of each participant (OD and OS separately).

As can be seen for the majority of the images, the hyper-reflective cell area was zero. Therefore, no statistical analyses were performed.

Table 32: Hyper-reflective cell areas (%) and standard deviations (SD) for central superficial epithelial images (OD and OS) of participants who were using the same lens type and the same type of solution for a prolonged period (A=FDA IV/PHMB, B=SiHy/PHMB, C=FDA IV/polyquaternium-1, D=SiHy/polyquaternium-1)

ID (group)	OD		OS	
	Hyper-reflective cell area	SD	Hyper-reflective cell area	SD
5 (A)	0.48	17.56	0	0
7 (A)	0	0	0	0
2 (B)	0	0	0	0
4 (B)	0.32	14.41	0.55	18.90
6 (C)	0	0	1.53	31.22
8 (C)	0	0	0.46	17.19
1 (D)	0	0	0	0
3 (D)	0	0	0	0

Conclusion

In this sample, the long-term wear of the same type of contact lens and long-term use of the same type of solution does not affect the superficial cellular appearance or result in hyper-reflective cells, and it also does not result in greater hyper-reflective cell areas in images of the superficial epithelium.

Effect of direct application of contact lens solution on the superficial corneal epithelium

The preceding experiments have shown that the combination of specific lenses and solutions were associated with hyper-reflective cells. As the hyper-reflective cells occurred predominately when the PHMB-based solution was used in combination with the lenses, it is suggested that the solution and not the lens was the triggering factor for hyper-reflective cell appearance. Therefore this control experiment was designed to investigate if the solution itself had an effect on the appearance of the superficial epithelium and if it was a predicting variable for hyper-reflective cell appearance.

Relevant data for this thesis were collected as part of a study conducted at the CCLR. The purpose of this experiment was to examine whether contact lens care regimens by themselves had any impact on the occurrence of hyper-reflective cells.

Objective

The specific objective of this experiment was:

- To determine if hyper-reflective cells occur when directly applying ReNu MultiPlus (Bausch & Lomb, test) and saline solution (control) onto cornea.

Methods

Participants

Ten non-contact lens wearing participants reporting no signs or symptoms of dry eye were recruited for the study.

Study specific inclusion & exclusion criteria

A person was eligible if he/she:

1. Was correctable to a visual acuity of 6/9 or better (each eye), with their habitual visual correction.
2. Had not worn contact lenses within the last six months.

A person was ineligible if he/she:

1. Had any signs or symptoms of dry eye.

Study solutions

Two ophthalmic solutions were used: ReNu MultiPlus (Bausch & Lomb) (Table 9 in Chapter 2 General Methods) and Minims (sodium chloride 0.9% w/v, Chauvin Pharmaceuticals Ltd., Kingston, England) unit dose preservative-free saline solution (Health Canada DIN # 02148501). No other ocular solutions or lubricants were used by the participants during the study. In order to ensure investigator masking, an assistant instilled all study solutions. Solutions were instilled directly from the bottle via sterilized pipettes, 100 μL of fluid to the lower fornix every 10 minutes, for a total of 60 minutes, after which measurements were taken.

Study design and study visits

Ethics clearance was obtained through the Office of Research Ethics at the University of Waterloo and informed consent obtained prior to enrolment.

This experiment was a non-dispensing study, using a randomized crossover design.

At an initial screening and baseline visit, participant eligibility based on ocular history, symptoms and slit lamp biomicroscopy was determined. On eligible participants baseline CS was recorded and baseline CM images of the corneal epithelium were taken at this visit

.

On the first day of each study, an assistant instilled one of the study solutions, ReNu MultiPlus or saline solution (randomly assigned), in each of the participants' eyes. 100 μL of solution was instilled directly into the lower fornix from the bottle via a sterilized pipette, every 10 minutes for 60 minutes. Immediately following this, CM and CS assessment were performed. The second study solution was instilled in the same manner on a different day during the second phase.

Procedures

CM was performed as explained in Chapter 2. Only one randomly selected eye underwent CM. In addition to examining the central and temporal cornea, the mid-peripheral cornea was also assessed. In order to obtain the mid-peripheral measurements, the fixation target, explained in Chapter 2 (General Methods), was set to 15 degrees.

Grading and Analysis

Images of the superficial epithelium were identified and the appearance of the cells was subjectively graded using the grading scale described in Chapter 2.

CS was graded according to the CCLR GSS (Chapter 2). CS during this experiment was graded by an investigator, not the author.

Image analysis was performed as described in Chapter 2.

Statistical analysis

CS data were analyzed using repeated measures ANOVA.

Results

Participants

Ten non-contact lens wearing participants were enrolled in the study (4 female, 6 male) and all participants completed the study. The mean age of the participants was 27.8 ± 11.8 years. Some of the characteristics of participants are shown in Table 33.

Table 33: Participants' dioptric characteristics (mean ± SD)

		OD	OS
K-readings	Flat K	43.1 ± 1.2	43.1 ± 1.3
	Steep K	43.4 ± 1.6	43.8 ± 1.5
Corneal cylinder		-1.21 ± 0.7	-1.10 ± 0.6
Refractive error	Sphere	0.10 ± 2.0	0.00 ± 2.1
	Cylinder	-0.90 ± 1.0	-0.85 ± 0.8

Superficial cellular appearance and presence of hyper-reflective cells

As shown in Table 34, the median grades of the appearance of the superficial epithelial cells after the application of ReNu MultiPlus and saline were 1 (presence of cells with more prominent margins) for all three positions. The median grades at baseline were also 1for the centre and 2 (presence of cells with prominent margins and contents) for the mid-periphery and periphery, respectively. The appearance of the superficial epithelial cells was graded as three (presence of hyper-reflective cells)

for participant ID#3 at baseline (centre) and after direct application of ReNu MultiPlus (centre), for participant ID#7 after direct application of ReNu MultiPlus (periphery), and for participant ID#10 at baseline (periphery). Figure 56 shows the differences in superficial appearance grade between the application of ReNu MultiPlus and baseline as well as after the application of saline and at baseline, respectively for the different corneal positions. Statistical analyses showed the differences in superficial appearance grades for all the various combinations were insignificant (Wilcoxon matched pairs $p>0.050$). As can be seen in Table 35, the numbers of observed hyper-reflective cells were small. For participant ID#3 the hyper-reflective cell density was 6 cells/mm^2 identified at baseline (centre) and after ReNu MultiPlus (centre). Participant ID#7 also showed a hyper-reflective cell density of 6 cells/mm^2 after direct application of ReNu MultiPlus (periphery). For participant ID#10 a hyper-reflective cell density of 13 cells/mm^2 was identified at baseline (periphery). Due to little variance in the data, statistical analyses on hyper-reflective cell counts were not performed.

Table 34: Appearance of the superficial epithelial cells at baseline and after application of ReNu MultiPlus and Saline*

	Baseline			ReNu MultiPlus			Saline		
ID	**C**	**M**	**P**	**C**	**M**	**P**	**C**	**M**	**P**
1	1	2	-	1	1	1	0	1	1
2	1	1	1	1	1	2	1	1	1
3	3	2	-	3	2	2	2	2	2
4	2	2	2	2	1	1	1	1	1
5	1	1	2	0	0	1	0	1	0
6	0	1	2	1	1	1	1	0	0
7	0	1	1	1	1	3	1	1	2
8	2	2	2	1	1	1	1	1	1
9	1	2	1	1	1	1	1	1	0
10	2	1	3	1	1	0	0	1	0
Median	1	2	2	1	1	1	1	1	1
Min.	0	1	1	0	0	0	0	0	0
Max.	3	2	3	3	2	3	2	2	2

*(C = centre, M = mid-periphery, P = periphery)

Table 35: Numbers of hyper-reflective cells (cells/mm^2) when grade 3 (presence of hyper-reflective cells) was given*

	Baseline			ReNu MultiPlus			Saline		
ID	**C**	**M**	**P**	**C**	**M**	**P**	**C**	**M**	**P**
1	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0
3	6	0	0	6	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	6	0	0	0
8	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0
10	0	0	13	0	0	0	0	0	0
Sum	6	0	13	6	0	6	0	0	0

*(C = centre, M = mid-periphery, P = periphery)

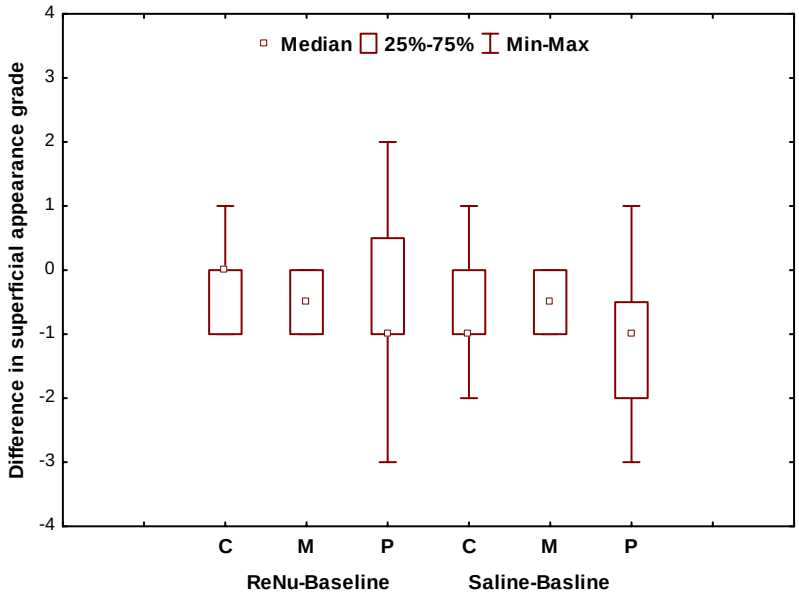


Figure 56: Differences for the superficial appearance grade (for the different corneal positions c=central, m=mid-peripheral, p=peripheral) between the application of ReNu and baseline as well as for the application of saline and baseline

Figure 57 is an example for the appearance of superficial cells for participant ID#6. Image A was obtained at the baseline visit and was graded as 0 (indistinctive cellular appearance), image B represents the superficial epithelium after the application of ReNu and image C shows the superficial epithelium after the application of saline. Grade 1 (presence of cells with more prominent margins) was given for image B and C, respectively.

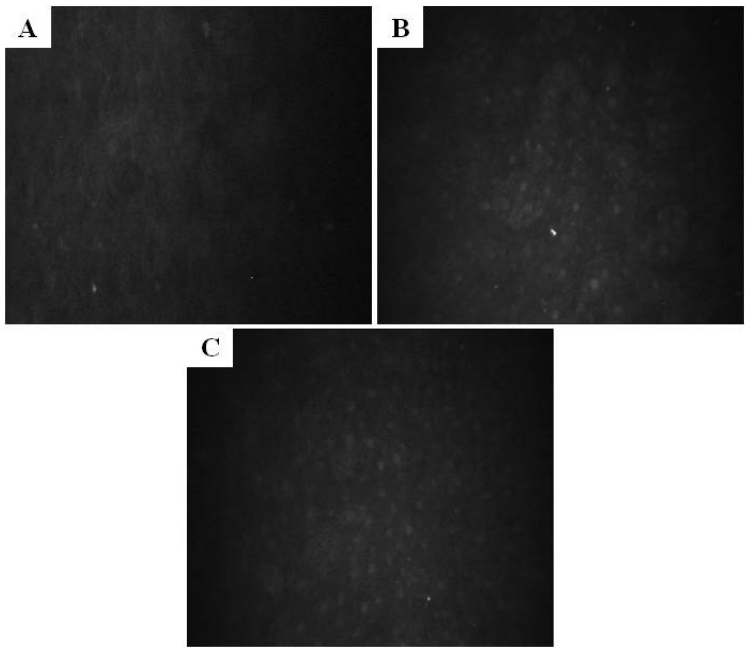


Figure 57: Examples of the superficial epithelium of one participant (ID#6) at baseline (A), after application of ReNu (B) and after application of saline (C)

Corneal staining (CS)

The data reported in Table 36 represent the mean (± SD) and the range of CS scores for the study eye. As shown in this table, no CS was found at baseline, except for one participant who had mild CS in the inferior quadrant. The central CS was minimal after application of both solutions.

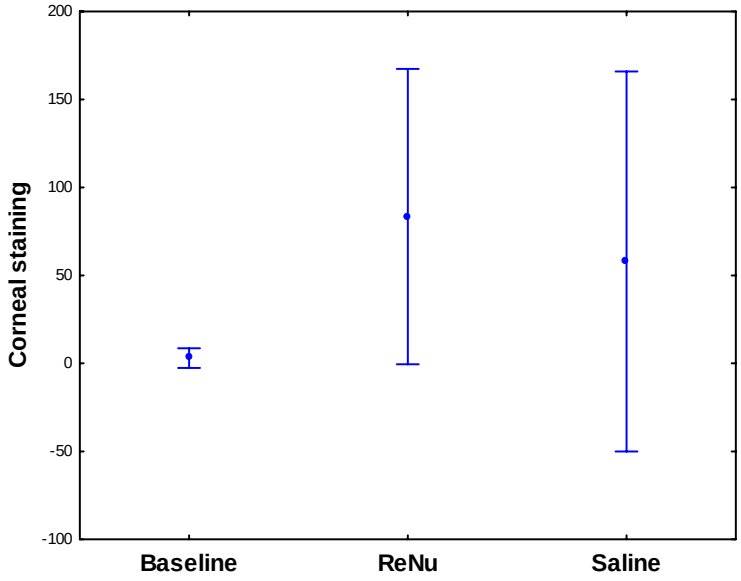
Table 36: Corneal staining (mean ± SD, range) at baseline, and after application of ReNu MultiPlus and Saline

	Visit/Solution		
	Baseline (mean ± SD) (range)	**ReNu MultiPlus (mean ± SD) (range)**	**Saline (mean ± SD) (range)**
Global CS Score (0-10 000)	3±7.9 (0-25)	84.5±117.3 (0-350)	58±150.9 (0-485)
Temporal	0.0±0.0 (0-0)	50±158.1 (0-500)	100±241.5 (0-750)
Superior	0.0±0.0 (0-0)	0.0±0.0 (0-0)	50±158.1 (0-500)
Nasal	0.0±0.0 (0-0)	62.5±135.0 (0-375)	50±158.1 (0-500)
Inferior	15±39.4 (0-125)	305±495.2 (0-1250)	85±195.5 (0-500)
Central	0.0±0.0 (0-0)	0.0±0.0 (0-0)	5±15.8 (0-50)

There was no statistically significant difference (RmANOVA p=0.297) in CS between solutions and across visits (Figure 58).

Corneal staining and presence of hyper-reflective cells

Table 37 lists the CS scores and the numbers of hyper-reflective cells (cells/mm^2) for those three participants (ID#3, ID#7 and ID#10) who received a grade 3 (presence of hyper-reflective cells). As can be seen, hyper-reflective cells were observed in small numbers and in no pattern. ID#3 was the only participant who manifested hyper-reflective cells during two visits (baseline and after direct instillation of ReNu Multiplus). Yet, only one cell at each visit was observed. No hyper-reflective cells were observed after the direct application of saline. No CS was observed for the three participants at the visit and location where hyper-reflective cells were found using CM.



Figu 58: Mean corneal staining across visits at baseline and after application of ReNu MultiPlus and saline

Table 37: Corneal staining (S) and numbers (cells/mm^2) of hyper-reflective cells (#) for those participants who exhibited hyper-reflective cells at the different visits and for the different corneal locations*

	Baseline						ReNu MultiPlus						Saline					
	C		M		P		C		M		P		C		M		P	
ID	S	#	S	#	S	#	S	#	S	#	S	#	S	#	S	#	S	#
3	0	6	--	--	--	--	0	6	--	--	--	--	--	--	--	--	--	--
7	--	--	--	--	--	--	--	--	--	--	0	6	--	--	--	--	--	--
10	--	--	--	--	0	13	--	--	--	--	--	--	--	--	--	--	--	--

* (C = central, M = mid-peripheral, P = peripheral)

Image analysis

Image analysis performed on the images resulted mainly in hyper-reflective cell areas of zero or close to zero. Due to this and therefore no measurable variance in the data for some points, statistical analysis was not performed. The hyper-reflective cell area data for each participant, each solution and

corneal location is listed in Table 38. The greatest hyper-reflective cell areas were measured for participant ID#8 in a peripheral image after application of saline.

Table 38: Hyper-reflective cell areas (%) of images of the superficial epithelium of participants who underwent direct application of different ophthalmic solutions. (BC=baseline/centre, BM=baseline/mid-periphery, BP=baseline/periphery, SC=saline/centre, SM=saline/mid-periphery, SP=saline/periphery, RC=ReNu/centre, RM=ReNu/mid-periphery, RP=ReNu/periphery)

ID	BC	BM	BP	SC	SM	SP	RC	RM	RP
1	0.02	0.13	--	0.00	0.00	0.51	0.00	0.82	0.38
2	0.00	0.17	--	--	0.89	2.33	--	--	0.42
3	0.00	0.00	0.63	0.00	0.00	--	0.00	0.00	0.00
4	0.00	0.00	0.41	0.00	0.00	0.00	0.00	0.00	0.00
5	--	0.00	1.31	0.00	0.00	1.44	0.00	1.07	0.05
6	0.00	0.21	0.68	0.00	0.00	0.00	0.00	0.00	--
7	0.00	0.00	0.00	0.23	0.14	0.15	0.00	0.00	0.00
8	0.00	0.00	0.03	0.00	--	4.79	0.00	0.00	1.46
9	0.00	0.00	0.00	0.00	--	0.00	0.01	0.05	0.10
10	0.00	--	0.00	--	0.00	0.00	0.00	0.00	0.00
Mean	**0.00**	**0.06**	**0.38**	**0.03**	**0.13**	**1.02**	**0.00**	**0.22**	**0.27**
SD	0.01	0.09	0.47	0.08	0.31	1.63	0.00	0.42	0.48
Min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Max	0.02	0.21	1.31	0.23	0.89	4.79	0.01	1.07	1.46

Conclusion

There was no difference in superficial cellular appearance at baseline and after direct application of ReNu Multiplus or saline for either corneal position. Small numbers of hyper-reflective cells were randomly observed for 3 participants. The observed CS was clinically insignificant and image brightness in superficial epithelial images did not seem to be affected when and where the images where taken.

General Discussion

The experiments conducted and described in this chapter, aimed to investigate qualitatively the superficial epithelium while and after being exposed to various conditions and specifically to determine if certain contact lens/solution combinations, prolonged wear of certain contact lens/solution combinations and contact lens solutions by themselves were associated with the appearance of hyper-reflective cells.

Effect of lens/solution combinations on the superficial corneal epithelium 1

The first experiment in this chapter was to compare the effect of specifically selected lens/solution combinations on the superficial epithelium. The experiment was to compare an investigational PHMB-based MultiPurpose solution to an already marketed polyquad-based product (RepleniSH). The two solutions were used in combination with four SiHy lenses (PureVision, Advance, Oasys and O_2Optix). The only information that was available for the investigational (test) solution was that it was a PHMB-based product. The amount of PHMB, as well as any other ingredients, was not disclosed. There were 100 participants who were randomized to wear any of the four lenses (four equal groups of 25 participants).

By Day 14, SICS was found to be significantly worse with the test solution when used in combination with Advance, Oasys and PureVision. The test solution in combination with O_2Optix did not result in high amounts of SICS as did the control solution (RepleniSH) in combination with the four different lenses. This result is in agreement with other studies that have shown that PHMB-based MultiPurpose solutions result in higher amounts of SICS, especially when used in combination with PureVision lenses, in comparisons to polyquad-based solutions.[29;31] The results in the present experiment appeared to confirm the proposal of Amos[125] that the specific disinfectant (PHMB) used in the solutions is not only implicated in SICS. He suggested that maybe additionally to the disinfectant, the combination and amount of other ingredients could also play a role in inducing SICS. There was significantly more CS on the temporal side than in the centre in the test solution group. This finding is also in agreement with a study showing that this type of CS follows an annular pattern.[29]

Hyper-reflective superficial cells were observed in some participants during both study visits (baseline and Day 14). However for the baseline visits, their counts were low and their presence

appeared to be unrelated with respect to specific participants, corneal locations (central and temporal) and lens/solution combinations. The same observation was made with RepleniSH in combination with the four different lenses and with the test solution in combination with O_2Optix at Day 14. On the other hand, the hyper-reflective cell counts at Day 14 with the test solution in combination with Advance, Oasys, and PureVision were significantly increased, in both central and temporal cornea, and were in the range observed in the study by Harvey.[1] Of interest was that O_2Optix was the only lens in combination with the test solution that did not result in high amounts of SICS and high counts of hyper-reflective cells. This was the only lens used in this experiment that has a special surface treatment (25nm plasma coating) that covers the entire lens, and this may have prevented ingredients of this specific solution to adsorb on the lens. The other three lenses used in this experiment either have no surface treatment or use plasma oxidation process that results in so-called glassy islands on the lens (Table 3, Chapter 1).

Image analysis was performed only on ideal superficial epithelial images obtained at the two week visit of the central and temporal cornea. Significantly greater hyper-reflective cell areas were measured for central and temporal superficial epithelial images from corneas exposed to the different test combinations, particularly with Advance, compared to the different control combinations. This observation suggests that white objects (cells) were probably present in some of those images and that the images were brighter on average. The observed standard deviations also indicate that there was spread in the measurements, indicating that the hyper-reflective cell areas in the measured images had a great deal of variability. Both of these observations were in agreement with the subjective grading of the central and temporal superficial epithelial images. Hyper-reflective cells were predominately noted with certain test combinations. However, not every participant who was exposed to these certain test combinations did manifest hyper-reflective cells. The highest counts of hyper-reflective cells were observed with the test solution in combination with Advance. This combination also had the greatest hyper-reflective cell areas. It has to be noted that the average hyper-reflective cell areas for superficial epithelial images of the control combinations was not 0. This, in addition to the observed spread in the data probably indicates that there may have also been luminous objects detected in images of corneas exposed to the control combinations. Those objects could have been hyper-reflective cells, as also identified with the subjective grading scale. The experiment showed that the solution or contact lens wear by itself was not simply associated with hyper-reflective cell occurrence but rather what specific lens/solution combination was used.

A criticism might be that prior to the CM procedure sodium fluorescein was used in order to obtain the CS scores. Therefore, sodium fluorescein residue may have influenced the cells imaged using CM. This idea is suggested by Mocan et al.[279] The authors have, during their experiment, observed hyper-reflective cells in patients with epithelial involvement and in particular in patients with keratoconus and a history of contact lens wear. Mocan et al.[279] hypothesized that a disruption of the tight junctions and a therefore associated rapid epithelial and stromal diffusion of fluorescein may have resulted in hyper-reflective cells. As a result of their[279] conclusion, the possible effect of sodium fluorescein on the superficial appearance will be addressed and investigated in Chapter 5 of this thesis.

Effect of lens/solution combinations on the superficial corneal epithelium 2

This control experiment in this chapter was to examine the effects of a different set of lens/solution combinations on the corneal epithelium. The solutions used were RepleniSH (polyquad-based) and Clear Care (peroxide hydrogen based). The latter is perhaps a "gold standard" solution as it is reported to not cause SICS.[31;178] The lenses worn during this study were Oasys and O_2Optix. 26 participants completed both study phases. CS scores for the different lens/solution combinations were very low and clinically insignificant. These findings are in accord with previous observations[31;178] suggesting that these lens/solution combination do not result in high amounts of SICS.

A grade of 3 (presence of hyper-reflective cells) was reported for a few participants and seemed to be independent of the lens/solution combination. Only participants in the O_2Optix/RepleniSH combination group did not manifest any hyper-reflective cells. The numbers of hyper-reflective cells observed ranged from 1-7 hyper-reflective cells per image (approx. 6 - 45 cells/mm^2) and were below the numbers reported by Harvey[1] as well as the numbers of hyper-reflective cells obtained during the previous experiment of this thesis. Also, the numbers of hyper-reflective cells in a single image varied widely between the participants. For the difference in proportions (0/24 and 2/24) of observed hyper-reflective cells to be significant at 80% power and with a 0.05 significant level an overall sample size of 82 participants would be required.

When comparing the individual CS scores to the number of hyper-reflective cells of those participants who had grade 3 for superficial epithelial cellular appearance, one participant had clinically

insignificant CS. This person had CS while being exposed to all lens/solution combinations. Again, these findings would suggest that, as the numbers of observed hyper-reflective cells were so low and that their occurrence was not related to SICS, their presence would be more related to a normal turnover process.[40]

Effect of long-term use of the same type of contact lens and contact lens solution on the superficial corneal epithelium

The second control experiment described in this chapter was to investigate if hyper-reflective cells were present in participants who had been wearing the same lens type and had been using the same type of solution for a prolonged period of time (at least two years).

The result of this experiment showed that in only one participant a small count of hyper-reflective cells (12 cells/mm^2) was found. That participant wore Acuvue 2 lenses (FDA Group IV) and used OptiFree Express (polyquad-based) for a prolonged period. The hyper-reflective cells were observed on the temporal side of the left eye only. However, the sample size of this experiment was only n=2 for the different groups. For the difference in proportions (1/2 and 0/2) of observed hyper-reflective cells to be significant at 80% power and with a 0.05 significant level a sample size of 11 participants in each group would be required.

No CS was observed for this participant at this position. This would suggest that the presence of these hyper-reflective cells could be a normal observation as it is reported in the literature and may be result of normal epithelial turnover.[40]

Effect of direct application of contact lens solution on the superficial corneal epithelium

The last control study was to examine if contact lens solution by itself was associated with hyper-reflective cell appearance.

In general, CM revealed no effects of ReNu MultiPlus or control solutions (saline) on the superficial epithelial morphology. The median grade of the appearance of the superficial epithelial cells after direct application of ReNu MultiPlus and saline was one (cells with more prominent margins) for all corneal positions. Interestingly, the grade at baseline in the mid-periphery and periphery was 2 (presence of cells with prominent margins and contents). Also, for three participants, hyper-reflective cells were identified at individual visits and in particular positions (baseline, centre and periphery,

and after direct application of ReNu MultiPlus, centre and periphery). However, the numbers of hyper-reflective cells in a given image were only 6 or 12 cells/mm^2 while the number of these cells in an image reported by Harvey[1] ranged between 8 - 25 (approx. 51 – 160 cells/mm^2) when using PureVision lenses and ReNu MultiPlus. Based on these observations with this experiment sample (n=10) there was no difference in observing hyper-reflective cells between the visits (solutions). For the difference in proportions (1/10 and 0/10) of observed hyper-reflective cells to be significant at 80% power and with a 0.05 significant level a sample size of 58 participants in each group would be required.

When looking at the CS scores at the specific positions for those participants in which hyper-reflective cells were observed, no CS was reported. This would indicate that the presence of those few bright, hyper-reflective superficial cells was not related to CS. This is also suggested as some of these cells were observed at the baseline visit.

The image analysis outcome seems to support the findings of the subjective grading of the superficial epithelium that the superficial epithelium after the direct application of ReNu MultiPlus or saline did not appear to be different from baseline. For the majority of participants, images at the different visits (baseline and exposure to two ophthalmic solutions) and corneal locations, showed hyper-reflective cell areas of either 0 (all black background) or close to 0.

The experiments conducted in this chapter of the thesis showed that hyper-reflective cells are associated with wearing specific lens/solution combinations. However, contact lens wear or solution use by itself, or wearing the same specific contact lens/solution combination for a period of time did not to result in the appearance of hyper-reflective cells.

Two possible variables that could have an effect on superficial cellular appearance or cause hyper-reflective cells are the use of sodium fluorescein and topical anaesthetics. The influence of both of these variables will be examined in the next chapter.

Chapter 5

Effect of Diagnostic Agents

General Introduction

One criticism of the study conducted by Harvey[1] was that the use of sodium fluorescein prior to CM to examine the cornea could have been responsible for the observation of hyper-reflective cells. This was also suggested by Mocan et al.[279] who concluded that sodium fluorescein had induced hyper-reflective cells in participants with keratoconus, possibly as a result of damaged epithelial tight junctions resulting in epithelial sodium fluorescein diffusion.

As proposed by Harvey[1] and as suggested as an outcome of the experiments in Chapter 4 the appearance of hyper-reflective cells may be associated to SICS which was caused by specific lens/solution combinations.[29-31;125] These combinations of contact lenses and lens care solutions interact to produce a characteristic pattern of corneal staining; typically punctate in appearance and more prominent in the periphery of the cornea, with only marginal central corneal involvement.[29] This typically asymptomatic SICS is most evident during the first two to four hours of contact lens wear, with reduced residual SICS after six hours of contact lens wear.[29;30] SICS can be visualized using sodium fluorescein and the hypotheses are that sodium fluorescein stains damaged cells, possibly living cells, penetrates intra-cellular spaces and/or pools in gaps of dropped out cells.[40;165] As in both Harvey's[1] study and during the experiments in this thesis, the corneas were examined prior to CM using sodium fluorescein, and it could have been possible that the observed hyper-reflective cells were sodium fluorescein induced artefacts. Therefore the experiments described in this chapter intended to investigate if sodium fluorescein was a predicting variable for observing hyper-reflective cells.

Another variable that could have been responsible for the appearance of hyper-reflective cells is the topical anaesthetic that was used during the CM. It has been reported[302;303] that topical anaesthesia can cause a certain surface toxicity and therefore possibly have an effect on the superficial appearance. The toxic effect of topical anaesthetics has been stated to inhibit the rate of corneal epithelial cell migration by disrupting the cytoplasmic action in filaments and to disrupt the superficial corneal

epithelial microvilli.[302;303] Therefore, in addition to sodium fluoresein one experiment in this Chapter was to investigate the effect of anaesthesia on hyper-reflective cell appearance.

Effect of sodium fluorescein

This experiment was designed to investigate the influence of sodium fluorescein used prior to CM on the superficial appearance in participants who were and were not exposed to manifest SICS.

Objective

The specific objective of this pilot experiment was:

- To investigate if the preceding use of sodium fluorescein affected the cellular appearance of superficial epithelial cells.

Methods

This control experiment consisted of two phases. In phase one, the superficial epithelium of participants was investigated before and after the application of sodium fluorescein. In phase two, SICS was provocatively induced and in both eyes and the superficial epithelium was then imaged. Only one eye was exposed to sodium fluorescein prior to CM.

Participants

For each phase, 10 participants were recruited.

Study specific inclusion & exclusion criteria

A person was ineligible if he/she:

1. Had a known sensitivity to the contact lens care solutions used in the study.

Study design and study visits

This experiment was conducted as a non-dispensing one day study. At an initial screening visit eligibility of participants was assessed.

Phase one

If participants were habitual contact lens wearers, they were asked to not wear contact lenses for 24 hours prior to the study visit. For phase one, the right eye of each participant underwent the procedures. Study sequence and procedures are shown in Figure 59.

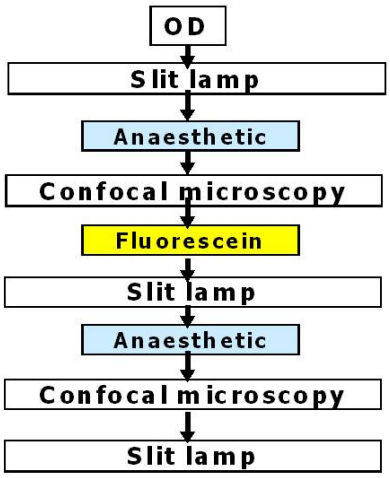


Figure 59: Study design for phase one

After the cornea was checked, without the use of sodium fluorescein, a drop of anaesthetic was instilled and CM was performed at the corneal apex. Then sodium fluorescein was instilled and the cornea re-examined. After seven minutes another drop of anaesthetic was instilled and CM was again performed at the corneal apex. Corneas were then re-checked.

Phase two

Figure 60 shows the study sequence and performed procedures during phase two of the experiment.

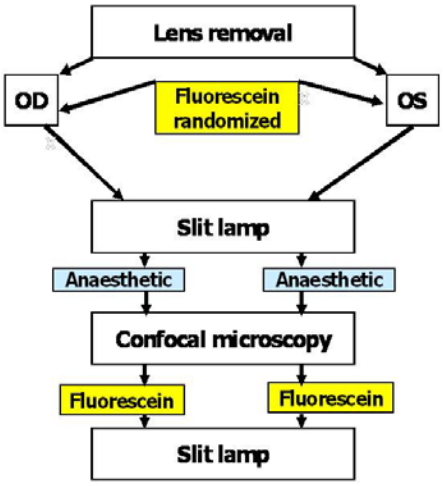


Figure 60: Study design for phase two

At the study visit of phase two, each participant used the previously optimally fitted PureVision lenses for each eye. The lenses were pre-soaked overnight in ReNu Multiplus. Participants wore the lenses for 2 – 3 hours. The lenses were then removed and both eyes were examined using a slit lamp, but sodium fluorescein was used on one eye only. Then a drop of anaesthetic was instilled onto both eyes and CM was performed on both eyes (central and temporal). After this, both corneas were re-examined using sodium fluorescein.

Participant eligibility for phase two was assessed at a screening visit. They were also trial fitted with Bausch & Lomb PureVision lenses to determine whether a good fit was achievable.

Study solution

During phase two of the study, lenses were pre-soaked overnight in ReNu MultiPlus (Bausch & Lomb). Details of the solution can be found in Table 9 (Chapter 2 General Methods). Lenses were stored in a Bausch & Lomb case for the ReNu Multiplus solution.

Study lenses

PureVision lenses (Bausch & Lomb) were used in this study (Table 8, Chapter 2 General Methods).

Procedures

Phase one

CM was performed only at the corneal apex of the right eyes, according to the procedures described in Chapter 2.

Phase two

CS was evaluated as described in Chapter 2. All observed CS during this experiment was graded by the thesis author. CM was performed according to the procedures illustrated in Chapter 2.

Grading and Analysis

Phase one and two

Images of the superficial epithelium were identified and the appearance of the cells subjectively graded using the grading scale described in Chapter 2.

CS was graded according to the CCLR GSS (Chapter 2).

Image analysis was performed as described in Chapter 2.

Statistical analysis

Hyper-reflective cell count was analyzed using Fisher's exact test (chi-square 2x2 contingency table). CS and image analysis data were analyzed using repeated measures ANOVA.

Results for phase one

Participants in phase one

Ten participants were enrolled in this study (one male). The mean age of the participants was 31.6 years (median 29.5 years, ranging from 24 years to 57 years).

Superficial cellular appearance and presence of hyper-reflective cells in phase one

Table 39 shows the grades given for superficial cellular appearance before and after the use of sodium fluorescein for each participant. As can be seen, grade 3 (presence of hyper-reflective cells) was not assigned. It can be seen in Figure 61 that there were, except for two participants, no differences (after-

before the use of sodium fluorescein) in superficial appearance grades before and after the use of sodium fluorescein.

Table 39: Superficial appearance grades for each participant before and after the use of sodium fluorescein (phase one)

Participant	before sodium fluorescein	after sodium fluorescein
1	1	2
2	1	1
3	0	0
4	2	2
5	1	2
6	0	0
7	2	2
8	1	1
9	1	1
10	0	0

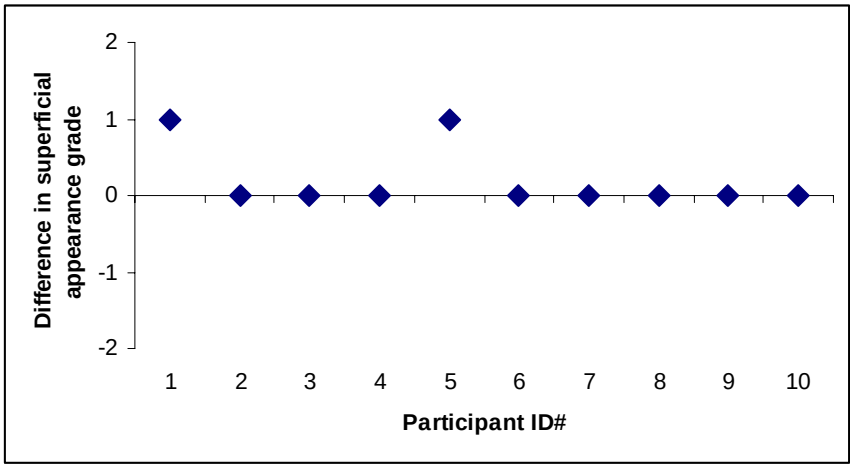


Figure 61: Differences in superficial appearance grade (after-before) in phase one for each participant (after sodium fluorescein and before sodium fluorescein)

Figure 62 is an example of images of the superficial epithelium of one participant. Image A represents the superficial cells prior to and image B represents the superficial cells after the instillation of

sodium fluorescein. As can be seen, the appearance of the cells is the same. Therefore, in both cases, grade 1 (presence of cells with more prominent margins) was given.

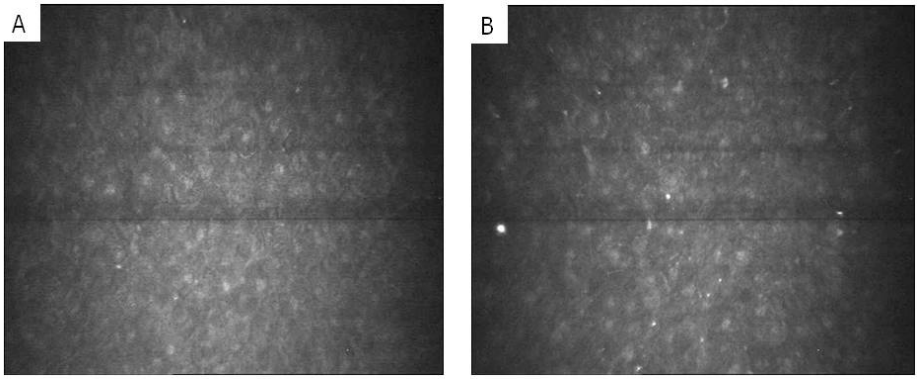


Figure 62: Example images of one participant for the appearance of superficial cells in phase one (A=before and B=after the use of sodium fluorescein). Grade 1 (presence of cells with more prominent margins) was given, respectively

Image analysis for phase one

Image analysis was performed on images obtained in both phases. Results will be shown in the results section of part two of this experiment.

Results for phase two

Participants in phase two

Ten participants were in enrolled in this part of the study (one male). The mean age of the participants was 40.2 years (median 33 years, ranging from 29 years to 61 years).

Superficial cellular appearance and presence of hyper-reflective cells in phase two

The superficial cellular appearance grades given for both eyes (no sodium fluorescein and sodium fluorescein) of each participant (both corneal locations) are listed in Table 40. As can be seen, assigned grades were similar with and without prior use of sodium fluorescein. This is also illustrated in Figure 63, where the differences in grades (sodium fluorescein-no sodium fluorescein) are plotted for both corneal positions. For two participants (ID#5 and ID#7) the grade of the central superficial

cells was higher without the use of sodium fluorescein. For ID#7, the temporal grade was higher with the use of sodium fluorescein. These three participants, were the only ones who did not receive a grade 3 (presence of hyper-reflective cells) for either corneal position (Table 40). Table 41 shows the numbers of hyper-reflective cells (cells/mm^2) for those participants who received grade 3.

Table 40: Superficial cellular appearance grade in phase two for both eyes of each participant and for corneal locations*

	No Sodium fluorescein		Sodium fluorescein	
ID	**C**	**T**	**C**	**T**
1	3	3	3	3
2	3	3	3	3
3	1	1	1	1
4	3	3	3	3
5	3	3	2	3
6	3	3	3	3
7	2	1	1	1
8	1	2	1	1
9	3	3	3	3
10	3	3	3	3

* C=central, T=temporal

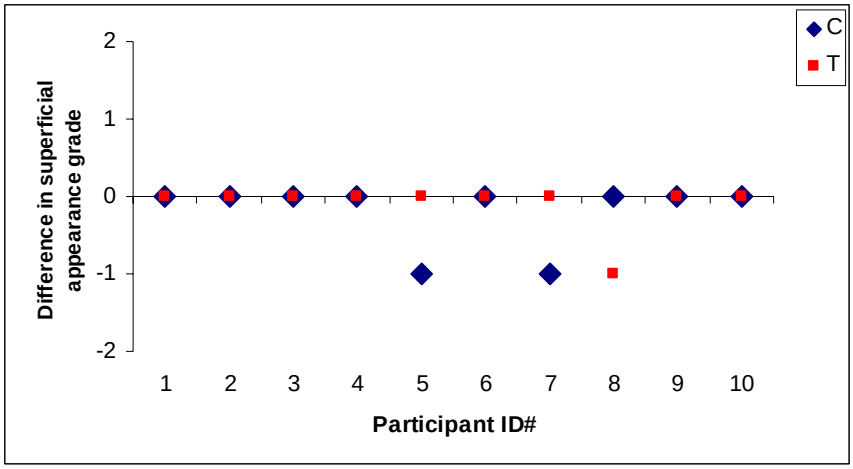


Figure 63: Differences in cellular appearance grade in phase two between the eye that had sodium fluorescein and the eye that had no sodium fluorescein prior to the CM procedure, for both corneal positions (c=central, t=temporal)

Table 41: Numbers of hyper-reflective cells (cells/mm^2) in phase two for each participant, when grade 3 (presence of hyper-reflective cells) was given*

	No Sodium fluorescein		Sodium fluorescein	
ID	**C**	**T**	**C**	**T**
1	26	38	32	26
2	19	26	26	19
3	0	0	0	0
4	86	96	58	77
5	77	86	0	13
6	38	45	32	45
7	0	0	0	0
8	0	0	0	0
9	32	58	64	51
10	15	70	86	96

* C=central, T=temporal

Statistical analysis showed that there was no statistically significant association (Fisher's exact test 2x2 contingency table p=0.857) between the number of hyper-reflective cells and whether sodium fluorescein was used prior to CM in participants with provocatively induced SICS.

Corneal staining (CS) in phase two

The CS scores for each participant are shown in Table 42. When looking specifically at the three participants (ID#3, ID#7 and ID# 8) who did not have hyper-reflective cells in either central or temporal position, it is interesting that ID#8 showed minimal CS for the temporal and no CS for the central position. For ID#7, the temporal CS score was higher but the central was low. For ID#3, CS scores were high for both corneal positions. ID#5 who had no central CS did not exhibit any hyper-reflective cells in the eye where sodium fluorescein was instilled prior to the CM procedure. However, cells were observed in the eye that did not have sodium fluorescein prior to the CM procedure.

Table 42: Corneal staining for each participant and for the eye that had sodium fluorescein prior to the confocal microscopy procedure during phase two

	Sodium fluorescein					
ID	**Temporal**	**Inferior**	**Nasal**	**Superior**	**Central**	**GSS**
1	750	1750	1000	1000	625	1025
2	625	75	375	250	125	425
3	2250	24000	2000	2250	1500	280
4	2125	2000	2000	1750	1500	1875
5	375	625	125	250	0	275
6	1750	1250	1750	1750	1250	1550
7	900	875	1050	50	125	640
8	125	250	0	125	0	100
9	1500	2100	1800	1580	1750	1730
10	1000	900	1500	1500	1000	1180
Average	**1140**	**1290**	**1160**	**1063**	**788**	**1088**
SD	**731**	**726**	**771**	**789**	**695**	**709**
Min	**125**	**250**	**0**	**125**	**0**	**100**
Max	**2250**	**2400**	**2000**	**2250**	**1750**	**2800**

Figure 64 shows images of the central superficial epithelium of a participant (ID#8) who had no central CS (Table 42). Image A is of the superficial epithelium of the eye that had sodium fluorescein prior to the CM procedure (grade 1, presence of cells with more prominent margins), and Image B is of the superficial epithelium of the eye that had no sodium fluorescein prior to the CM procedure (grade 0, indistinctive cellular appearance).

Images of the superficial epithelium of a participant (ID#10) who had a high score of central CS (Table 42) are presented in Figure 65. Image A is the superficial epithelium of the eye that had sodium fluorescein prior to the CM procedure (grade 3, presence of hyper-reflective cells), and Image B is from the eye that had no sodium fluorescein prior to the CM procedure (grade 3).

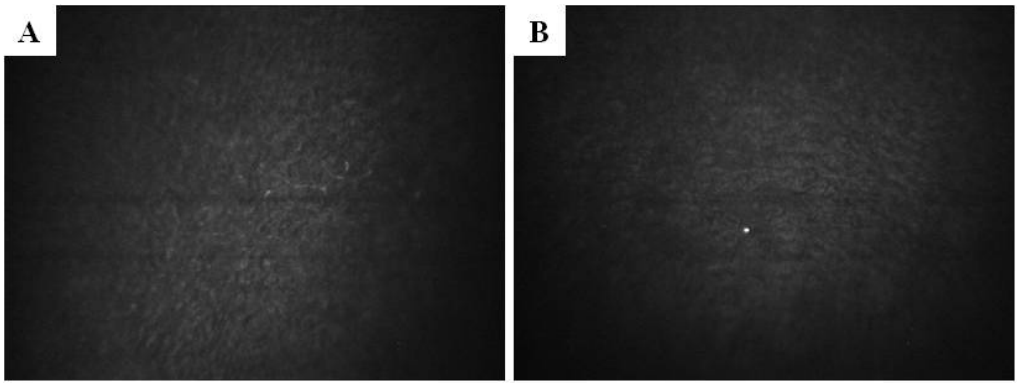


Figure 64: Images of the central superficial epithelium of a participant (ID#8) in phase two who had minimal CS (Table 42) and no hyper-reflective cells, image A=with sodium fluorescein and image B=without sodium fluorescein

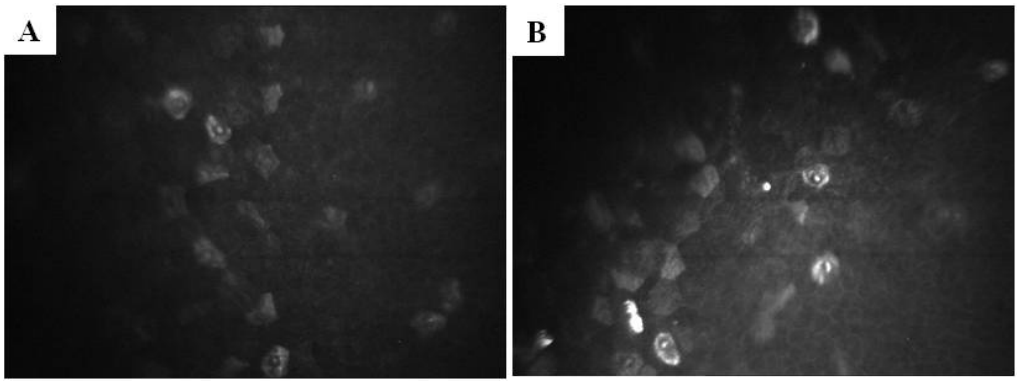


Figure 65: Images of the superficial epithelium of a participant (ID#10) in phase two who had high amounts of CS (Table 42) and exhibited hyper-reflective cells, image A=with sodium fluorescein and image B=without sodium fluorescein

Image analysis for phase one and two

Statistical analysis was combined for both phases. The sample size was n=14.

Figure 66 illustrates that images of the superficial epithelium of participants who were provocatively exposed to exhibit SICS showed statistically significantly greater (RmANOVA p=0.019) hyper-reflective cell areas than participants who were not exposed to the lens/solution combination.

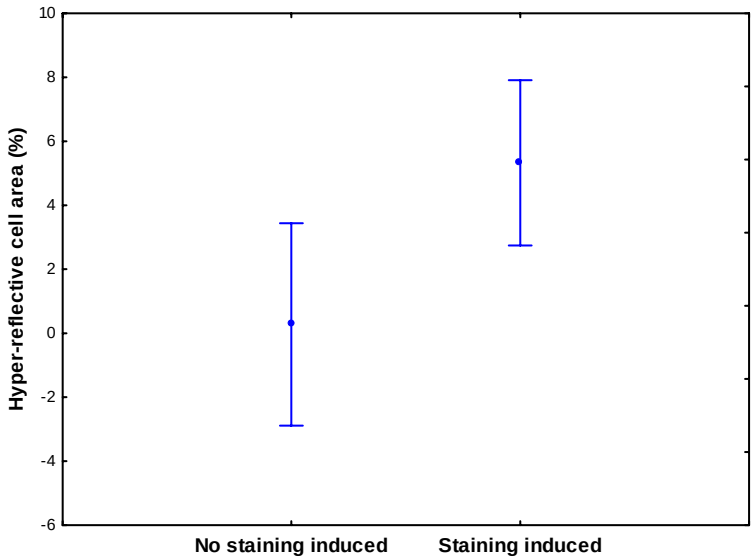


Figure 66: Hyper-reflective cell areas (%) of superficial epithelial images for participants who did not have SICS and participants who were provocatively exposed to exhibit SICS

Analysis of the standard deviations of images of the superficial epithelium in participants who provocatively exhibited SICS were also statistically significantly higher (RmANOVA p=0.026, Figure 67).

The effect of sodium fluorescein on the hyper-reflective cell areas of superficial epithelial images was not statistically significant (RmANOVA p=0.919). This is illustrated in Figure 68. This observation is also supported when analyzing the standard deviations. No statistically significant difference was found (RmANOVA p=0.710).

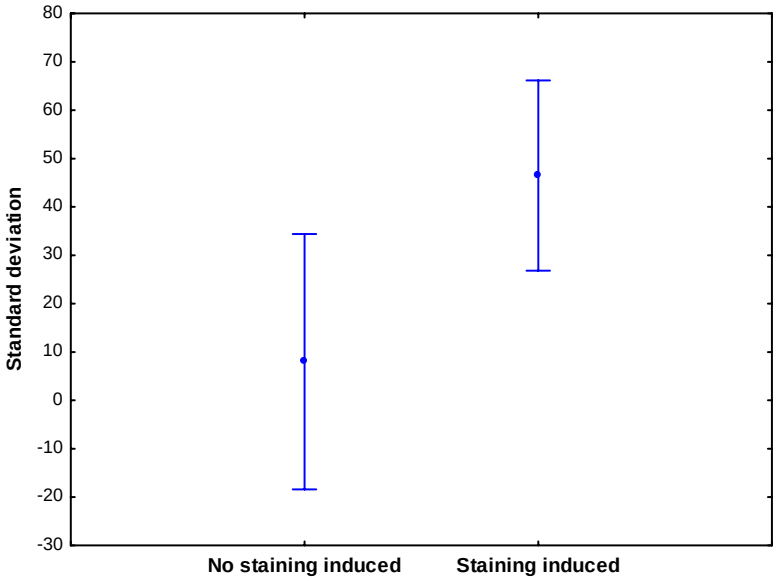


Figure 67: Standard deviations of superficial epithelial images for participants who did not have SICS and participants who were provocatively exposed to exhibit SICS

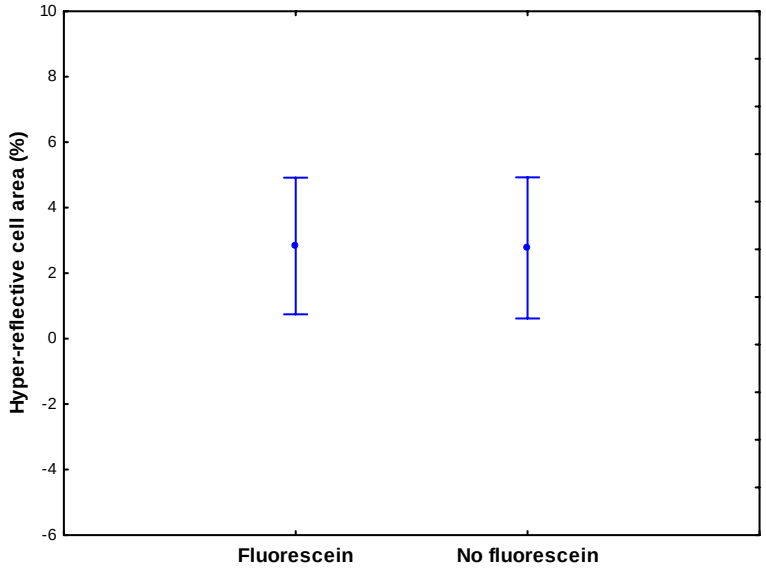


Figure 68: Hyper-reflective cell areas (%) of images of the superficial epithelium with prior use of sodium fluorescein and with no use of sodium fluorescein

Figure 69 shows that, although hyper-reflective cell areas appeared greater if SICS was induced (independent of the use of sodium fluorescein), the difference in hyper-reflective cell areas was not statistically significant (RmANOVA p=0.539). Analysis of the standard deviations was similar with p=0.167 (RmANOVA).

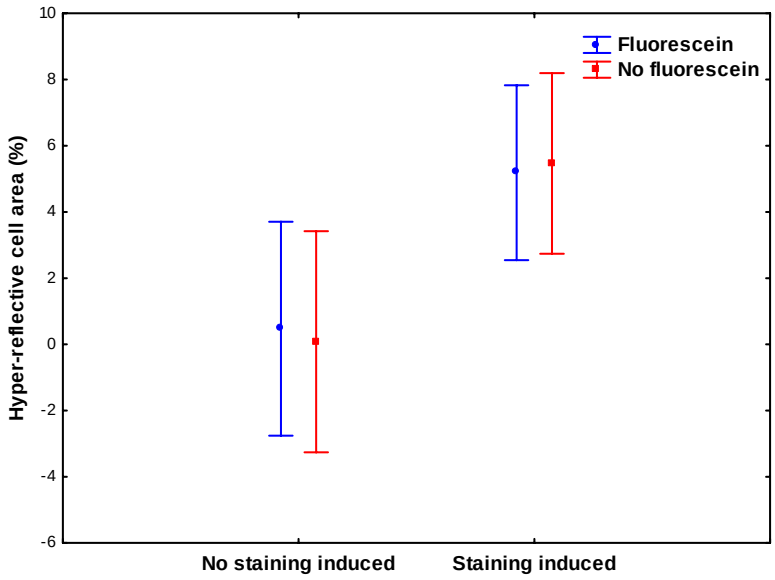


Figure 69: Hyper-reflective cell areas (%) of superficial epithelial images of participants who were and were not exposed to exhibit SICS as well as used or did not use sodium fluorescein prior to the CM procedure

Conclusions for phase one and two

Phase one of this experiment suggests that the use of sodium fluorescein prior to CM does not have an effect on the normal superficial cellular appearance and does not produce hyper-reflective cells.

Phase two of the study confirms that even after provocatively inducing SICS, the use of sodium fluorescein prior to the CM procedure is not responsible for the presence of hyper-reflective cells.

Image analysis showed that there was no difference in brightness of superficial epithelial images if sodium fluorescein was used prior to the CM procedure or not. However, the presence of SICS results in increased image brightness in images of the superficial epithelium.

Effect of anaesthetic and sodium fluorescein

The previous experiment showed that sodium fluorescein was not associated with the presence of hyper-reflective cells. It did show that hyper-reflective cells seemed to be associated with the presence of SICS induced by a specific lens/solution combination. Therefore the present experiment was designed to examine the corneal epithelium after provocatively inducing a SICS response. Specific interest was to observe the appearance of the superficial epithelium over time as SICS is reported to be most prominent after 2 – 4 hours of lens wear.[29;30] In order to be minimally invasive, sodium fluorescein was not instilled prior to the CM procedure and contact lenses were not removed during the CM procedure. The purpose of the latter was to eliminate the effect of anaesthetics on the superficial epithelium. CM with the lens *in situ* has to the best of my knowledge not been mentioned in the literature.

Objectives

The specific objectives of this experiment were:

- To investigate the prevalence of hyper-reflective cells with while using ReNu MultiPlus (ReNu, Bausch & Lomb) and Clear Care (CIBA Vision) in phase one, and SoloCare Aqua (CIBA Vision) and control soltion Clear Care in phase two, while eliminating the use of sodium fluorescein and anaesthetics.
- To characterize the temporal change and appearance of hyper-reflective cells.
- To compare the presence of hyper-reflective cells the central and peripheral cornea.

Methods

Participants

Four participants, who demonstrated a positive SICS reaction with the PureVision/ReNu combination (phase one) after two hours of lens wear were enrolled in the study. The same four participants were then enrolled in phase two.

Specific inclusion & exclusion criteria

A person was eligible if he/she:

1. Was a current soft contact lens wearer.
2. Had a positive SICS reaction with the PureVision/ReNu combination.
3. Had a refractive power within the range of the available lenses (+6.00D to -8.00D).

A person was ineligible if he/she:

1. Had any signs or symptoms of dry eye.

Study solutions

The test solutions that were used in this experiment were ReNu MultiPlus (Bausch & Lomb) and SoloCare Aqua (CIBA Vision) and the control solution was Clear Care (CIBA Vision). All care systems are commercially available and approved by Health Canada. For details to the different care systems, the reader is referred to Table 9 in Chapter 2 (General Methods).

Study lenses

The contact lenses used in this study were PureVision (Bausch and Lomb), currently commercially available and approved by Health Canada. Lens details have already been described previously in Table 8 (Chapter 2 General Methods).

Study design

This protocol was approved by the Office of Research Ethics at the University of Waterloo.

The study consisted of two phases and data were collected at two scheduled appointments, one screening and fitting visit and one assessment visit per study phase (Figure 70 represents, phase one and Figure 71 represents phase two). The first visit was followed by 24-hour washout period during which participants wore their spectacles and no contact lenses.

Participant eligibility was determined at a screening appointment. Informed consent was obtained for all participants prior to their enrolment in the study.

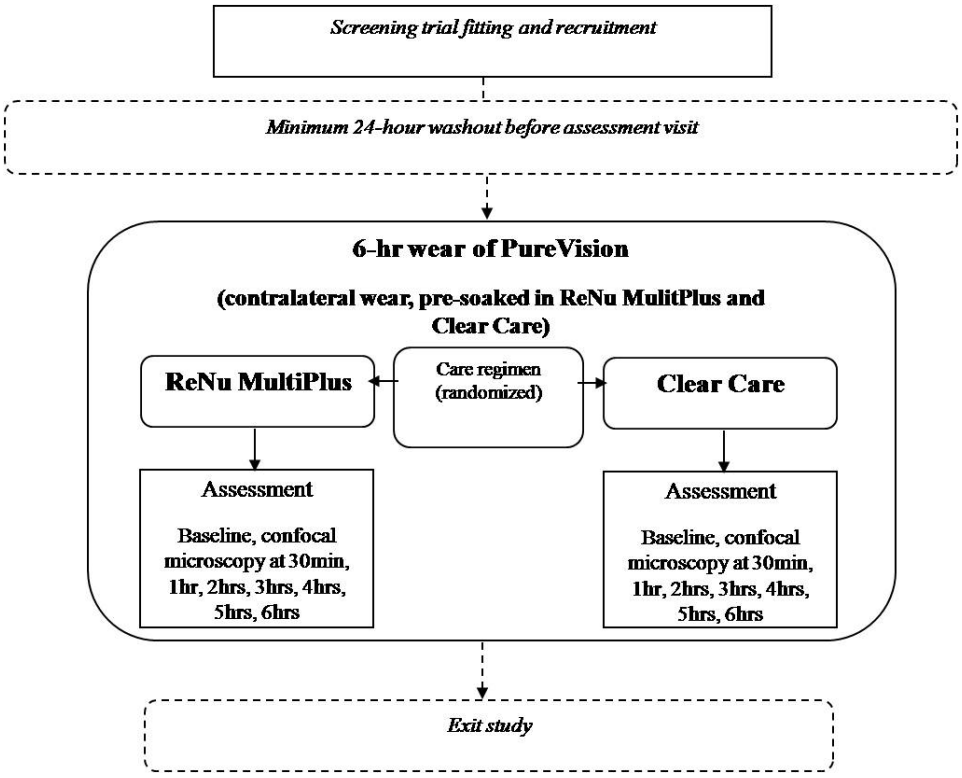


Figure 70: Study design of phase one

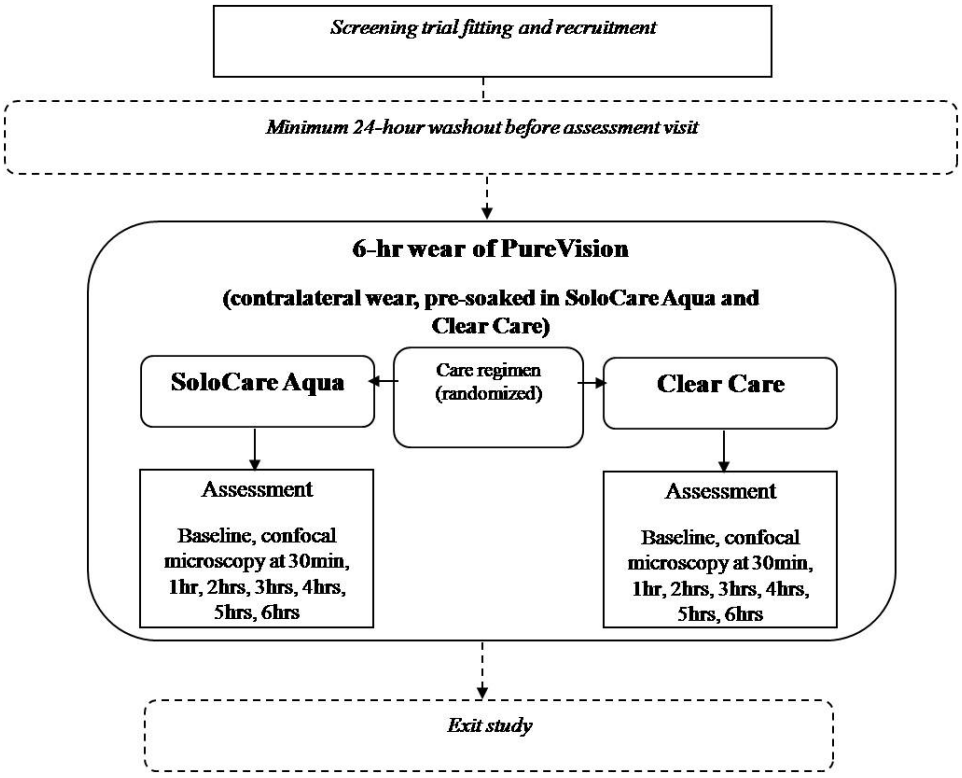


Figure 71: Study design of phase two

Study visits

Screening/baseline visit

Each participant read and signed the information consent letter.

Both study phases included a screening/baseline measurement: For phase one, participants were fitted with PureVision lenses pre-soaked overnight in ReNu Multiplus to provoke a physiological response. For phase two, they were fitted with PureVision lenses pre-soaked overnight in SoloCare Aqua. They were asked to wear these lenses for two hours. After two hours of lens wear, the lenses were removed and their corneas were assessed for CS using slit lamp biomicroscopy and sodium fluorescein. Eligibility was based on ocular history, symptoms and a slit lamp biomicroscopy following the provocative lens fit. Eligible participants (i.e. those who stained) were enrolled in the study.

Assessment visit

Before lens insertion, slit lamp biomicroscopy was performed without the use of sodium fluorescein. The study lenses, according to the study phase, were then inserted.

After 30 minutes of lens wear, CM was performed on the temporal side and central area of each eye without removal of the contact lens. This procedure was repeated after one, two, three, four, five and six hours of lens wear. Participants' corneas were re-inspected using the slit lamp biomicroscope (no sodium fluorescein) after each CM measurement.

Following the last CM measurements the contact lenses were removed and slit lamp biomicroscopy using sodium fluorescein and visual acuity measurements were performed.

Procedures

CM was performed as explained in Chapter 2. However, the lenses were not removed. Therefore, the use of anaesthetic was not necessary. Also, prior to the CM procedure no sodium fluorescein was instilled.

Grading and Analysis

Images of the superficial epithelium were identified and the appearance of the cells subjectively graded using the grading scale described in Chapter 2.

CS was graded according to the CCLR GSS (Chapter 2). All observed CS during this experiment was graded by the thesis author.

Image analysis was performed as described in Chapter 2.

Statistical analysis

Hyper-reflective cell count was analyzed using Fisher's exact test (chi-square 2x2 contingency table). Superficial cellular appearance was analyzed using Wilcoxon matched pairs test and Friedman ANOVA. CS and image analyses data were analyzed using repeated measures ANOVA and Tukey HSD (post hoc).

Results

Participants

Five female participants were enrolled in phase 1 of the study. The mean age of the participants was 37.2 years (median 31 years, ranging from 21 years to 61 years). Only four out of those five enrolled participants were able to complete both phases of the study. The mean age of the participants completing phase 2 was 41.2 years (median 40 years, ranging from 24 to 61 years). Table 43 shows some characteristics of the participants.

Table 43: Participants' (n=5) dioptric characteristics (mean ± SD)

		OD	OS
K-readings	Flat K	43.9 ± 2.1	44.2 ± 2.2
	Steep K	45.0 ± 2.3	45.2 ± 2.3
Corneal cylinder		-0.76 ± 0.3	-1.01 ± 0.4
Refractive error	Sphere	-3.38 ± 4.0	-2.60 ± 3.7
	Cylinder	-0.74 ± 0.7	-0.91 ± 0.8

Discontinuations

After successfully completing the screening visit, one participant asked to be discontinued from the study as it was difficult for her to schedule the assessment visit. Also due to scheduling problems, one participant was not able to complete phase two of the study. To ensure four participants completed both phases of this study, a further participant was recruited to complete both phases. Data for phase one will be reported for n=5, whereas data for phase two and comparisons between the two phases will be reported with n=4.

Superficial cellular appearance and presence of hyper-reflective cells

Table 44 andTable 45 show the grades of superficial cellular appearance for each participant at each time-point and corneal location (phases one and two, respectively).

For phase one (Table 44) a grade 3 (presence of hyper-reflective cells) was assigned for the majority of the participants, which was in most cases for the eye that was exposed to the test combination (PureVision/ReNu (R)).

Table 45 shows the superficial cellular appearance over time for phase two. As can be seen for the majority of the participants, grade 1 or 2 was given at different times and positions. Grade 3 was only given a few times.

Figure 72 shows the differences in cellular appearance grade (test solution – control solution), for both central and temporal cornea in phase one and phase two. Statistical analyses showed that the difference in cellular appearance grades were higher centrally and temporally inphase one (Wilcoon matched pairs test $p<0.001$ and $p<0.001$, respectively).

Table 44: Grading of superficial cellular appearance over time, phase one (ReNu (R) and Clear Care (C), n=5)

	30 min				1 hour				2 hours				3 hours				4 hours				5 hours				6 hours			
ID	**R**		**C**		**R**		**C**		**R**		**C**		**R**		**C**		**R**		**C**		**R**		**C**		**R**		**C**	
	C	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**
1	3	1	2	0	3	3	0	0	3	3	0	0	3	3	0	0	3	3	0	0	3	3	0	0	2	2	0	0
3	3	2	1	1	3	3	0	1	3	3	2	2	3	3	2	2	3	3	2	1	3	3	2	2	3	3	2	2
4	2	1	1	1	3	3	1	3	3	3	2	2	3	3	1	1	2	3	1	1	2	2	2	2	2	3	1	2
5	2	3	3	2	2	2	1	1	3	3	1	1	3	3	1	2	3	3	1	1	3	3	2	2	2	3	1	2
6	1	1	0	0	1	3	0	0	2	3	0	0	3	3	0	0	2	2	0	0	3	3	0	0	1	2	0	0
Mean	**2**	**2**	**1**	**1**	**2**	**3**	**0**	**1**	**3**	**3**	**1**	**1**	**3**	**3**	**1**	**1**	**3**	**3**	**1**	**1**	**3**	**3**	**1**	**1**	**2**	**3**	**1**	**1**
SD	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	1	0	1	1	0	0	1	1	1	1	1	1
Max	3	3	3	2	3	3	1	3	3	3	2	2	3	3	2	2	3	3	2	1	3	3	2	2	3	3	2	2
Min	1	1	0	0	1	2	0	0	2	3	0	0	3	3	0	0	2	2	0	0	2	2	0	0	1	2	0	0
Med	2	1	1	1	3	3	0	1	3	3	1	1	3	3	1	1	3	3	1	1	3	3	2	2	2	3	1	1

Table 45: Grading of superficial cellular appearance over time for phase two (SoloCare Aqua (S) and Clear Care (C) n=4)

	30 min				1 hour				2 hours				3 hours				4 hours				5 hours				6 hours			
ID	**S**		**C**		**S**		**C**		**S**		**C**		**S**		**C**		**S**		**C**		**S**		**S**		**S**		**C**	
	C	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**
3	2	2	2	2	3	3	2	2	1	3	2	2	1	3	2	1	3	3	1	2	1	1	2	2	1	3	2	2
4	1	1	1	1	2	1	1	1	2	2	1	2	2	1	1	1	1	2	1	1	2	2	1	2	3	2	1	2
5	1	0	1	1	1	2	1	2	2	2	1	2	3	3	3	2	2	2	2	2	2	2	3	2	-	-	-	-
6	1	3	1	1	1	3	1	2	1	2	2	1	1	2	2	2	1	3	2	1	2	3	2	1	1	3	2	2
Mean	**1**	**2**	**1**	**1**	**2**	**2**	**1**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**3**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**3**	**2**	**2**
SD	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
Max	2	3	2	2	3	3	2	2	2	3	2	2	3	3	3	2	3	3	2	2	2	3	3	2	3	3	2	2
Min	1	0	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	2	1	2
Med	1	2	1	1	2	3	1	2	2	2	2	2	2	3	2	2	2	3	2	2	2	2	2	2	1	3	2	2

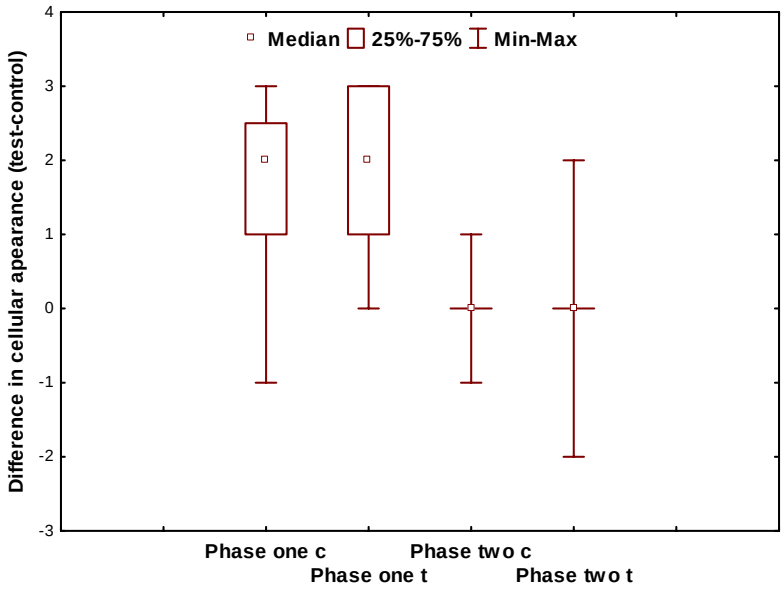


Figure 72: Differences (test-control) in cellular appearance grade for central and temporal cornea in phase one and phase two (averaged over time)

Analyses of the differences in cellular appearance grade over time were performed for phase one. Figure 73 shows the differences for the central cornea and Figure 74 for the temporal cornea. Differences in cellular appearance grade approached statistical significance for both the central and temporal cornea (Friedman ANOVA p=0.054 and p=0.065, respectively).

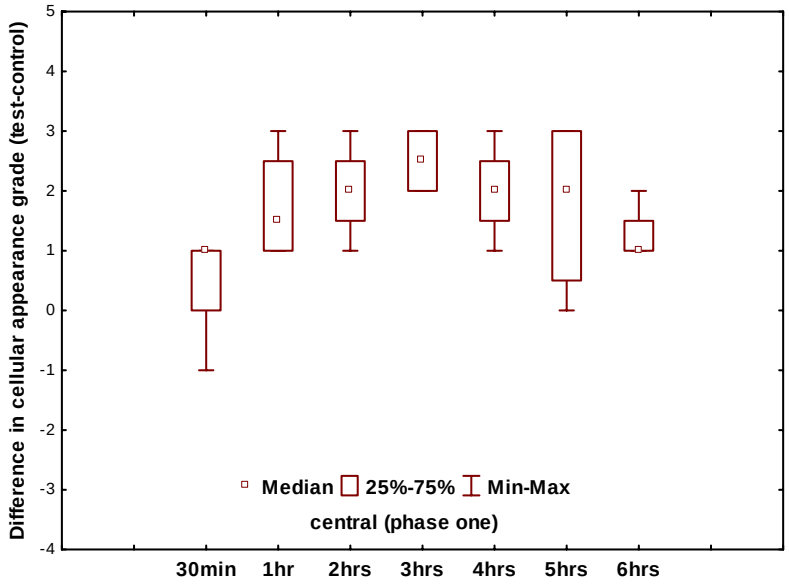


Figure 73: Differences (test-control) in cellular appearance grade over time for the central cornea in phase one

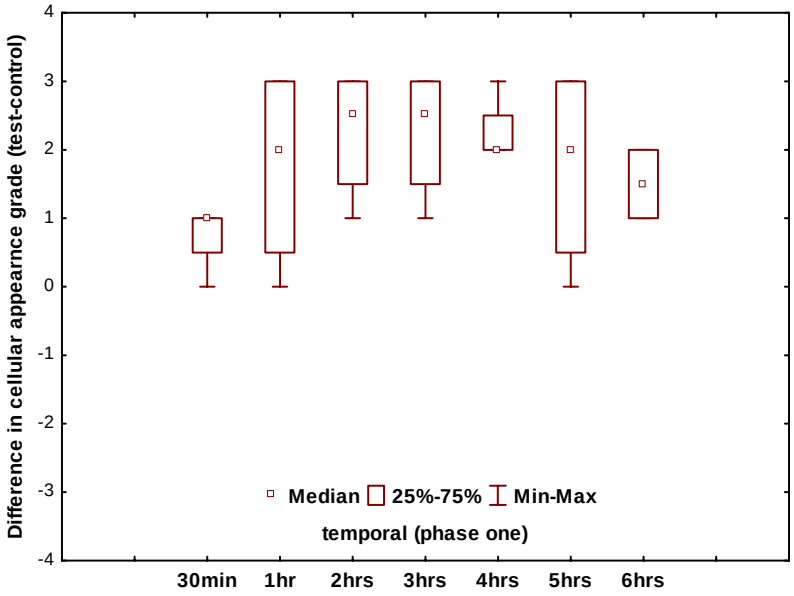


Figure 74: Differences (test-control) in cellular appearance grade over time for the temporal cornea in phase one

Hyper-reflective cells were counted in images with a cell appearance grade of 3. An example of a CM image obtained through a contact lens, with hyper-reflective cells is in Figure 75.

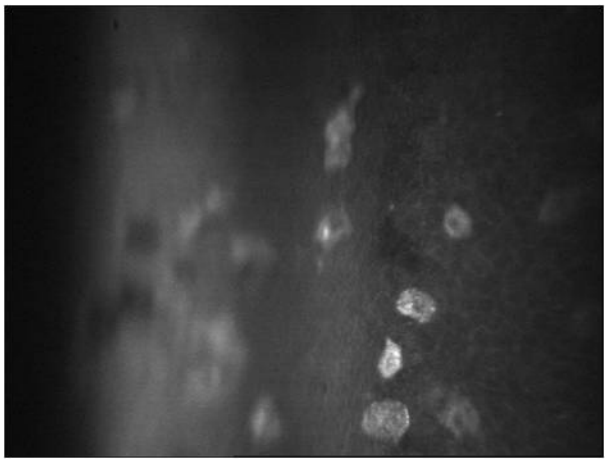


Figure 75: CM image of hyper-reflective cells, imaged through a contact lens

Table 46 shows the numbers of hyper-reflective cells (cells/mm^2) present for each participant, each time-point, and each corneal location (central and temporal) in phase one of the study. Hyper-reflective cells were present mainly in the eye that was exposed to the test combination (PureVision/ReNu (R)). The number of hyper-reflective cells appearas to peak between two and four hours, after which it deacreases. The number of hyper-reflective cells present varied between participants.

Table 47 shows the numbers of hyper-reflective cells (cells/mm^2) for each participant, each time-point and each corneal location in phase two of the study. In this phase, very few hyper-reflective cells were observed in the eye that was exposed to the test combination (PureVision/SoloCare Aqua (S)).

Table 46: Numbers of hyper-reflective cells (cells/mm^2) over time, phase one (ReNu (R) and Clear Care (C), n=5)

ID	30 min				1 hour				2 hours				3 hours				4 hours				5 hours				6 hours			
	R		C		R		C		R		C		R		C		R		C		R		C		R		C	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
1	19	0	13	0	26	19	0	0	26	38	0	0	19	32	0	0	13	32	0	0	13	13	0	0	0	0	0	0
3	38	0	0	0	51	38	0	0	45	26	0	0	26	6	0	0	6	32	0	0	13	32	0	0	6	26	0	0
4	0	0	0	0	32	70	0	38	70	58	0	0	45	45	0	0	0	19	0	0	0	0	0	0	0	6	0	0
5	0	26	6	0	0	0	0	0	19	19	0	0	26	6	0	0	6	19	0	0	6	32	0	0	0	19	0	0
6	0	0	0	0	0	6	0	0	0	32	0	0	19	32	0	0	0	0	0	0	6	13	0	0	0	0	0	0
SUM	58	26	19	0	109	134	0	38	160	173	0	0	134	122	0	0	26	102	0	0	38	90	0	0	6	51	0	0
Mean	12	5	4	0	22	27	0	8	32	35	0	0	27	24	0	0	5	20	0	0	8	18	0	0	1	10	0	0
SD	17	11	6	0	22	28	0	17	27	15	0	0	11	17	0	0	5	13	0	0	5	14	0	0	3	12	0	0
Max	38	26	13	0	51	70	0	38	70	58	0	0	45	45	0	0	13	32	0	0	13	32	0	0	6	26	0	0
Min	0	0	0	0	0	0	0	0	0	19	0	0	19	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Med	0	0	0	0	26	19	0	0	26	32	0	0	26	32	0	0	6	19	0	0	6	13	0	0	0	6	0	0

* C=central, T=temporal

Table 47: Numbers of hyper-reflective cells (cells/mm^2) over time, phase two (SoloCare Aqua (S) and Clear Care (C), n=4)*

ID	30 min				1 hour				2 hours				3 hours				4 hours				5 hours				6 hours			
	S		C		S		C		S		C		S		C		S		C		S		C		S		C	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
1	0	0	0	0	6	26	0	0	0	32	0	0	0	26	0	0	13	32	0	0	0	0	0	0	0	26	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	6	19	6	0	0	0	0	0	0	0	6	0	0	0	0	0
6	0	19	0	0	0	26	0	0	0	0	0	0	0	0	0	0	0	13	0	0	0	13	0	0	0	13	0	0
SUM	0	19	0	0	6	51	0	0	0	32	0	0	6	45	6	0	13	45	0	0	0	13	6	0	32	38	0	0
Mean	0	5	0	0	2	13	0	0	0	8	0	0	2	11	2	0	3	11	0	0	0	3	2	0	8	10	0	0
SD	0	10	0	0	3	15	0	0	0	16	0	0	3	13	3	0	6	15	0	0	0	6	3	0	16	12	0	0
Max	0	19	0	0	6	26	0	0	0	32	0	0	6	26	6	0	13	32	0	0	0	13	6	0	32	26	0	0
Min	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Med	0	0	0	0	0	13	0	0	0	0	0	0	0	10	0	0	0	6	0	0	0	0	0	0	0	6	0	0

* C=central, T=tempora

Figure 76 shows the sums of hyper-reflective cells over time for the different solutions. The sum of hyper-reflective cells for all participants (both eyes, central and temporal together) are shown for each solution and time point. As can be seen, more hyper-reflective cells can be seen with the PureVision/ReNu combination, compared to the other solutions. A peak in hyper-reflective cell count can be observed between one and three hours of lens wear, followed by a gradual decrease in the number of hyper-reflective cells towards six hours of lens wear.

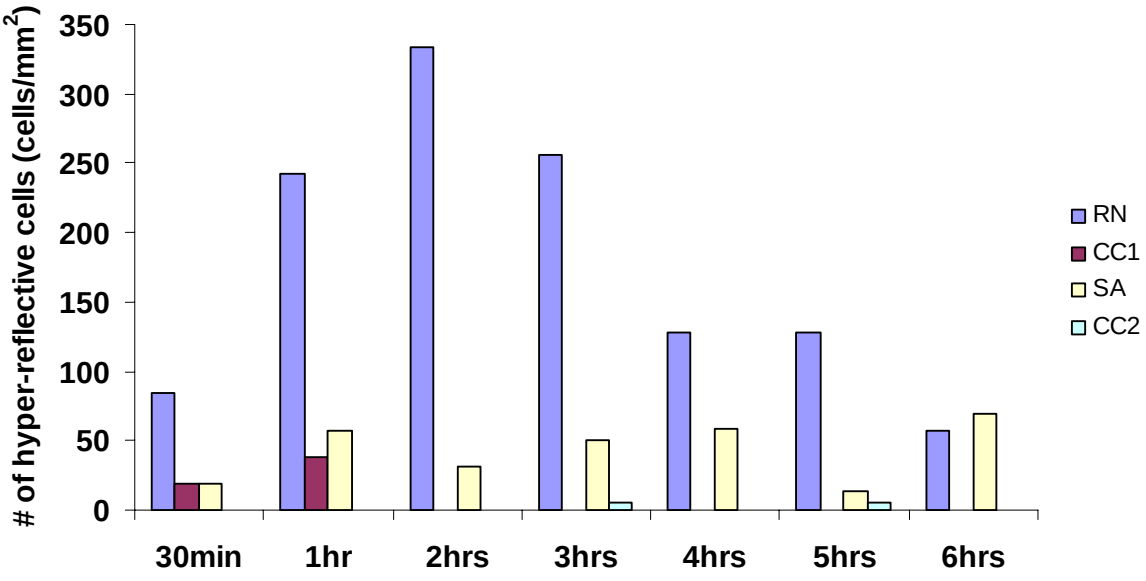


Figure 76: Numbers (cells/mm^2) of hyper-reflective cells (sum of central and temporal cornea for all participants) over time for the different solutions (RN=ReNu, CC1=Clear Care in phase one, SA=SoloCare Aqua, CC2=ClearCare in phase two)

Fisher's exact test showed that for both phases, a statistically significant relationship existed between the number of hyper-reflective and the lens/solution combination ($p=0.021$ for phase one and phase two). In both phases more hyper-reflective cells occurred with the test combination (PureVision/ReNu in phase one and PureVision/SoloCare Aqua in phase two) than with the control combination (PureVision/Clear Care). No statistical significant difference in numbers of hyper-reflective cells between the PureVision/ReNu and the PureVision/SoloCare Aqua combination were found for the different time-points (all Sign-test $p>0.050$).

Corneal staining (CS)

Table 48 and Table 49 show CS results obtained at the screening visit for each study phase. Table 48 lists the mean CS scores (OD and OS separately) prior to lens insertion and Table 49 lists the mean CS scores (OD and OS separately) on lens removal after two hours of lens wear.

For analyses, the CS scores (average of all five zones, a maximum score of 10,000) for each eye was used and the baseline and two-hour visits were compared for both study phases. After two hours of lens wear, there was significantly higher CS (RmANOVA $p<0.001$) with the PureVision-ReNu combination compared to the PureVision/SoloCare Aqua combination (Tukey HSD, $p<0.001$) (Figure 77). Figure 78 illustrates the statistically significant visit-phase-eye interaction (RmANOVA $p<0.001$). Tukey post hoc testing revealed that there was significantly more CS (Tukey HSD $p<0.001$) on the right eye at the two-hour visit of phase two.

Table 48: Corneal staining (mean ± SD, range) at screening visit, prior to lens insertion, for both study phases

	Phase one (n=5) (RenNu) (mean ± SD) (range)		**Phase two (n=4) (SoloCare Aqua) (mean ± SD) (range)**	
	OD	**OS**	**OD**	**OS**
Total CS score (sum of 5 zones)	1200 0 - 750	1525 50 - 925	950 50 - 300	1900 25 - 1000
Temporal	75 ± 87 0 - 125	25 ± 56 0 - 125	75 ± 119 0 - 250	86 ± 111 0 – 250
Superior	20 ± 27 0 - 50	10 ± 22 0 - 50	31 ± 38 0 - 75	13 ± 25 0 – 50
Nasal	25± 56 0 - 125	75 ± 168 0 - 375	44 ± 59 0 - 125	119 ± 173 0 – 375
Inferior	150 ± 105 0 - 250	185 ± 179 0 - 375	75 ± 61 0 - 125	250 ± 204 0 – 500
Central	0 ± 0 0 - 0	10 ± 22 0 - 50	13 ± 25 0 - 50	6 ± 13 0 - 25

Table 49: Corneal staining (mean ± SD, range) at screening visit after 2 hours of lens wear, for both study phases [α]

	Phase one (n=5) (RenNu) (mean ± SD) (range)		Phase two (n=4) (SoloCare Aqua) (mean ± SD) (range)	
	OD	OS	OD	OS
Total CS score (sum of 5 zones)	46500 [α] 0 - 2500	47625 [α] 0 - 2500	13075 0 - 1625	1575 0 - 250
Temporal	3208 ± 3218 625 - 2500	1950 ± 737 750 - 2500	1270 ± 1183 50 - 1500	88 ± 43 50 – 125
Superior	1875 ± 893 375 - 2500	1950 ± 665 875 - 2500	688 ± 415 125 - 1000	94 ± 120 0 – 250
Nasal	1925 ± 716 750 - 2500	1975 ± 731 75 - 2500	825 ± 604 50 - 1500	75 ± 61 0 – 125
Inferior	1950 ± 873 500 - 2500	2050 ± 758 750 - 2500	825 ± 661 50 - 1625	138 ± 83 50 – 250
Central	1625 ± 1031 0 - 2500	1600 ± 912 0 - 2250	138 ± 132 0 - 250	0 ± 0 0 - 0

[α] After 2 hours statistically significant higher CS for ReNu than SoloCare Aqua

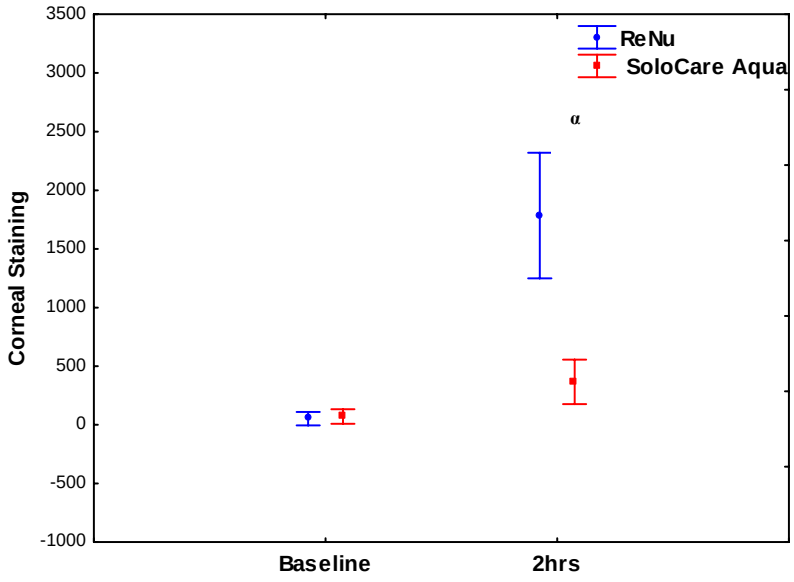


Figure 77: Corneal staining for the two test combinations (phase one and two)
[α] After 2 hours statistically significant higher CS for ReNu than SoloCare Aqua

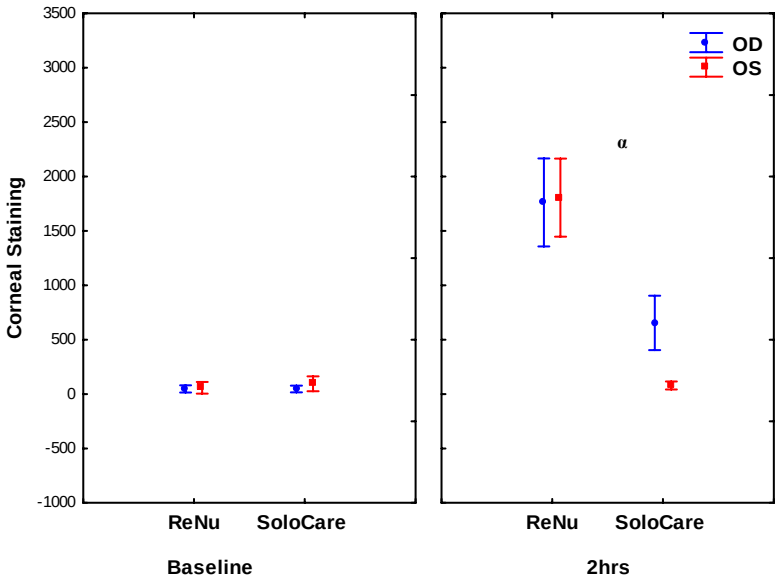


Figure 78: Corneal staining at baseline and after two hours of lens wear in right and left eyes
[α] After 2 hours statistically significant higher CS for OD

Analysis showed significantly more CS at the temporal cornea (Figure 79) in comparison with the central cornea after two hours wearing the PureVision/ReNu combination (RmANOVA p=0.008).

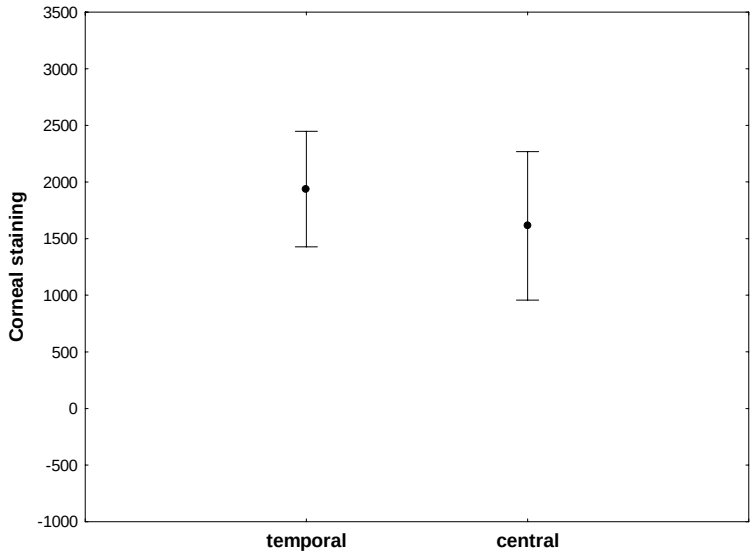


Figure 79: Corneal staining after two hours of lens wear (PureVision/ReNu) for central and temporal cornea (n=5)

Table 50 lists CS (mean± SD, range) for all study participants recorded after six hours of contact lens wear.

Table 50: Corneal staining (mean ± SD, range) scores for study visits (phase one and two) after six hours of lens wear

	Phase one (n=5) (RenNu) (mean ± SD) (range)		Phase two (n=4) (SoloCare Aqua) (mean ± SD) (range)	
	ReNu	**Clear Care**	**SoloCare Aqua**	**Clear Care**
Total CS score (sum of 5 zones)	9150 0 - 1400	5225 0 - 2250	3100 0 - 1600	2250 0 - 1000
Temporal	225 ± 185 0 - 500	75 ± 112 0 - 250	106 ± 181 0 - 375	163 ± 293 0 – 600
Superior	275 ± 224 0 - 500	145 ± 203 0 - 500	119 ± 94 50 - 250	38 ± 25 0 – 50
Nasal	225 ± 205 0 - 500	185 ± 236 0 - 500	44 ± 59 0 - 125	38 ± 25 0 – 50
Inferior	730 ± 488 250 - 1400	495 ± 982 0 - 2250	488 ± 748 50 - 1600	306 ± 464 50 – 1000
Central	375 ± 369 0 - 900	145 ± 260 0 - 600	19 ± 24 0 - 50	19 ± 24 0 - 50

CS after six hours of lens wear was analyzed separately for phase one and phase two. Analysis showed that in phase one CS approached significance (RmANOVA p=0.051); CS scores were slightly higher with Clear Care (Figure 80). In phase two (Figure 81), no significant differences in CS scores were found (RmANOVA p=0.491). There was also no significant difference (RmANOVA p=0.197) in CS when comparing the eye that was exposed to ReNu for six hours to the eye that was exposed to SoloCare Aqua (Figure 82).

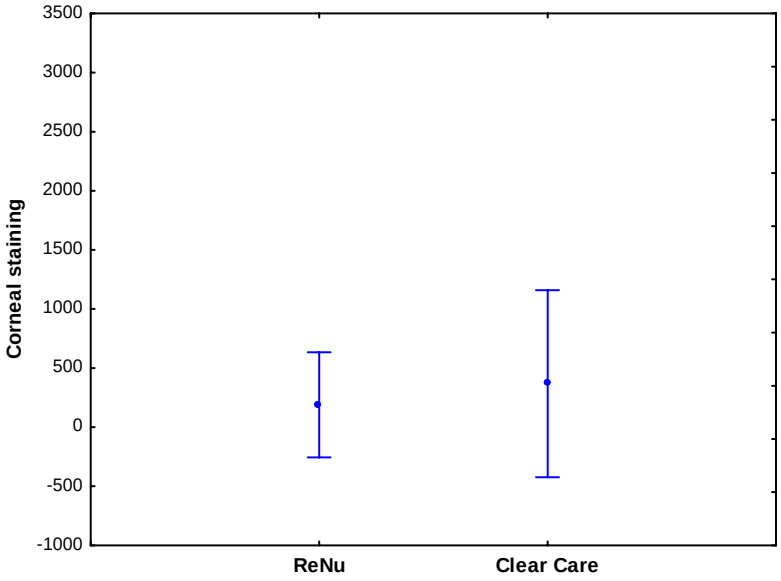


Figure 80: Corneal staining after six hours in phase one (n=5)

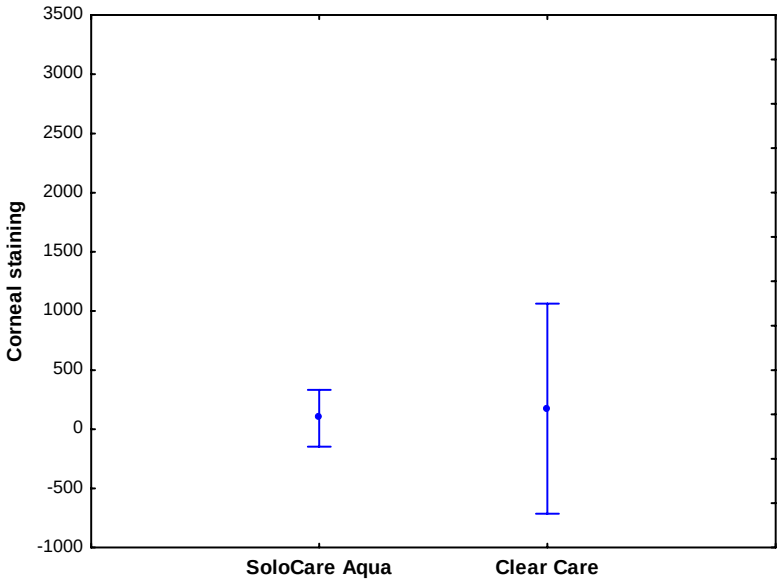


Figure 81: Corneal staining after six hours in phase two (n=4)

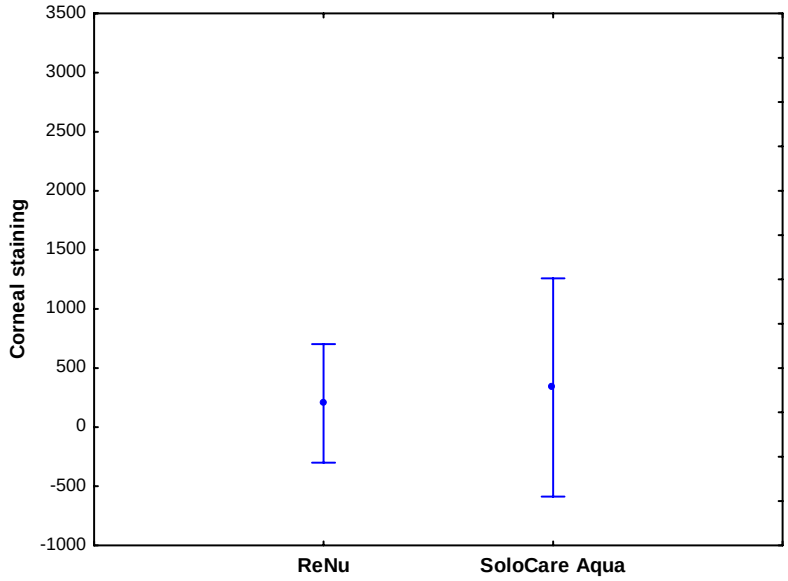


Figure 82: Corneal staining after six hours, ReNu vs. SoloCare Aqua (n=4)

Image analysis

Sample size for each phase was n=4. In order to include as many complete data sets as possible, data were analyzed separately for phase one and phase two.

Figure 83 shows that the hyper-reflective cell areas of central images of the superficial epithelium (phase one) of eyes exposed to the PureVision/ReNu combination were statistically significantly greater (RmANOVA p=0.007) than for superficial epithelial images of eyes exposed to PureVision/Clear Care. Also, significantly higher standard deviations were observed for PureVision/ReNu compared to PureVision/Clear Care (Figure 84). There was also a statistically significant change (RmANOVA) in hyper-reflective cell areas (Figure 85) and standard deviations (Figure 86) over time (RmANOVA p=0.012 and p=0.014, respectively). Post-hoc testing revealed that images obtained at 3hrs showed significantly greater hyper-reflective cell areas than images obtained at 30min (Tukey HSD p=0.007).

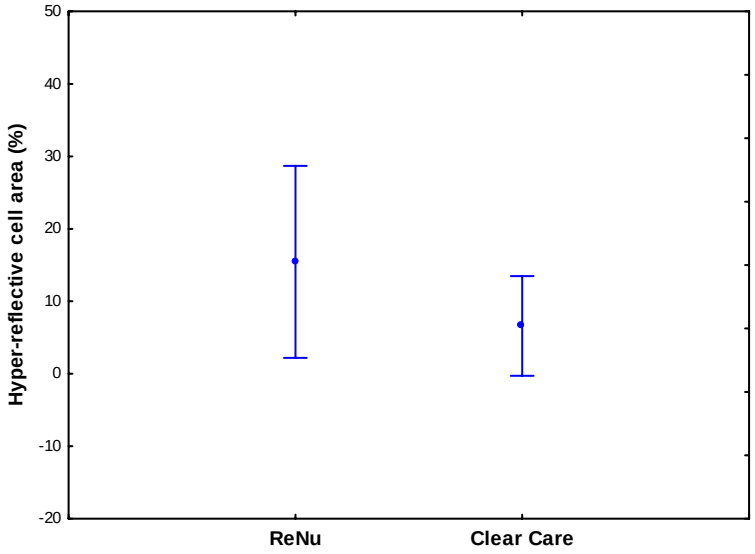


Figure 83: Hyper-reflective cell areas (%) of superficial epithelial images of corneas exposed to ReNu and Clear Care (central)

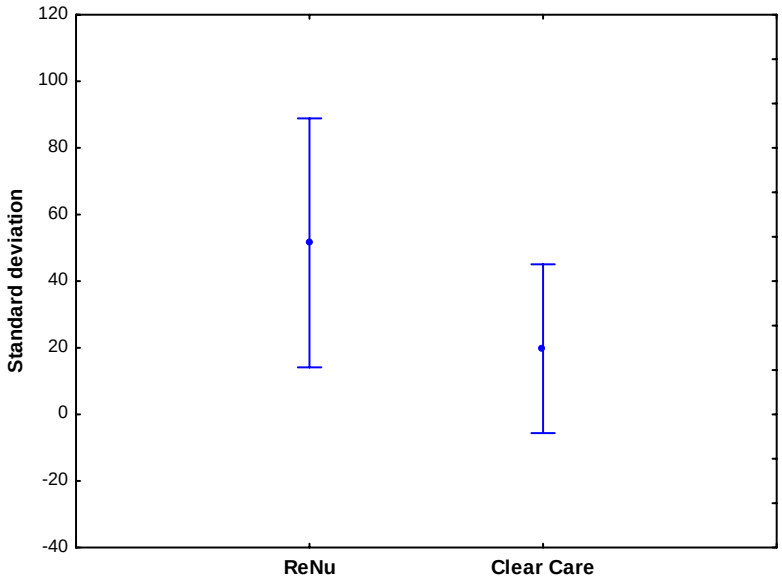


Figure 84: Standard deviations of superficial epithelial images of corneas exposed to ReNu and Clear Care (central)

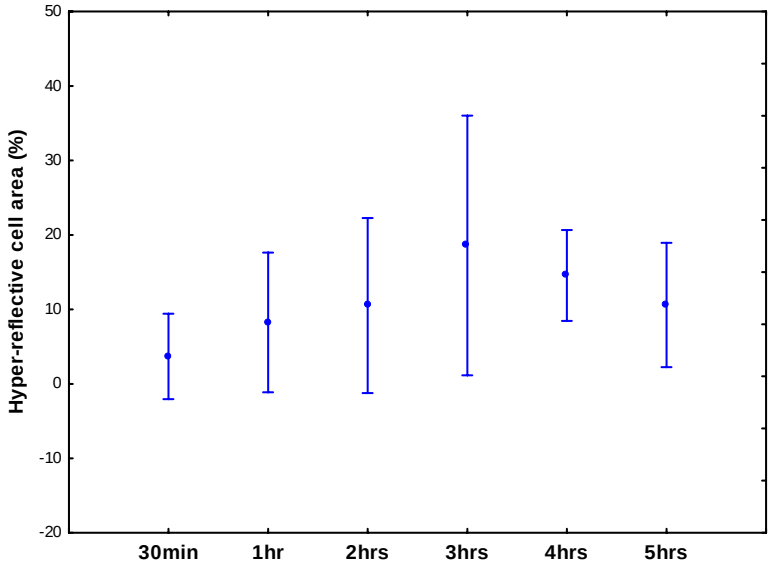


Figure 85: Hyper-reflective cell areas (%) of superficial epithelial images over time (central)

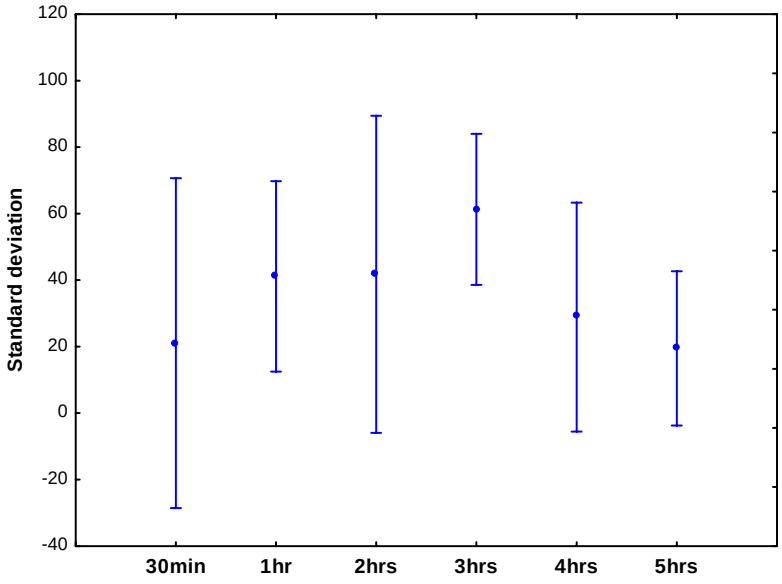


Figure 86: Standard deviations of superficial epithelial images over time (central)

No statistically significant differences (RmANOVA p=0.632) in hyper-reflective cell areas could be shown for the different lens/solution combinations over time (Figure 87), as well as no differences (RmANOVA p=0.067, Figure 88) in standard deviations for the lens/solution combinations over time.

However, post-hoc testing (Tukey HSD) showed statistically significant differences in hyper-reflective cell areas for certain combinations. Images taken at 3hrs of corneas exposed to the PureVision/ReNu combination were significantly brighter than those images collected at 30min of corneas exposed to the PureVision/ReNu combination (Tukey HSD p=0.012) as well as images exposed to the PureVision/Clear Care combination at 30min, 1hr, 2hrs and 5hrs (Tukey HSD p=0.062, p=0.001, p=0.024 and p=0.017, respectively). Hyper-reflective cell areas were also statistically significantly greater for the PureVision/ReNu combination at 4hrs, when compared to images exposed to the PureVision/Clear Care at 30min and 1hr (Tukey HSD p=0.038 and p=0.037). Post-hoc testing (Tukey HSD) also showed statistically significant differences in standard deviations for certain combinations. Images taken at 3hrs of corneas exposed to the PureVision/ReNu combination had significantly higher standard deviations than those images of corneas exposed to the same combination at 30min and at 6hrs (Tukey HSD p=0.015 and p=0.028). Images of the same combination (PureVision/ReNu) at 3hrs had also significantly higher standard deviations than images of the PureVision/Clear Care combination at 30min, 1hr, 2hrs, 4hrs, 5hrs and 6hrs (Tukey HSD p=0.030, p=0.029, p=0.007, p=0.001, p=0.001 and p=0.002). Standard deviations of images with the PureVision/ReNu combination at 1hr were significantly higher than those with the PureVision/Clear Care combination at 4hrs, 5hrs and 6hrs (Tukey HSD p=0.016, p=0.001 and p=0.015). Also, standard deviations of images with the PureVision/Renu combination at 2hrs were significantly higher than those with of the PureVision/Clear Care combination at 4hrs, 5hrs and 6hrs (Tukey HSD p=0.026, p=0.015 and p=0.028).

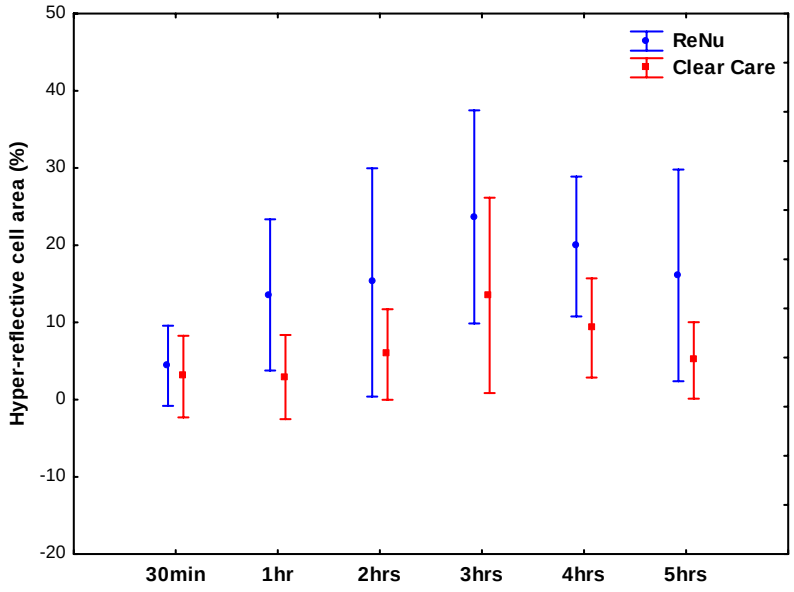


Figure 87: Hyper-reflective cell areas (%) for the two lens/solution combinations (phase one) and over time (central)

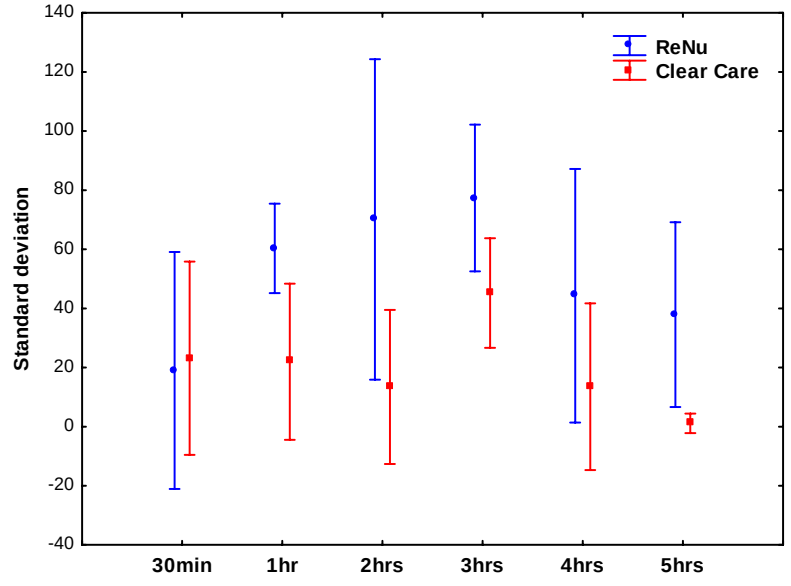


Figure 88: Standard deviations for the two lens/solution combinations (phase one) and over time (central)

Hyper-reflective cell areas of images of the central superficial epithelium of corneas that were exposed to PureVision/SoloCare Aqua and PureVision/Clear Care (phase two) can be seen in Figure 89. No statistically significant difference (RmANOVA p=0.100) in hyper-reflective cell areas between the two combinations were found. This was also true for standard deviations (RmANOVA p=0.142, Figure 90).

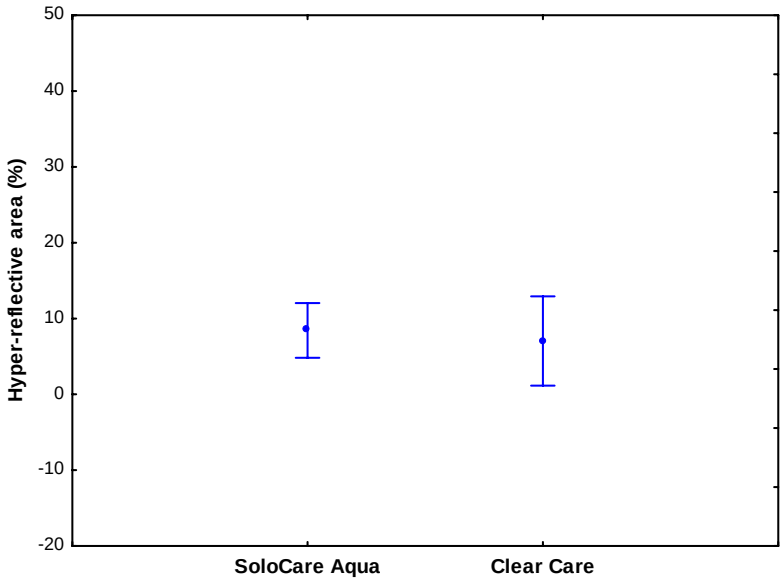


Figure 89: Hyper-reflective cell areas (%) of superficial epithelial images of corneas exposed to SoloCare Aqua and Clear Care (central)

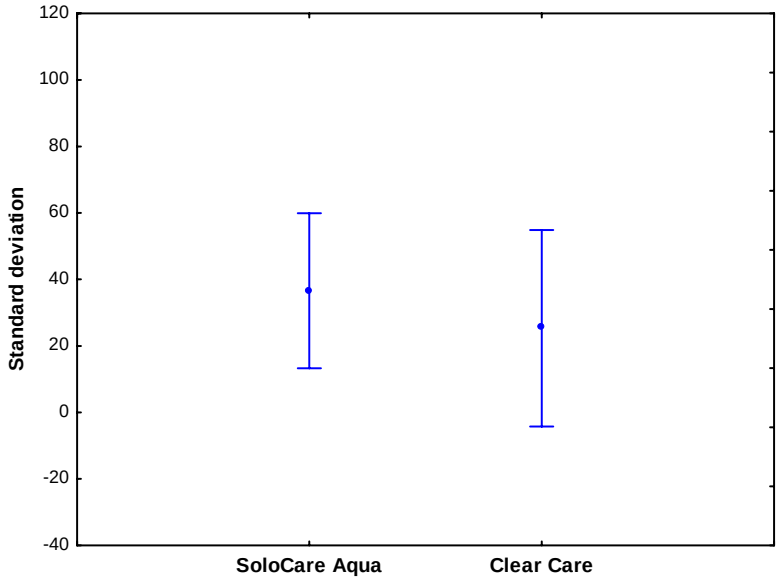


Figure 90: Standard deviations of superficial epithelial images of corneas exposed to SoloCare Aqua and Clear Care (central)

No statistically significant differences in hyper-reflective cell areas (RmANOVA p=0.577) or standard deviations (RmANOVA p=0.955) were found over time. Also no statistically significant interaction in hyper-reflective cell areas (RmANOVA p=0.556, Figure 91) or standard deviations (RmANOVA p=0.746, Figure 92) for lens/solution combination and over time were found.

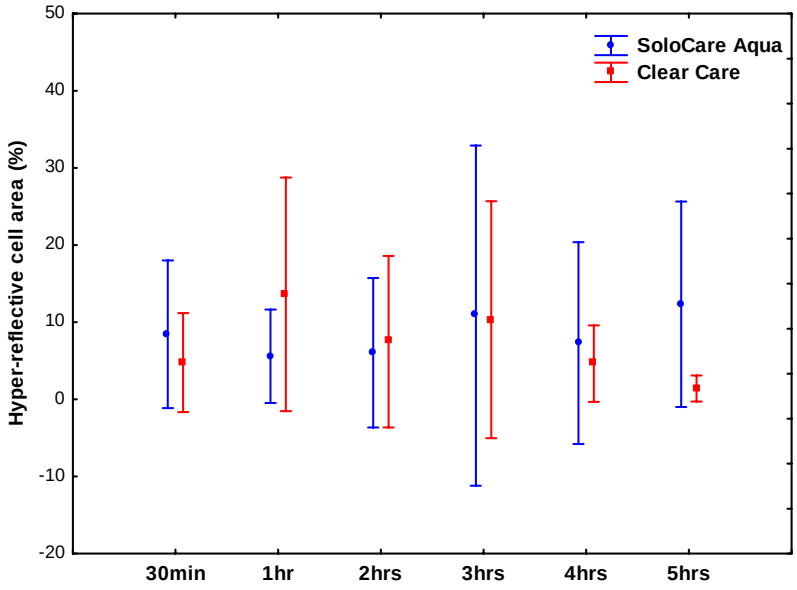


Figure 91: Hyper-reflective cell areas (%) of the two lens/solution combinations (phase two) and over time (central)

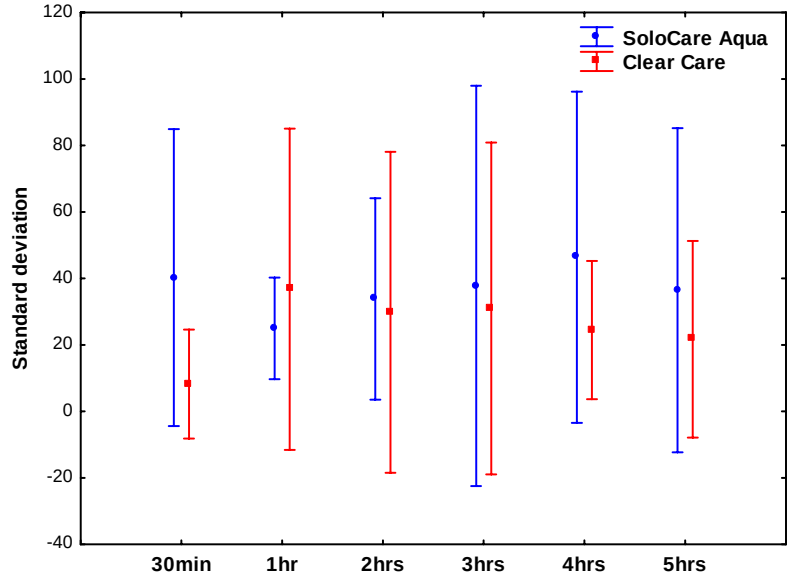


Figure 92: Standard deviations of the two lens/solution combinations (phase two) and over time (central)

Conclusion

The results of this study suggest that the presence of hyper-reflective cells is not a result of the prior use of sodium fluorescein or anaesthetics. There were more hyper-reflective cells in participants having SICS and wearing PureVision lenses in combination with ReNu. The numbers of observed hyper-reflective cells seemed to peak after two hours of lens wear.

Superficial epithelial images obtained from participants who were exposed to the PureVision/ReNu combination were brighter than those from participants who were exposed to PureVision/SoloCare Aqua combination as well as the PureVision/Clear Care combination. Also, the hyper-reflective cell areas in images of participants exposed to PureVision/ReNu changed over time, in the same way as the subjective scores and cell counts.

General Discussion

The objectives of the experiments conducted in this chapter were to investigate the effect of sodium fluorescein and the topically used anaesthetic on the superficial epithelium and to observe the occurrence of hyper-reflective cells.

Effect of sodium fluorescein

The first experiment in this chapter determined the effect of sodium fluorescein prior to the CM procedure on the superficial epithelium. The first part included non contact lens wearers or participants who had not worn lenses for at least 24 hours prior to CM. No hyper-reflective cells were observed in the eye that had sodium fluorescein prior to the CM imaging and there were no hyper-reflective cells found in the eye that had no sodium fluorescein. The cellular appearance in eyes that had sodium fluorescein and those with no sodium fluorescein was similar for the individual participants. This would imply that sodium fluorescein or sodium fluorescein residue is not associated with the presence of hyper-reflective cells. This is in contrast to the study conducted by Mocan et al.[279] who suggested that the prior use of sodium fluorescein resulted in the superficial cells becoming

more prominent and visible and was also responsible for the presence of hyper-reflective cells in eyes with keratoconus.

The second part of this experiment was to investigate whether hyper-reflective cells would be visible with and without the use of sodium fluorescein prior to CM, when SICS was present. SICS was provocatively induced using PureVision lenses in combination with ReNu MultiPlus[29;31] on ten contact lens wearing participants. Eight out of those ten people did show a corneal reaction in both eyes. The study showed that those people who did exhibit this SICS did also have hyper-reflective cells. This was not dependent on whether the eyes had sodium fluorescein prior to CM. The participants who did not have CS did not have hyper-reflective cells in either eye. Hyper-reflective cells were subjectively noted in images of participants who exhibited SICS, but not in images of participants who did not exhibit SICS. The use of sodium fluorescein prior to the CM procedure did subjectively not affect the appearance of the superficial epithelial cells. The outcome of this experiment therefore was that hyper-reflective cells co-occur with SICS and the cells are not the direct result of the use of sodium fluorescein. For the difference in proportions (4/10 and 3/10) of observed hyper-reflective cells to not be significant at 80% power and with a 0.05 significant level a sample size of 356 participants in each group would be required.

The results that there is no difference in superficial appearance independent of if sodium fluorescein is used or not were supported by the image analysis data. This again is in contrast to the results obtained by Mocan et al.[279]

Effect of anaesthetic and sodium fluorescein

The objective of the second experiment described in this chapter was to use CM to investigate possible alterations of the corneal epithelium (hyper-reflective superficial cells) associated with SICS over time and to eliminate the effect of anaesthetics and sodium fluorescein.

This experiment demonstrated significantly higher CS with the PureVision/ReNu combination than with either the PureVision/SoloCare Aqua combination or the PureVision/Clear Care combination. This finding is in accord with various other studies reporting that PureVision lenses, in combination with ReNu, produce higher amounts of CS.[29-31;125;179;304] There was a significant increase in SICS after

two hours of lens wear compared to baseline. This finding also supports outcomes reported in other studies that SICS seems to peak at two to four hours of lens wear.[29;30] It was also shown that SICS was significantly higher in the temporal compared to the central cornea when participants were exposed to the PureVision/ReNu combination for two hours. This too supports the result[29] that SICS had an "annular-shaped" appearance.

It has been reported[196] that although participants used the same lens/solution combination in both eyes, for some participants only one eye showed CS. The amount of CS also varied widely between participants. It was unclear why this was the case. It was also reported[196] that the extent and severity of CS present in participants wearing the same lens/solution combination varied greatly. This intersubject variability of SICS is in accord with the observations made in the present experiment.

The results of the present experiment confirm the findings reported in the MSc Thesis conducted at the University of Waterloo by Harvey.[8] During this MSc project, there was an unexpected "ancillary" finding, that some participants showed bright, reflecting superficial cells. These so called "hyper-reflective" cells appeared to be intact and seemed to occur primarily in corneas exposed to a specific lens/solution combination. Harvey[1] suggested that the appearance of these hyper-reflective cells could be associated with SICS. This association was also suggested in Chapter 4 of this thesis. This present experiment confirms this hypothesis for the first time. Hyper-reflective cells seem to occur when SICS is present, and a higher number of these cells are associated with higher CS scores. The literature shows[29;31] that SICS peaks at approximately two to four hours of lens wear and then decreases with only residual CS left after six hours of lens wear. This pattern seems to correlate with the presence of a higher number of hyper-reflective cells present between two and five hours, as demonstrated in this experiment. The expectation, however, that since CS was greater temporally than centrally and therefore more hyper-reflective cells would be visible in the temporal cornea was not confirmed in this experiment. Explanations for this could include low statistical power, large intersubject variation in the effect of the lens/solution combination and possibly that the presence of hyper-reflective cells is indicative of another factor (such as epithelial stress). Another aspect could be that as the density of superficial cells is greater in the centre when compared to the periphery,[40] which could indicate that the likelihood of observing more hyper-reflective cells in the centre could just be greater.

CM, as a research and clinical tool, has provided us with the opportunity to investigate *in vivo* changes to the cornea on a cellular level that occur during many aspects of contact lens wear.[48;86] It has been reported that contact lens wear and the use of contact lens care solutions affect the corneal epithelium on a deeper, cellular level than can be visualized with the slit lamp.[86] The use of CM in this present experiment has confirmed these observations.

To best of my knowledge, all data published using CM have been obtained using a topical anaesthetic. One study conducted by Lohman et al.[305] reported the use a soft lens instead of anaesthetics to investigate the corneal epithelium using specular microscopy. In the present experiment, the contact lens was *in situ* while CM was performed, so that a topical anaesthetic was not necessary. The observed hyper-reflective cells could therefore not have been induced by the topical anaesthetic.

A literature search revealed no mention of a connection between hyper-reflective superficial cells and SICS. However, reports on the presence of brighter superficial or hyper-reflective cells do exist.[243;278;279] This observed hyper-reflectivity of cells has been described as being possibly due to dry eyes or normal cell turnover (desquamated cells). The reported connection between hyper-reflective cells and the prior use of sodium fluorescein by Mocan et al.[279] was not replicated. Hyper-reflective cells were observed during this present experiment even though no sodium fluorescein was used prior to the procedure.

In summary, the results of experiments conducted in this thesis support the observation made in the previous chapter and as suggested by Harvey[1] that hyper-reflective cells occur when specific contact lens/solution combinations were used. The use of sodium fluorescein and/or topical anaesthetics however, was shown to not be a variable that was associated with hyper-reflective cells.

Chapter 6

Overall Discussion

Hyper-reflective superficial epithelial cells were observed using CM, on a number of participants during an MSc project conducted by Harvey[1]. She[1] suggested that the appearance of the hyper-reflective cells may be linked to SICS which was observed in those participants. This idea, however, had never been mentioned in the literature. Generally, reports on hyper-reflective cells in the literature are mainly limited to " additional observations" and their presence has been hypothesized to be part of normal epithelial turnover[40] and also related to dry-eye symptoms[278]. The reason hyper-reflective cells are seldom reported in the literature could be that they have not been specifically looked for or that their occurrence is rare and even perhaps that images with hyper-reflective cells are not part of "normal epithelial appearance" protocols, so the occasional images containing hyper-reflective cells are discarded. Therefore the purpose of the present thesis was to further investigate the appearance of these hyper-reflective cells. The different chapters in this thesis were designed to define the normal appearance of superficial epithelial cells, to examine various possible predicting variables that may be associated with the occurrence of hyper-reflective superficial epithelial cells and lastly to try confirm the proposed connection[1] between wearing a specific lens/solution combination and the occurrence of hyper-reflective cells. The predicting variables that were considered to be possibly associated with the appearance of hyper-reflective cells included: age, dry eyes, contact lenses, wear, contact lens solutions, certain lens/solution combinations that result in SICS, prolonged use of certain lenses of certain contact lens solutions, application of anaesthetic and the use of sodium fluorescein.

Experimental outcomes

Examining and defining the superficial epithelium of this sample of normal, non-contact lens wearing participants showed that the appearance of the superficial epithelium in CM images is similar to the description and observation of the normal superficial epithelium obtained in previously conducted CM studies.[48;86;87;208;216] The superficial cellular appearance was graded at a baseline visit and then again nine months after this visit. Similar superficial cellular appearance was reported for both visits,

suggesting that the appearance of superficial cells does not change over time. Comparison of the appearance of the superficial cells between the different participants showed that the cells looked similar, but that their appearance was subject dependent. Small numbers of hyper-reflective or bright cells were randomly noticed for a few participants but their presence seemed to be independent of eye or corneal position. No or clinically insignificant CS was observed for the few participants who manifest these hyper-reflective cells. This finding supports reports that CS in normal, non-contact lens wearing people is non-existent or minimal.[56;299;300] Based on this, the presence of the few hyper-reflective cells that were observed were probably not associated with CS, but their appearance could be explained as being part of the normal epithelial turnover and the reported differences in brightness of superficial cells represent different stages in the cell life cycle.[85;243] It has been suggested that the light cells are the youngest of the superficial cells, having just arrived at the surface.[85] Therefore, the dark cells would represent hyper-mature cells that are in the process of being desquamated.[76] Also, different shades of superficial cells, have been identified by scanning electron microscopy as well as CM, perhaps reflecting different amounts and patterns of microvilli and microplicae. Dark cells possess fewer surface features, e.g. loss of microvilli and microplicae: Their surface is less rough than that of light cells.[40;85]

In an attempt to examine the effect of age, I was able to collect data in a group of older females. The investigation was of the appearance of superficial epithelial cells of dry eyed post-menopausal women and non-dry eyed post-menopausal women and there were no differences in superficial cellular appearance between the two groups. The observed superficial cellular appearance compared to previous descriptions of superficial cells in controls imaged using CM.[48;87;208;216] The small number of hyper-reflective cells that was observed in a few participants of the dry eye group, also reflects findings of previous studies. [243;278;295] Few highly reflective superficial cells observed in dry eyed people have been mentioned in a few reports.[243;278;295] However, no suggestions or explanations on their origin have been made. In the present experiment, significantly more CS was observed in the dry eye group compared to the non dry eye group and this finding supports the report in the literature in which staining is suggested to be one of the methods to diagnose dry eye disease.[298] However, no or clinically insignificant CS was observed in those participants who had hyper-reflective cells. This suggests that CS was not a strong indicator for their occurrence. The presence of these few hyper-reflective cells was probably a result of the normal turnover mechanism of the cornea,[40;85] or perhaps related more strongly to their dry eye disease.

Another predicting variable of the appearance of hyper-reflective cells that was of interest was the effect of different lens/solution combinations. An investigational PHMB-based MPS was compared to OptiFree RepleniSH (polyquad-based). These two solutions were used in combination with four SiHy different SiHy lenses (PureVision, Advance, Oasys and O_2Optix) and it was shown that after 14 days, statistically significant more SICS was found with the test solution when used in combination with Advance, Oasys and PureVision when compared to the control combinations. This result is in agreement with other studies that have shown that PHMB-based MultiPurpose solutions (especially ReNu Multiplus), compared to polyquad based solutions, result in higher amounts of SICS when used in combination with PureVision lenses.[29-31;178] Although the exact ingredients of the test solution were not disclosed, the results would confirm the hypothesis of Amos'[125] that it is not only the specific disinfectant (PHMB) used in the solutions that is responsible for causing SICS, but the combination and amount of other different ingredients. It was also shown that neither the solution itself nor the contact lenses themselves induced SICS, but that it was the specific lens/solution combinations. The observed SICS with the test solution was also significantly more on the temporal than in the central cornea. This finding is in agreement with a study that said that this type of CS follows an annular pattern.[29] During this experiment, hyper-reflective superficial cells were observed in some participants during both study visits (baseline and Day 14). However for the baseline visits, their counts were low and their presence appeared to be unrelated to specific participants, corneal locations (central and temporal) and lens/solution combinations. The same observation was made with RepleniSH in combination with the four different lenses and with the PHMB-based test solution in combination with O_2Optix at Day 14. On the other hand, the hyper-reflective cell counts at Day 14 with the test solution in combination with Advance, Oasys, and PureVision were significantly increased, in both central and temporal cornea, and were in the range observed in the study by Harvey.[1] It has to be noted that for both hyper-reflective cell count and SICS a high degree of inter-subject variability existed. Not everybody who was exposed to those combinations actually manifest staining or hyper-reflective cells.

In another experiment, the superficial epithelium was examined when using other lens/solution combinations. These were RepleniSH (polyquad-based) and Clear Care (peroxide hydrogen based). The latter is the "gold standard" solution for not causing SICS,[31;178] and was used with Oasys and O_2Optix lenses. SICS observed for the different lens/solution combinations was thought to be clinically insignificant and the typical appearance[29] for SICS was not noted. These findings are in

accord with previous observations,[31;178] that suggest that these lens/solution combinations do not result in high amounts of SICS. Hyper-reflective cells were reported for a few individual participants and their appeared to be random and the numbers of observed hyper-reflective cells were well below the numbers reported by Harvey.[1] Again, the findings of this experiment suggest that the presence of hyper-reflective cells would be related to a normal turnover procedure,[40;85] and was not associated with what was inducing staining.

A possible predicting variable for observing hyper-reflective cells was the prolonged use of the same type of contact lens and same type of solution. Results from the experiment investigating this variable showed that only one out of the eight participants showed two hyper-reflective cells. This particular participant wore FDA Group IV lenses (Acuvue 2, Johnson & Johnson) in combination with a polyquad-based solution (OptiFree Express, Alcon). The hyper-reflective cells were observed on the temporal side of the left eye only and there was no CS in this participant at this position. These two observed hyper-reflective cells could again be a result of normal epithelial turnover.[40;85] However, the small sample size (n=8) of this experiment could have possibly biased the study outcome. As was noted in an earlier experiment where hyper-reflective cells were observed, presence of hyper-reflective cells was also subject-dependent and had a great deal of inter-subject variability in terms of counts of hyper-reflective cells. Therefore, potentially, as only one of the eight participants during this experiment manifested two hyper-reflective cells (12 cells/mm^2), this could mean that the other participants who were enrolled into the experiment were people who by chance did not manifest hyper-reflective cells at all.

The effect of direct application of ReNu and saline on the superficial epithelium was the focus of another experiment. CM did not show changes to the superficial epithelium after applying ReNu or saline when compared to baseline measures. For three participants, hyper-reflective cells were identified at individual visits and in particular positions. The numbers of hyper-reflective cells in a given image for these participants were very small (6 – 12 cells/mm^2) compared to the number of hyper-reflective cells reported by Harvey[1] (8-25 in an image represents approx. 51 – 160 cells/mm^2). CS was not noted at the specific positions for those participants in the same corneal positions where the hyper-reflective cells were observed. This indicates that the presence of those few hyper-reflective superficial cells was not related to CS.

The use of sodium fluorescein prior to CM was a potential confounder in the study conducted by Harvey[1] and in many of the experiments reported in this thesis. Sodium fluorescein may have been potentially responsible for inducing the hyper-reflective cells. Therefore, the objective of another experiment was to investigate if sodium fluorescein prior to CM was responsible for the appearance of hyper-reflective cells. The superficial epithelium of non-contact lens wearers and of participants who were provocatively exposed to induce SICS was examined. The result of the experiment with the non-contact lens wearer was that no hyper-reflective cells were observed in the eye that was exposed to sodium fluorescein prior to CM. Also, no hyper-reflective cells were found in the eye where no sodium fluorescein had been instilled. This suggests that sodium fluorescein or sodium fluorescein residue did not cause hyper-reflective cells. This is in contrast to the study conducted by Mocan et al.[279] who suggested that the prior use of sodium fluorescein would make the superficial cells hyper-reflective, especially in eyes with a combination of keratoconus and a history of contact lens wear.

When SICS was provocatively induced in participants using PureVision lenses in combination with ReNu[29;31] SICS was observed in both eyes in the sample of eight of the ten participants and hyper-reflective cells were present even when sodium fluorescein was not used. Participants who did not have SICS, also did not show hyper-reflective cells in either eye. This outcome suggests that hyper-reflective cells may occur when SICS is present and not because of the use of sodium fluorescein. This again is in contrast to the results obtained by Mocan et al.[279] They observed that these hyper-reflective cells or as they referred to as intracytoplasmic and nuclear staining occured in participants with a history keratoconus and contact lens wear. They hypothesized that this was from a disruption of epithelial tight junctions which lead to an epithelial and stromal diffusion of sodium fluorescein that resulted in these hyper-reflective cells. They also proposed that this observed intracytoplasmic and nuclear staining may be indicative of an increased corneal turnover. An increased corneal turnover in keratoconic patients has also been suggested by others.[306-309] Weed et al.[309] for example observed desquamating superficial cells in keratoconic eyes that had bright boundaries. But as in the present experiment, hyper-reflective cells also occurred when no sodium fluorescein was used, so it is likely that the hypothesis of damaged epithelial tight junctions cannot be simply applied to explain the presence of hyper-reflective cells, but much rather that the cause of the hyper-reflectivity may be something different.

The last experiment in this thesis was intended to study the effect of different lens/solution combinations over time on the appearance of the epithelium, while eliminating sodium fluorescein and anaesthetics as possible confounding variables. To achieve this, no sodium fluorescein was used prior to CM and images of the central and temporal cornea where obtained without removing the contact lens after 30min, 1hr, 2hrs, 3hrs, 4hrs, 5hrs, and 6hrs of lens wear. The purpose of leaving the contact lenses on the eye during CM was to make the use of anaesthetics unnecessary. There was significantly higher CS with the PureVision/ReNu combination than with either the PureVision/SoloCare Aqua combination or the PureVision/Clear Care. This finding is in agreement with various other studies reporting that the PureVision/ReNu combination results in higher amounts of SICS.[29-31;125;178;179] SICS was significantly higher after two hours of lens wear when compared to baseline. This too is in agreement with study outcomes reporting that SICS seems to peak after approximately two to four hours of lens wear.[29;30] It was also shown that SICS was significantly higher temporal than central partly confirming the reports on an annular appearance of SICS.[29] The extent and severity of SICS varied widely between participants. There were high counts of hyper-reflective cells with the PureVision/ReNu combination and only a few seemingly random occurring cells with the other lens/solution combinations. This showed that the occurrence of hyper-reflective cells in the study conducted by Harvey[1] was reproducible when using PureVision lenses in combination with ReNu. It also confirmed the suggestion made by her[1] that the presence of these cells may be associated with SICS. For the first time, this study unambiguously confirmed the hypothesis that hyper-reflective cells seem to occur when SICS is present. As mentioned before, the literature showed[29;30] that SICS peaks at approximately two to four hours of lens wear and then decreases with only residual CS left after six hours of lens wear. This pattern seemed to also occur with the presence of hyper-reflective cells. A higher number of hyper-reflective cells were observed at around 2 hours. The expectation that since SICS was greater temporally than centrally more hyper-reflective cells would be visible in the temporal cornea was not confirmed in this study. This could be the result of the small sample size and that therefore no difference in the numbers of hyper-reflective cell centrally and temporally could be shown. Another explanation could be that superficial cell density is greater centrally than temporally and therefore the likelihood of observing hyper-reflective cells in the centre may just be higher. Also, the results of this and previous experiments where hyper-reflective cells were observed showed that a great deal of inter-subject variability existed for counts of hyper-reflective cells. On the other hand, it is also possible that the expected assumption is wrong and that the occurrence of hyper-reflective cells is non-systematic.

Conclusions obtained during the different experiments in this thesis show that variables, such as age, dry eye, sodium fluorescein, anaesthetic, solution or contact lens wear itself and prolonged wear of the same lens type and solution were found to not be linked to the appearance of hyper-reflective cells and that hyper-reflective cells seemed to occur when SICS was present.

SICS is often explained as being the result of toxic exposure to the corneal epithelium.[29-31;123] However, the cause of this toxic reaction is unclear. It is suggested that certain components within a solution, mainly the preservatives, are being adsorbed onto the contact lens surface and then slowly released during lens wear, causing this toxic reaction.[29] A recent paper (2009) by Martone et al.[280] reported that the superficial epithelial cell layer showed hyper-reflective cell bodies with less prominent cellular outlines in glaucoma patients using preserved therapy compared to a group using preservative-free therapy and controls. These findings are in agreement with observations made by Ichijima et al.[203] and Labbe et al.[202] Ichjijima et al.[203] who used CM to investigate the rabbit cornea treated with BAK. They suggested that application of as little as 0.005% BAK causes a toxic reaction that lead to swelling and desquamating of superficial epithelial cells and that these cells had a brighter, hyper-reflective appearance. Labbe et al.[202] compared the toxicity of BAK and polyquaternium-1 (PQ-1) on rats using CM. They showed that rats exposed to BAK had more hyper-reflective superficial cells without visible nuclei when compared to rats that were exposed to PQ-1. These findings would suggest that the reason for hyper-reflective cells occurring during some experiments in this thesis is that cells were also exposed to a toxic environment. This toxicity could have possibly been the result of an interaction of a specific lens/solution combination. However, why this toxicity only affects some superficial cells and not the entire superficial epithelium is unclear. An explanation for this could be that maybe cells that are already at the end of their life cycle are more receptive to changes to their environment. Also interestingly, not all participants manifest hyper-reflective cells, even though they are exposed to the specific lens/solution combination. This indicates even more complexity of the mechanisms. It is likely that the healthy tear film, and particularly as it is containing enzymes and proteins that may protect the ocular surface cells from oxidative stress,[35] might also play an important role. Perhaps people, with a tear film containing a reduced amount of enzymes and proteins and therefore with decreased protection of the ocular surface cells are more likely to manifest hyper-reflective cells.

Confocal microscopy (CM)

During the different experiments in this thesis, CM was used to image the superficial epithelium. CM is a fast and non-invasive technique to evaluate the different corneal layers.[48;86;87;208;216] Efforts were made, such as using a fixation target, to obtain images consistently of the approximately same corneal area for each participant and for the different time points. However, a weakness of this thesis is still the technical difficulty of obtaining CM images of the superficial epithelial cells at the same location. This problem is present with *in vivo* CM. The lack of landmarks on the normal corneal epithelium, in combination with the high magnification, as well as slight movements of participants, complicates returning to the exact same corneal area. For this thesis the superficial epithelial images were obtained using the ConfoScan3.[281] This instrument does not utilize a corneal stabilization device such as both the ConfoScan 4 (Nidek Technologies, Gamagori, Japan) and the HRT (Heidelberg Engineering, Heidelberg, Germany) do. These instruments stabilize the cornea, and therefore stabilize for movements, using a z-ring[310] and a PMMA cap[231] for the ConfoScan 4 and HRT, respectively. A stabilization feature on the used CM could have been helpful during the experiments in this thesis to improve the confidence of re-examining the same corneal area of participants.

Another point to consider is that the image acquisition with CM is en face.[87;208;216] Therefore, if a proper perpendicular positioning of the objective lens is not achieved, for example during eye movements, the contents in the obtained images appear to be on an angle. The result of this is a slice-like appearance of the cornea with more than one corneal layer visible in an image. A proper placement of the objective lens was also very important to obtain images of the superficial epithelial cell layer without surface reflections. Surface reflections on images were specifically a problem in the study where CM images were obtained with the contact lens *in situ*. These reflections probably occurred on the additional contact lens/air interface, especially when a proper perpendicular positioning of the objective lens was not achieved. Therefore, CM images of the superficial epithelium obtained during the experiment with the lens *in situ* were qualitatively not as clear as CM images obtained during the other experiments where no additional interface was involved.

Defining and quantifying superficial cellular appearance

Another difficulty that had to be overcome was the quantification of the superficial cellular appearance, in particular the presence of hyper-reflective cells. CM images of the superficial epithelium seemed to vary between participants, visits and studies. Even though no grading scale for the superficial cellular appearance exists in the literature, the appearance of the normal superficial epithelium observed with CM has been described.[48;86;87;231] Based on these descriptions, and to be able to compare the superficial appearance between studies, a subjective grading scale of appearances of the superficial epithelium was developed.[282] The subjective grading scale consists of four levels (0=indistinctive cellular appearance, 1=presence of cells with more prominent margins, 2=presence of cells with prominent margins and contents, 3=presence of hyper-reflective cells) and is shown in Figure 93.

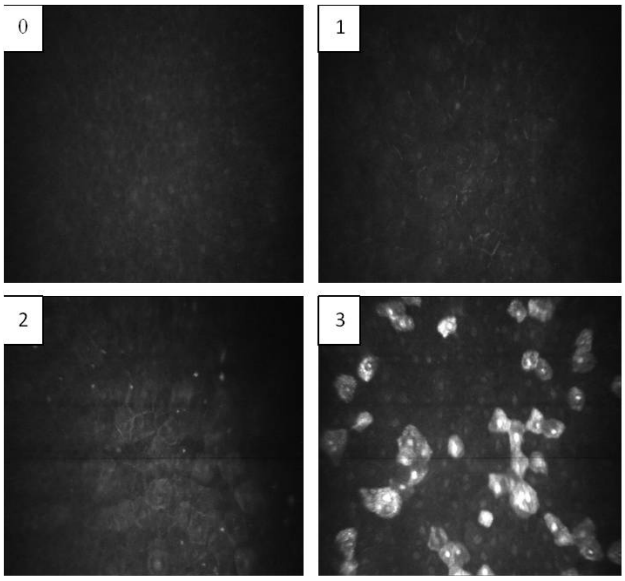


Figure 93: Examples for the different grades for superficial cellular appearance (0=indistinctive cellular appearance, 1=presence of cells with more prominent margins, 2=presence of cells with prominent margins and contents, 3=presence of hyper-reflective cells)

A criticism of this grading scale is that the increments actually do not represent a continuous progress from normal superficial cellular appearance to the presence of hyper-reflective cells. Particularly, if grade 1 (presence of cells with more prominent margins) and grade 2 (presence of cells with

prominent margins and contents) really do characterize differences from the "normal" superficial cellular appearance.[40;53] Superficial epithelial cells show brightness variations depending on where they are in their life cycle.[40] Therefore, it could be argued that only two increments, indistinctive cellular appearance and presence of hyper-reflective cells, may have been enough.

Grading of the superficial cellular appearance was performed by an experienced investigator. A decision on a grade was based on the appearance of the superficial epithelium in one image. However, in order to stabilize grading, prior to decision making, the entire CM scan from which the image to be graded was selected, was viewed to ensure that the image that was chosen was representative of the superficial cell layer and that its appearance did not vary within a scan.

Also, it can be argued that the brightness settings of the CM in the different experiments could have been different which could have influenced the appearance of the superficial epithelium and therefore biased the grading. However, in order to provide a consistent environment, the brightness settings on the CM were fixed prior to starting the experiments and the settings were not changed during the different experiments. It has to be noted, that the aging process of the illumination source was not taken into account.

Another drawback of the grading scale is that it is subjective and therefore a method to objectively identify cellular images was developed. In particular, the idea was that the presence of hyper-reflective cells in an image would increase the level of image brightness, when compared to images that did not contain hyper-reflective cells. A low-pass filter[311;312] was applied to blur and to remove low contrast, narrow cell walls and other small artefacts. Images were then turned into binary images using local thresholding by Bernsen.[287] Local thresholding is generally performed to apply different thresholds on different sections of an image[311] and a number of statistics in an elliptical region of interest (ROI) were obtained. As the cornea is curved, an elliptical ROI was chosen to address the problem of the curvature of field in CM images with blurred or out of focus image edges.

Before performing the described image processing and analyzing method to obtain results for this thesis, the practicality and validity of the method was tested on a sub-set of images. This was done by a different, independent investigator who was given a set of 30 images, including 15 images with and 15 images without hyper-reflective cells. Statistical analysis of the graded and processed images

showed that there was a statistically significant difference in hyper-reflective cell areas (Two sample t-test, $p=0.004$) between the two groups (images with and without hyper-reflective cells). A Levene's test also showed that there was a statistically significant difference ($p<0.001$) in the deviations of the intensities of the images indicating that one group had more variance in the data than the other group.

Based on these previous results, applying the previous described image analysis procedures to the superficial epithelial images of the different experiments in this thesis, seemed to provide a satisfactory method to show objectively that if hyper-reflective cells were subjectively identified in an image and that they were measureable. Figure 94 and Figure 95 are Bean plots[313] and show the measured intensities and the standard deviations of 575 images, obtained from the experiments in this thesis, plotted against the subjective appearance grade of the superficial epithelium. Figure 94 shows that the means of the measured intensities increased systematically with increasing grade levels. It can also be seen that for the majority of images that were graded 0 (indistinctive cellular appearance), the measured intensities in those images were 1 or very close to 1($\log^{10}$ scale). As is most clearly illustrated in the filled in density functions in Figure 94 and Figure 95, the shift to higher measurements for higher grades occurs for both intensities and standard deviations. The figures perhaps also indicate that the subjective grading scale had construct and face validity if objective measures were used as the reference but also that the objective measures can perhaps be used as a prediction for a grade.

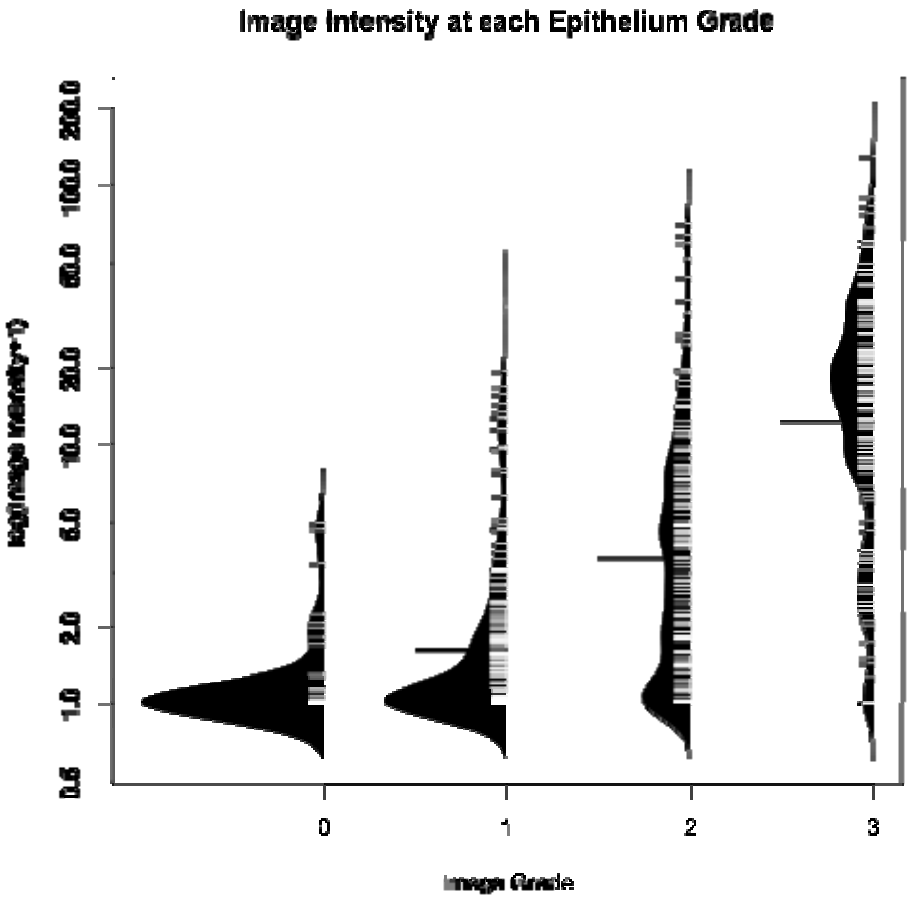


Figure 94: Intensities for respective epithelial grades from all experiments

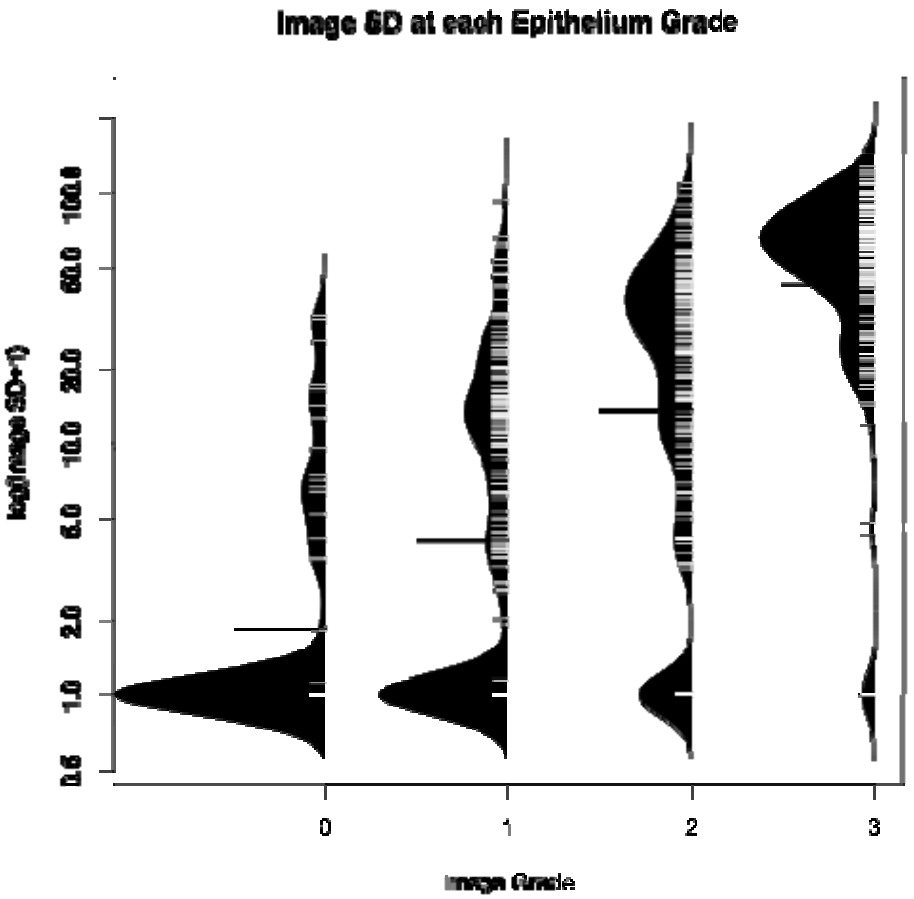


Figure 95: Standard deviation for respective superficial appearance grade for all experiments

However, as can be also seen in Figure 94 and Figure 95, even though for the majority of the images the method appears to reflect the appearance, there were also some unexpected measured intensities (for example intensity and/or SD ≈ 1). In these few images the image contrast and brightness was high enough to be rated clinically as so, but was filtered out and the image was converted to black. At the other end of the spectrum another possibility was that some images had cell artefacts and noise and that these appeared as white areas in the images that were clinically judged to not be hyper-reflected cells but were not filtered out and therefore were measured to not be zero. Excluding the experiment where CM was performed with the contact lens *in situ*, image analysis was completed on 575 images. 111 of these images showed the cell edge artefacts. When a contact lens was in place,

221 out of 241 images had cell edge artefacts or bright areas perhaps due to specular reflection from the lens' polished surface. Figure 96 is an example that illustrates these cell artefacts that occurred in some of these images (1A=original superficial epithelial image, 1B=after application of the low-pass filter, 1C=after local thresholding).

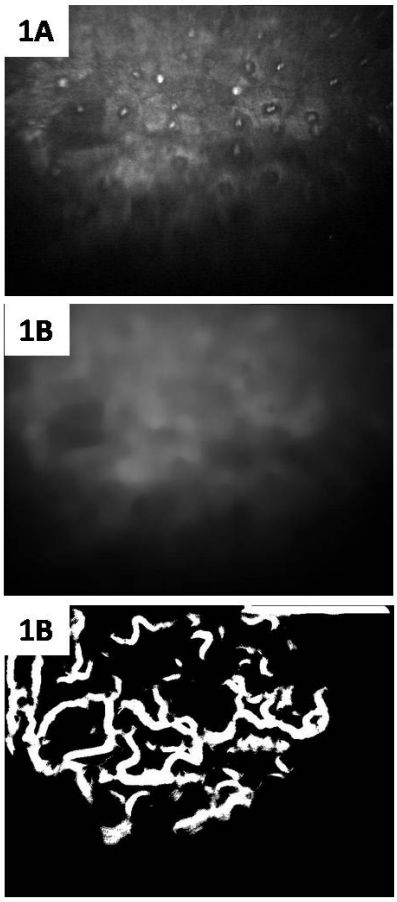


Figure 96: Example of an image (superficial epithelial grade 2) where cell artefacts occurred after image processing (1A=original superficial epithelial image, 1B=image after applying low-pass filter, 1C=image after local thresholding)

Since the process was automated and non-selected these artefactually high measures were not excluded in any of the reported objective analyses and contributed a source of measurement noise. Despite this, it should be emphasized that the results of the objective analyses were supported by the

grading results, although, as might be expected the objective data were more sensitive in revealing differences, occasionally not present in the subjective results.

The idea that increased image brightness due to higher amounts of reflected or backscattered light can be used to measure a specific variable has been previously reported in the literature.[314-319] Patel et al.[314] developed a technique to measure backscattered and reflected light from normal human corneas and corneas with posterior lamellar keratoplasty *in vivo* at different depths using a scatterometer (modified photographic slit lamp). They used a solid piece of fluorescent glass with a convex anterior surface as a reference to standardize the intensity of images, and corneal intensities were multiplied by a certain factor to adjust for variation in the light source and sensitivity of the camera. As the different layers of the cornea cause differences in backscatter, the authors[314] modified each horizontal scan line in the images using a previously described technique.[320] Images were also straightened by a second order polynomial and the intensity profile of an entire corneal image was calculated using the mean grayscale intensity of corresponding pixels in all horizontal scan lines. In order to compare the results between images they converted the intensities to arbitrary "scatter units" using a commercial turbidity standard solution (AMCO Clear) as the reference. With this method the authors[314] were able to provide an objective and repeatable method for measuring corneal backscatter from different depths of the cornea. However, for the present experiment to objectively identify hyper-reflective cells in images Patel et als.[314] method may not be applied. First, image intensity is not used to quantify density. It is used (after thresholding) as a simple metric of the number of maximum intensity pixels and therefore hyper-reflective cells. Hyper-reflective cells were considerably brighter compared to the normal superficial cells and were also of notable size. Therefore the blurring by the median filter removed noise and disruptive edges .Using local thresholding, images were turned into binary images where hyper-reflective cells were visible as white areas on a black background. This thresholding of the images enabled calculation of the coverage (%) of hyper-reflective cells in the ROI and this was used to objectively quantify the relative area of hyper-reflective cells. This is different to Patel et als.[314] method who measured density of backscatter in grayscale units. In addition, the images obtained by Patel et al. were cross-sectional images, whereas images obtained with the CM are *en face* and therefore quantifying reflections and backscatters from different corneal layers was not necessary as only images of the superficial epithelium were chosen for image analysis.

Possible improvements in order to avoid interfering artefacts could be made by adjusting the acquisition of images and the characteristics of the low-pass filter and the sensitivity of the adaptive

thresholder. The low-pass filter that was used to blur the images had a radius of 15 pixels and as the purpose of this was to remove disruptive non-hyper-reflective, low contrast cell edges. This radius could have been too high for some images removing hyper-reflective cell information. Conversely, the radius could have been too small, not completely removing a disruptive pattern, cell details and noise. A further improvement could be that after placing the ROI-measuring tool over the desired area where image analysis is going to be performed, a shape recognition algorithm application could be helpful to differentiate between cells and cell artefacts. This could be particularly helpful for the study that included acquiring CM images with the lens *in situ* when large specular reflections from the polished lens surface were present.

The initial idea to provide a completely automated and objective method to identify and quantify hyper-reflective cells in an image was not possible to achieve. There was subjective (CM operator) involvement including the placement and size of the ROI for measuring the hyper-reflective cell area. Another fundamental operator requirement was the acquisition/selection of optimal images. This was not always possible, given the constraints of the actual data collection process. For example, immediate evaluation of quality of all images in the acquired stack was simply not possible.

Count of hyper-reflective cells

The hyper-reflective cells that were present in a given CM image of the superficial epithelium were counted. If each cell's brightness represents a distinct fraction of the entire image brightness we can analyze the results differently. The more cells present in an image the brighter the image would be because counts are measures of image brightness. This count as a measure for hyper-reflectivity is based on the theory of measurement introduced by Helmholtz.[321,322] His theory was further developed by Hoelder[323] and Helmholtz's and Hoelder's theory of measurement was related to "physical measurements, which are based on additive quantities and quantities derived from them".[322]
In Chapter 5, Figure 76 suggests that there are differences between lens/solution combinations and the number of cells present, but this was not supported by the (lower power) analyses of cell counts. On the other hand, if we apply the theory that counts are measurements of brightness, Figure 97 shows how these measures differ from Figure 76 in Chapter 5. For the PureVision/ReNu combination a statistically significant difference (RmANOVA, p=0.015) in "numbers of hyper-reflective cells" over time can be found with significantly more hyper-reflectivity at two hours compared to six hours

(Tukey HSD, p=0.027). The difference between central and temporal cornea was not significant (RmANOVA, p=0.116).

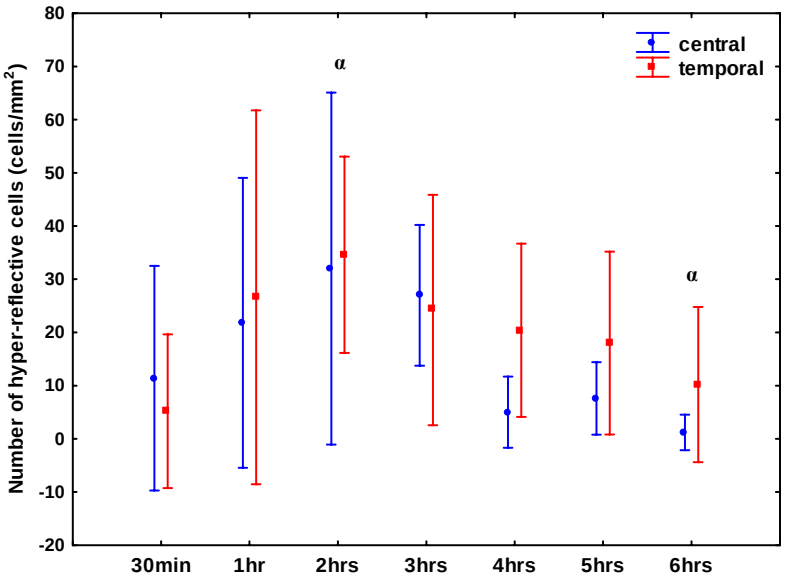


Figure 97: Number of hyper-reflective cells over time, central and temporal cornea (PureVision-ReNu)

($^{\alpha}$ Statistically significant difference (p<0.050) in hyper-reflective cells between 2hrs and 6hrs)

Speculations on why cells could become hyper-reflective

The purpose of this thesis was to identify variables that would predict the appearance of hyper-reflective cells. The only variable that seems to be consistently associated with hyper-reflective cell appearance was a specific contact lens/solution combination. Hyper-reflective cells seem to co-occur with SICS that is present with this specific lens/solution combination. However, I can only speculate on what exactly happens to make some cells hyper-reflective. Therefore, the following hypotheses are offered as potential mechanisms that could result in hyper-reflectivity of cells.

The cornea as a transparent structure[40] reflects approximately 1% of the incident light due to its special orderly structure.[48] The main sources for backscattered light in the cornea are tissue interfaces between cells and intra cellular matrix or tissues and fluid, where changes in refractive index occur.[324]

However, the backscatter provided by these sources does, under normal circumstances, not affect the transparency of the corneal tissue, but it is sufficient to allow instruments that work on the basis of reflected light to visualize certain structures. The observation of superficial epithelial cells with CM is because more light gets reflected from epithelial cells. Boehnke and Masters[87] describe the normal superficial epithelial cells, when imaged with CM, as "multicornered in shape and with different levels of reflectivity" having a dark visible nucleus with occasionally a small bright reflex.

In broad terms, optics is described as the study of light, including its interaction with matter. Light coming from a source into a homogeneous medium travels unhindered until hits some sort of a barrier. A barrier in the context of my experiments, is a region within which refractive index varies rapidly and therefore may behave like a mirror that reflects light.[325] Such barriers could be responsible for causing hyper-reflectivity. The following figures are intended to illustrate scenarios that could lead to a rapid change of refractive index and could therefore turn normal superficial cells into hyper-reflective cells.

Figure 98 shows rays of light that hit a cellular surface where for example the protective mucus layer is partly missing and therefore the underlying cell is exposed. This cell could have a different refractive index and causes light to get reflected. Another explanation could be that the entire cell is missing and a cell from the wing layer below is visible because it has a different refractive index or a smoother surface. In Figure 99 it is illustrated what could happen at a rough surface. Such an interface could potentially occur when microvilli are lost or if the top or parts of the cell are ripped off or disrupted, for example parts of the membrane, and therefore areas with different refractive indices are exposed. Lastly, increased reflectance because of intracellular areas with different refractive indices is illustrated in Figure 100.

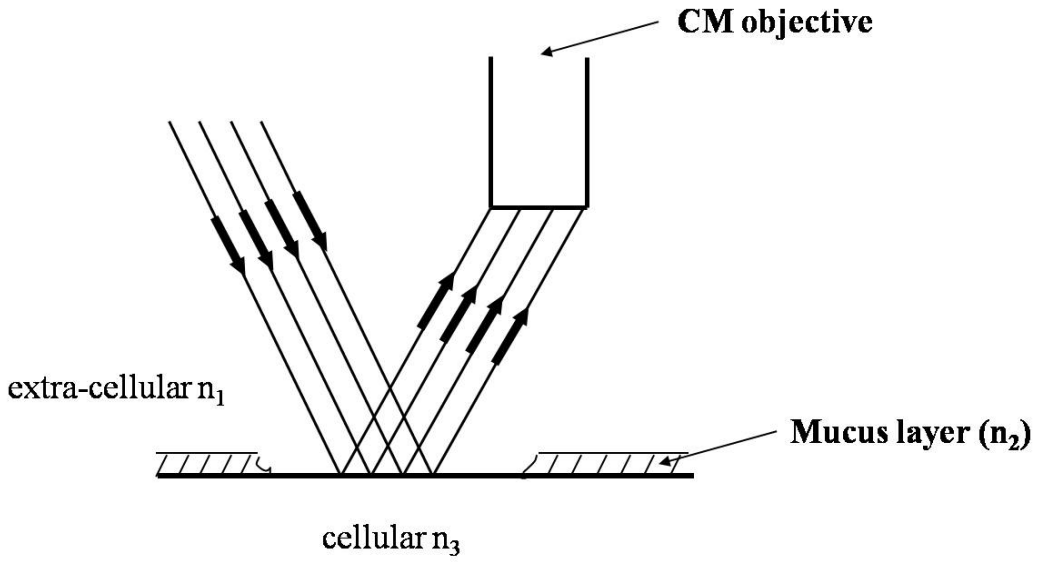


Figure 98: Light may get reflected because a disruption of the protective mucus layer could expose underlying cell (different refractive index)

* Extra-cellular medium n_1 would be tear film or optical coupling medium used during CM

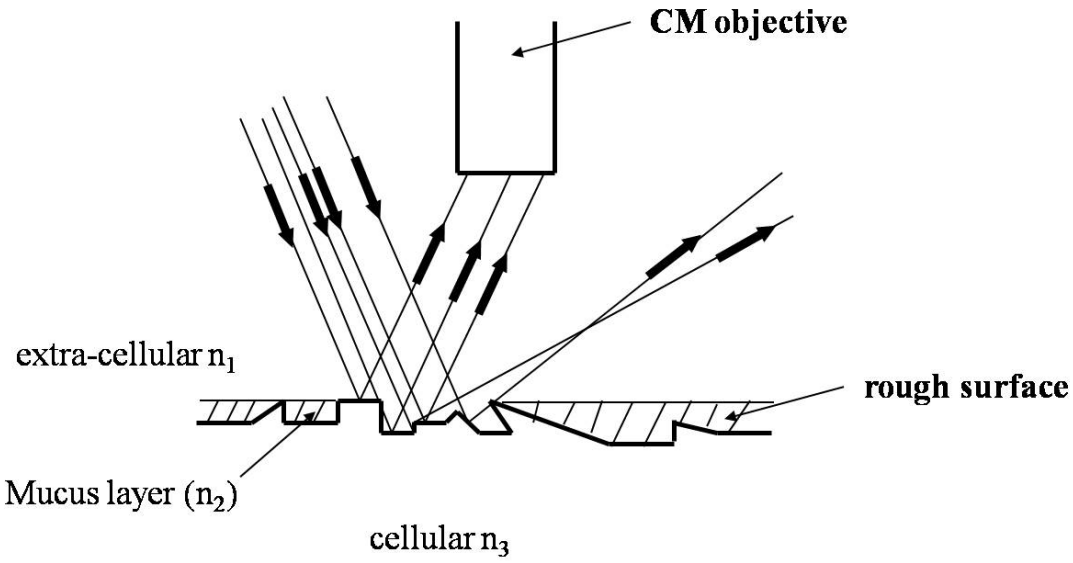


Figure 99: Some light may be reflected at a rough surface that is potentially exposed by disrupted mucus layer

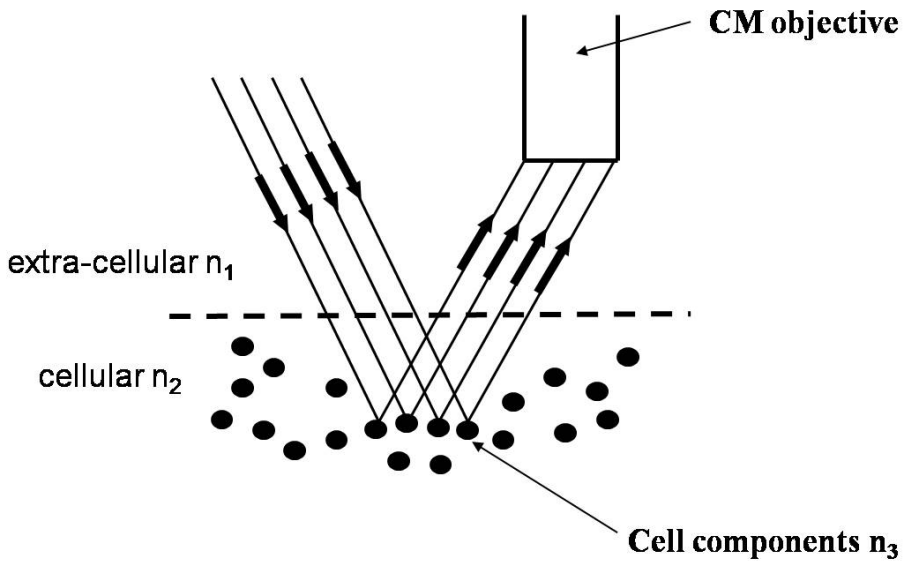


Figure 100: Light may be reflected by areas with different refractive indices within the cell

Another aspect which involves interference is mentioned when studying scatter in biological tissue.[325] Backscatter enhancement, enhanced backscattering or coherence enhanced backscatter[326-330] is a phenomenon that occurs from constructive interference in elastic light scattering and results to enhanced scattered intensity in the backward direction.[327] It occurs when photons in a plane wave that is scattered from the medium in the backward direction has a time-reversed photon that travels the same path in the opposite direction. Both of these photons have the same phase and hence interfere constructively. It is reported[326] that enhanced backscatter may be observed when the wave field gets disrupted by chaotically distributed scatterers or by a rough surface.[326] Another requirement in order for enhanced backscatter to occur is that the region occupied by the scatterers is large relative to the wavelength.[326] This idea of enhanced backscatter may be applied on explaining hyper-reflective cells. If, for example it is assumed that the orderly structure within a cell is disrupted which leaves chaotically distributed scatterers, e.g. by fluid entering the cell, and enhanced backscatter might occur. Similar, disruption of the surface could also perhaps result in enhanced backscatter.

The following table lists possible situations in which a superficial cell could theoretically become hyper-reflective (Table 51).

Table 51: Possible situations in which a superficial epithelial cell could become hyper-reflective

Situation	Possible cause	Condition/consequence
- Loss of microvilli and microplicae (Figure 98)	- Toxic effect?	- disruption of cellular surface →rougher surface?
- Partial or entire loss of cell (Figure 99)	- Toxic effect?	- Disruption of mucus layer, gap fills with tear film → exposure of a lower stratum wing cell → different refractive index?
- Loosening or loss of tight junction between cells	- Toxic effect?	- Tear film can enter between cells → additional interface → change in refractive index? → change in refractive index gradient?
- Cell swells (Figure 100)	- Hypoxia? - Damaged cell → fluid enters	- Disruption of orderly arrangement to maintain cells transparency → change in refractive index?
- Changes to biochemistry or genetics of cells, e.g. corneal crystallins[331] (Figure 100)	- Activation of cell defence mechanisms	- Loss of transparency → change/ disruption of cellular structure and composition

The situations outlined above in the table may theoretically lead to hyper-reflectivity of superficial cells, but they do not explain why all of the cells *are not* hyper-reflective. A suggestion could be that those cells that are already close to the end of their life cycle may be specifically susceptible to trauma or changes to their environment, which could result in the speedier death (necrosis/apoptosis).

Another interesting point is that not every participant who was exposed to the specific lens/solution combinations did actually manifest SICS and hyper-reflective cells. It is likely that the tear film composition also plays a role; perhaps, for example, a higher concentration of proteins and enzymes in their tear film is more effective in protecting the cornea from a toxic environment.

The actual cause or causes of the hyper-reflectivity of superficial epithelial cells however are likely the result of complex biochemical mechanisms becoming manifest as a biophysical phenomenon – the hyper-reflectivity. These biochemical effects will remain hidden, until the pathways leading to the structural change/ breakdown of the cells have been fully elucidated.

Chapter 7

Conclusion and Further Work

Conclusions

The specific aims of this thesis were: (1) to characterize the appearance of the normal superficial epithelial cells using CM, (2) to investigate variables that could influence the appearance of hyper-reflective superficial cells, and (3) to try and establish a relationship between the appearance of hyper-reflective superficial cells and SICS.

The first part of Chapter 3 of this dissertation addressed the first research aim. The superficial epithelial appearance in younger non-contact lens wearers and in post-menopausal dry-eye symptomatic and asymptomatic participants was characterized. Low numbers of hyper-reflective cells were observed in a few participants in the non-contact lens wearing group and in 3 people of the dry-eye symptomatic group.

Variables that could possibly be associated with the appearance of hyper-reflective superficial cells were examined in the second part of Chapter 3, in Chapter 4 and in Chapter 5 of this thesis. These variables included the effect of age and dry eye, as well as the effect of contact lenses and solutions and the effect of diagnostic agents (sodium fluorescein and anaesthetics). The conclusions drawn from the different experiments are shown in Table 52.

The results obtained from this thesis indicate that hyper-reflective superficial epithelial cells occur when wearing specific lens/solution combinations. This, for the first time confirms the suggestions made by Harvey[1] who reported hyper-reflective cells in her MSc thesis and that these hyper-reflective cells occurred when a specific lens/solution combinations was used. However, bright superficial cells were occasionally observed during almost every experiment of the present dissertation. This is in agreement with the literature reporting that different shades, from light to dark, of superficial epithelial cells can be observed, perhaps representing different stages of normal corneal cell turnover.[40;243]

Table 52: Possible variables that may induce the appearance of hyper-reflective cells and the conclusion drawn from the conducted experiments

Variables that may cause hyper-reflective cells	Conclusion
Contact lens solution	x
Lens/solution combinations	x
Specific lens/solution combinations that induce SICS	✓
Contact lens wear	x
Age	x
Dry eye symptom	x
Sodium fluorescein	x
Anaesthetics	x
Long-term use of same type of contact lens and solution	x

* x = negative, ✓ = positive

The third research aim was to try and establish a relationship between the appearance of hyper-reflective cells and SICS. This issue was addressed in an experiment in Chapter 5 of this dissertation and the results of this experiment suggest that large numbers of hyper-reflective cells can be observed primarily when SICS is present. Also in this study, it was shown that it is possible to perform CM with the contact lens *in situ* and therefore eliminating the use of anaesthetics.

Future Work

Why we see hyper-reflective cells and what makes them hyper-reflective, is not known. If these cells are dead or still viable and what exactly happens to them over time, is something that needs further investigation. Determining if the cells are still attached to the ocular surface or are just floating in the tear film and are possibly trapped by the lens could be an easily achieved experiment by performing CM over the contact lens and then again immediately after removal of the lens. Also, an experiment that would allow the harvest of hyper-reflective cells in particular, either by removing them off the ocular surface or from the contact lens, and then examining their viability would be valuable. Proteomic analyses on the cells might be a method to determine possible genetic alterations. Another experiment to monitor the same area of cells over time would also be valuable. This would allow

determining, whether cells actually returned to their normal, non-hyper-reflective state or if they are shed and flushed out with the tear film.

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