THE PREDICTIVE ABILITY OF CLINICAL TESTS FOR DRY EYE IN CONTACT LENS WEAR

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This PhD is in commemoration of my father,

and dedicated to my lovely wife Britta and both of my wonderful

children, Franziska and Maximilian

II. Summary:

Thirty to fifty percent of contact lens wearers discontinue contact lens wear[1]. Therefore it is essential for the clinician to have a predictive method that can forecast the development of dryness symptoms in contact lens wear.

Contact lens wear induces tear film instability leading to increased tear film evaporation and its associated hyperosmolarity. This in turn causes ocular surface inflammation resulting in the release of cytokines, impacting tear mucin production, which further destabilises the tear film[2].

Since this mechanism can only be fully evaluated by an intensive laboratory-based approach, the clinician must rely on assessing those factors easily accessible within the practice situation: tear film stability and mucin loss/epithelial damage. These two factors are inter-related, and investigating one also provides information about the other.

For this PhD, these two components of the mechanism have been investigated.

This PhD has found that:

(1) Bulbar and limbal redness are inter-related; using the CCLRU grading scales, a limbal redness grade above 2.5 or a bulbar redness grade above 3.0 may be considered abnormal.

(2) Symptomatic, experienced soft contact lens wearers exhibit significantly more lid wiper epitheliopathy (LWE) and lid parallel conjunctival folds (LIPCOF) but not corneal staining, bulbar hyperaemia or decreased Pre-lens break up time (PLBUT). LWE and LIPCOF are significantly correlated, suggesting that both are related to mechanical forces during blinking caused by a deficiency of the mucin layer.

(3) LIPCOF and LWE are also positively correlated with symptoms amongst non-contact lens wearers.

Using these two tests as a surrogate, the clinician has, for the first time, a useful indication of the mucin layer in contact lens patients.

Returning then to the fundamental question – "Can the development of dryness symptoms in soft contact lens wearers be predicted?" This PhD has found that the clinician can use a combination of currently available tests to meet this question. The optimum combination of tests was found to be LIPCOF Sum plus NIBUT plus OSDI, termed the P-Test. The P-Test shows outstanding[3] potential as a discriminator and predictor of contact lens induced dry eye.

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III. Abbreviations

ANOVA	Analyses of Variances
AUC	Area Under Reciver Operating Characteristic Curve
BUT	Tear break-up time
CCH	Conjunctivochalasis
CCLRU	Cornea and Contact Lens Research Unit
CL	Contact lens
CLDEQ	Contact Lens Dry Eye Questionnaire
CLIDE	Contact lens induced dry eye
DEWS	International Dry Eye Workshop
Dk	Oxygen permeability
Dk/t	Oxygen transmission
DMA	N-dimethylacrylamide
DTT	Dithiotreitol
FDA	Food and drug administration
GPC	Giant papillary conjunctivitis
HEMA	2-hydroxyethyl methacrylate
HLA	Human leukocyte antigen
ICAM	Intercellular adhesion molecule
IL	Interleukin
KCS	Kerato conjunctivitis sicca
LIPCOF	Lid parallel conjunctival fold
LLT	Lipid layer thickness
LR	Likelihood ratio

LWE	Lid wiper epitheliopathy
MA	Methacrylic acid
MGD	Meibomian gland disease
MMP	Matrix metallopeptidase
mPDMS	Monofunctional polydimethylsiloxane
MUC	Mucin
NIBUT	Non-invasive break-up time
O	Observer
OA	overall accuracy
OCI	Ocular Comfort Index
OCT	Optical coherence tomography
OSDI	Ocular Surface Disease Index
PhD	Doctor of Philosophy
PHMB	Polyhexylmethyl biguanide
PLBUT	Pre-lens break-up time
PLH	Positive likelihood ratio
PLTF	Pre-lens tear film
PPV	Positive predictive value
PRTT	Phenol red thread test
P-Test	Pult-Predictive-Test
PVP	Polyvinyl pyrolidone
ROC	Receiver Operating Characteristic Curve
SD	Standard deviation
TEGDMA	Tetraethyleneglycol dimethacrylate

TF	Tear film
TMH	Tear meniscus height
TNF	Tumor necrosis factor
TRIS	Trimethylsilyl
TTR	Tear turnover rate
UK	United Kingdom
USA	United States of America
WHS	Women's Health Study

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1. Introduction

Even with the progressive improvement in contact lens materials, there are still a considerable number of patients who stop wearing their lenses. This 'drop-out rate' varies with the lens design and lens wear modality, but a major reason is subjective discomfort[4-6] while wearing the contact lens. Contact lens wearers are five times more likely to experience discomfort than spectacle wearers[7]: 30% of all contact lens wearers in Germany drop out in the first year due to symptoms of dryness[8], 72% of contact lens wearers in the USA and 53% in the UK claim the primary reason of discontinuation as discomfort[6]. Discomfort may be due to a tear film problem, lens mechanical irritation or an inherent change in the contact lens itself. About a quarter to half of non-lens wearing asymptomatic patients experience symptoms of dryness when in contact lens wear[7,9-12]. This condition is named contact lens induced dry eye (CLIDE). Unfortunately, although there is a wide range of tear film tests and contact lens types and materials, clinicians are not successful in predicting which patients will have problems before fitting them. Current tests have a poor correlation with symptoms[13,14], and, indeed, may not be assessing the correct parameters.

It is known that contact lenses affect the tear film[15,16] and that the tear film is associated with comfort, so perhaps if the test or tests which best describe the patient at risk of discomfort can be determined, then a way to avoid or treat the problem can be found.

This PhD investigates the development and evaluation of an easy-to-use and valuable test to predict dry eye symptoms in lens wearers in clinical practice. The overall aim of the thesis can be summarised as being:

Is it possible to predict symptoms of dryness in soft contact lens wearers?

2. Literature Review

2.1 Physiology of the tear film

The tear film protects the eye against chemical, mechanical, bacterial, and viral attack, maintains an optically uniform interface between the air and cornea, and lubricates the eye to ensure a smooth movement of the eyelids over the globe during blinking[17]. For safe and comfortable contact lens wear, maintaining the integrity of the tear film is important. To understand the interactions between the tear film, the ocular surface and contact lenses and how these factors relate to dry eye symptoms in contact lens wear, a fundamental knowledge of tear film physiology is indispensable.

2.1.1 Tear film structure

The tear film has three main components, each of which has been classically described as a separate layer that performs a specific function. The boundaries and thicknesses of these layers are under discussion. Wolff[18] suggested a tri-laminar structure that is about 7μm thick and is composed of an outer lipid layer (approximately 0.1μm thick), an intermediate aqueous phase (7μm), and an inner mucous layer (0.05μm). Tiffany et al[19] confirmed the same basic structure, but argued that the interfaces between the air, lipid, aqueous, mucous and epithelium each have their own peculiar physio-chemical properties, and that the tear film should be considered as composed of six layers. Prydal and Campbell[20,21] criticised the proposed thickness of the mucous layer of the Wolff model and argued that the tear film should be thought of as being 34-45μm thick, but with the same tri-laminate structure. From a theoretical standpoint (based upon the appearance of oil slicks on the ocean as viewed from space), Baier and Thomas argued that the tear film structure is the reverse of the Holly's model - the outer layer of the tear film is a mucinous glycoprotein gel, with an inner lipid layer in contact with the

epithelium[22]. Hodson and Earlam[23] assumed that the tear film had no defined structure, but had the composition of a loose fibronectin gel in which the lipid, mucous and aqueous components are intermixed. King-Smith et al [24] used reflectance spectra from the cornea and measured a tear film thickness of only 3μm. This was confirmed by Wang et al[25] using optical coherence tomography (OCT). However, setting aside the uncertainty as to its true thickness in recent models, the general opinion is that the tear film is composed of an outer lipid layer, a mucous-aqueous layer and an underlying mucous-layer (glycocalyx) that covers the corneal and conjunctival epithelium (Figure 2.1)[26-28].

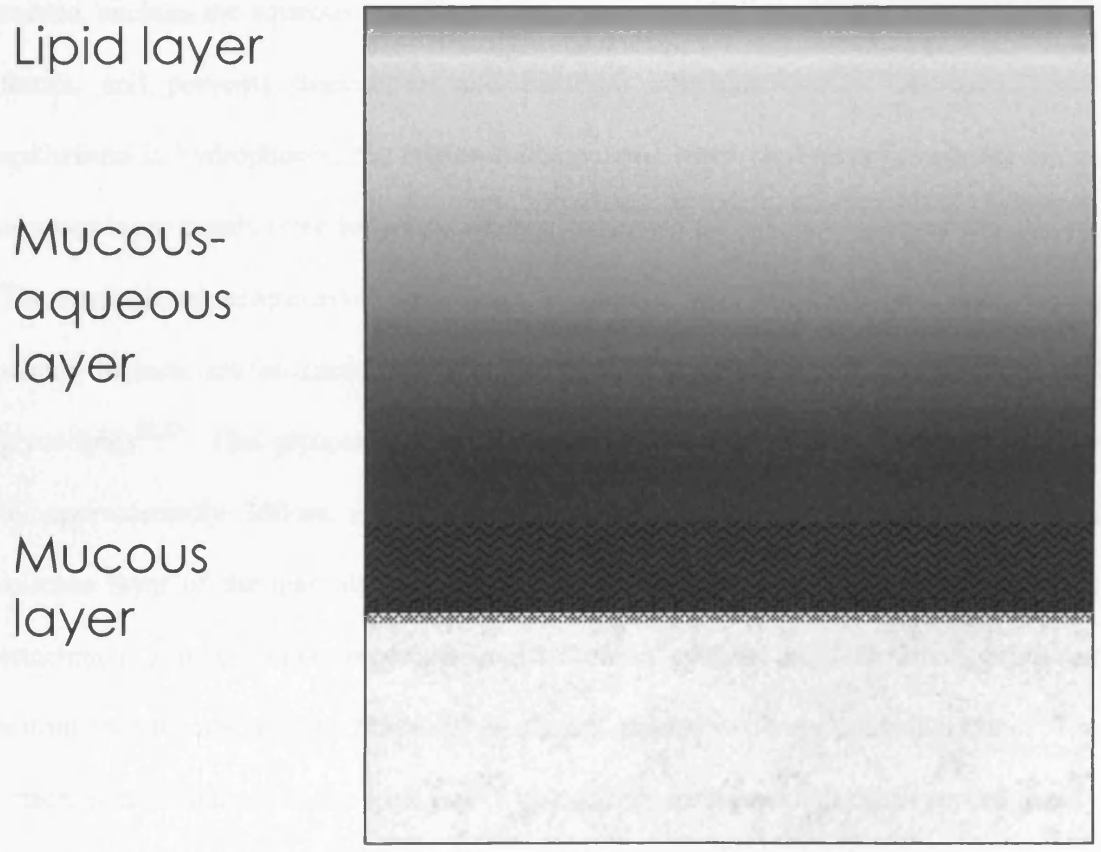


Figure 2.1: Model of tear film structure.

2.1.2 Composition of the tear film

The tear film is a complex structure composed of water, salts, enzymes, proteins, immunoglobulins, lipids, several metabolites, and exfoliated epithelial and polymorphonuclear cells[29]. It is a highly dynamic fluid, making it impossible to define its exact composition at a particular point in time, since the specific content will vary depending upon the environmental challenges that the ocular surface has to deal with[29].

2.1.2.1 Mucous layer

The ocular mucous layer is composed of mucins, immunoglobulins, urea, salts, glucose, leukocytes, cellular debris and enzymes[30]. The mucous layer lubricates and protects the cornea, anchors the aqueous tear film to the corneal epithelium protecting it from shear forces, and prevents desiccation and bacterial contamination[31]. As the corneal epithelium is hydrophobic, the hydrophilic mucous layer facilitates the spread of the aqueous layer evenly over the ocular surface.

The corneal and conjunctival epithelium is covered with microvilli and microplicae, which, in turn, are covered by a glycocalyx that is composed of glycoproteins and glycolipids[32,33]. This glycocalyx extends anteriorly from the microvilli and microplicae by approximately 300nm and can extend laterally between the microvilli[34]. The mucous layer of the tear film attaches to the carbohydrate-rich glycocalyx[26,35]. This attachment of mucous may protect the epithelium by causing the shear forces, produced within the mucous layer by blinking, to occur further away from the cell surface. The attachment of mucous to the glycocalyx also allows the aqueous layer to spread evenly over the corneal epithelium[36].

The corneal and conjunctival epithelia synthesise mucins at the apical surface of the epithelium to constitute the glycocalyx[35]. These membrane-bound mucins (MUC1, 4 and 16) are important in tear film spreading and are essential for proper ocular surface wetting. Membrane-bound mucins have a hydrophobic amino acid segment that spans the plasma membrane, allowing the mucin core protein to remain intimately associated with the epithelial cell[37]. The secretory mucins (MUC2, 5AC, 5B and 6) are very large molecules that contribute to the gel-forming aspect of the tear film. These mucin types form a gel overlying the epithelial surface that provides lubrication and protection to the cells. Gel-forming mucins form networks of tangled linear polymers that are responsible for the non-Newtonian thixotropic, or viscoelastic, properties of mucin gels. These help to avoid shearing damage during rapid relative movements of the lids and globe[35,38]. The mucous layer is secreted mostly by the conjunctival goblet cells. However, the corneal and conjunctival epithelia also contribute to the mucous layer. Conjunctival goblet cells are secretory cells, and these may be stimulated to secrete mucin by histamine, antigen, immune complexes or mechanical forces (i.e. blinking)[39]. Sensory, sympathetic and parasympathetic nerves innervate the conjunctiva surrounding the goblet cells[40,41]. Thus, stimuli from the cornea and conjunctiva can indirectly induce goblet cell mucin secretion[40,41].

A deficiency of aqueous tears, damage to the epithelium or glycocalyx, or an increase in epithelial cell loss allows mucous to adhere to itself or to the epithelium causing mucous clumping and leads to tear film instability and corneal damage[42].

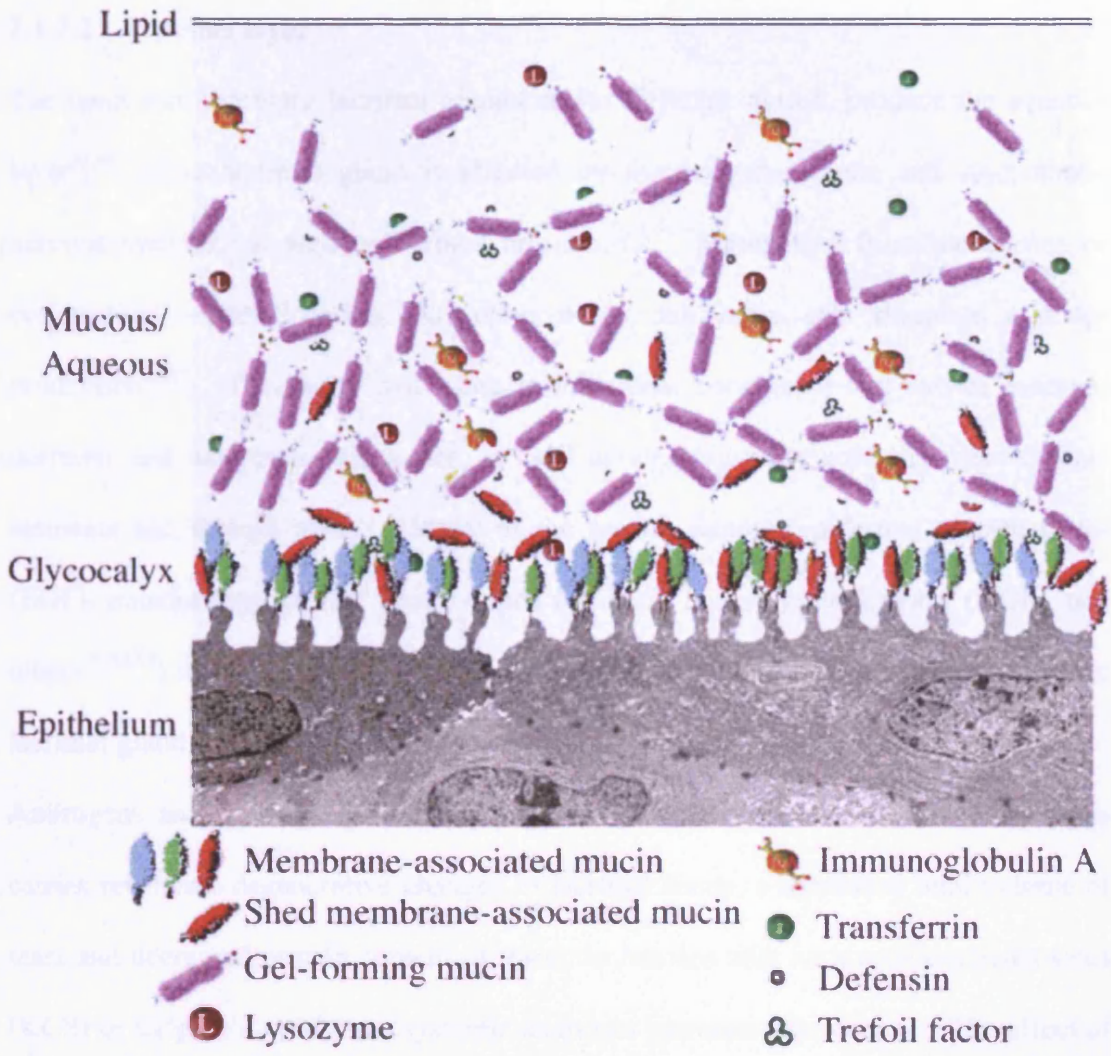


Figure 2.2: The composition of the tear film and the mucous layer[26].

2.1.2.2 Aqueous layer

The main and accessory lacrimal glands, under different stimuli, produce the aqueous layer[43,44]. The lacrimal gland is affected by the parasympathetic and sympathetic nervous systems, as well as various hormones[45,46]. Stimulation from the cornea or conjunctival sensory nerves, the optic nerve and brain can stimulate aqueous production[37,47]. This layer is the largest individual component and carries essential nutrients and oxygen to the cornea, as well as washing away epithelial debris, toxic elements and foreign bodies. Many of the growth factors (epidermal growth factor (EGF), transforming growth factor –alpha (TGF–α), human growth factor (HGF), and others[29,48,49]) that are present in the aqueous phase of the tear film are derived from the lacrimal gland tissue.

Androgens and oestrogens modulate lacrimal gland secretion. A lack of androgen causes reversible degenerative changes in lacrimal tissue, a decreased total volume of tears and decreased protein content of tears. In humans with keratoconjunctivitis sicca (KCS) or Sjögren's syndrome, systemic androgen increases tear volume. The effect of oestrogen on the lacrimal gland is controversial. Oestrogen deficiency has been linked to the development of KCS as well as degeneration of the lacrimal gland[50]. Other studies have shown no change in the lacrimal gland or tear film with oestrogen deficiency[51].

Decreased aqueous tear production results in a decreased growth factor concentration in the tears[52], with subsequent effects on ocular surface health. Many pro-inflammatory factors (e.g. human leukocyte antigen (HLA) DR, interleukin 6 (IL-6) and IL-8) are found in the aqueous phase, where they modulate the eye's response to changes in the condition of the ocular surface[53,54].

2.1.2.3 Lipid layer

The lipid layer is produced by the oily secretion from the meibomian glands, located in the tarsal plates of the lids. The lipid layer prevents the evaporation of tears[55-57] and enhances the stability of the tear film. It is assumed that the blink reflex is important in the release of secretions from the meibomian glands[29]. Rapid and forceful blinking, perhaps in response to a foreign body, increases the thickness of the lipid layer[58]. Conversely, office eye syndrome (a surface pathology with clinical subjective and objective signs very similar to dry eye that occurs in some office workers) appears to be associated with prolonged inter-blink periods and corresponding thinning of the lipid layer[59].

Although meibum consists of polar and non-polar lipids, it is the non-polar sterol esters and wax that predominate[60-62]. The polar fraction of the meibomian layer acts like a surfactant, comprised mostly of phospholipids, and spreads over the aqueous layer of the tear film, while the non-polar fraction of the meibomian layer lies more superficially[60,61] (Figure 2.3). The surface tension of the tear film decreases when lipids spread over the surface and this reduction of surface tension draws water into the tear film and thus increases the film thickness. The reduction of surface tension also allows the lipids to continue to spread during blinking[60]. If the lipid layer is removed, it would lead to evaporation of the tear film, resulting in decreased tear film break-up time and increased tear osmolarity[60,63]. Hyperosmolarity is believed to be relevant in the pathogenesis of various dry eye conditions[63].

The meibomian glands are sebaceous glands. Androgens are known to regulate other non-ocular sebaceous glands, usually in hair-covered areas. Androgen receptor mRNA and protein have been localised to rat, rabbit and human meibomian acinar epithelial cells[51,64]. In those studies it was determined that androgens modified the lipid

production of the meibomian gland[51,64]. It is assumed that androgens stimulate meibomian secretion, whereas oestrogens reduce secretion[62,64]. The meibomian glands have autonomic innervation, and contain various neuropeptides; however, no direct evidence exists of either sympathetic or parasympathetic control of secretion[60].

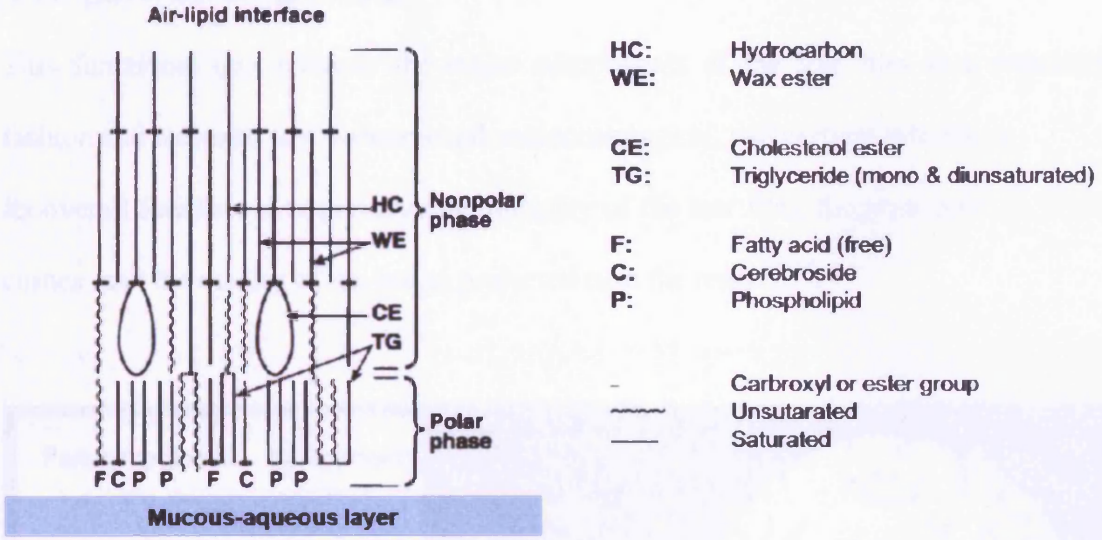


Figure 2.3: Composition of the lipid layer[65].

2.1.3 Regulation of tear production

The production of tears necessary for ocular surface homeostasis and repair is regulated by a reflex loop involving the ocular surface (conjunctiva, cornea) and the main lacrimal glands[29] (Figure 2.4). Stimulation of nerves at the ocular surface or in the nasal mucosa generates a reflex response via nerves passing to the lacrimal glands. Nerve impulses generated by emotional stimuli also feed into this reflex loop.

Trigeminal sensory fibres (fifth cranial nerve) arising from the ocular surface run to the superior salivary nucleus in the pons, from whence efferent fibres pass, in the nervus

intermedius, to the pterygopalatine ganglion. Here, post-ganglionic fibres arise, which terminate in the lacrimal gland, nasopharynx and vessels of the orbit. Another neural pathway controls the blink reflex, via trigeminal afferents and the somatic efferent fibres of the seventh cranial nerve (nervus facialis). Higher centres feed into the brainstem nuclei, and there is a rich sympathetic supply to the epithelia and vasculature of the glands and ocular surface.

This functional unit controls the major components of the tear film in a regulated fashion and responds to environmental, endocrinological, and cortical influences.

Its overall function is to preserve the integrity of the tear film, the transparency of the cornea, and the quality of the image projected onto the retina[66 67].

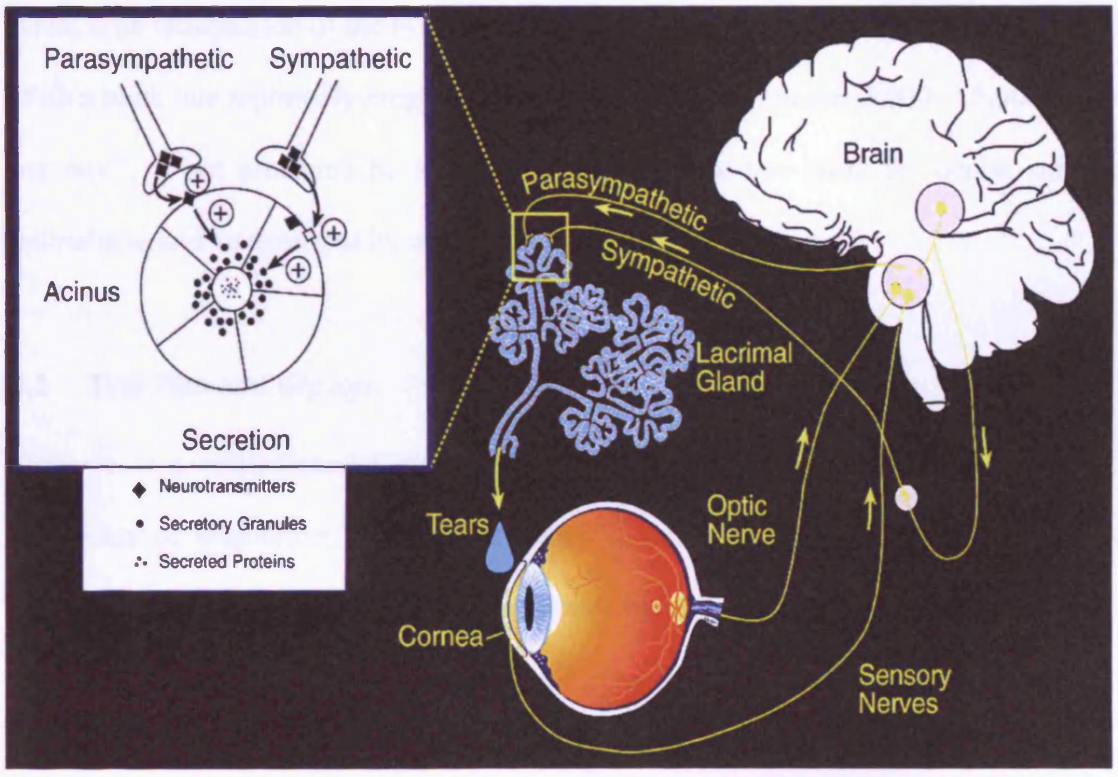


Figure 2.4: Schematic representation of the neural regulation of the lacrimal gland in the normal state[68].

Ocular surface irritation, from excessive evaporation, low humidity, or contact lenses, may result in chronic afferent stimulation and increased lacrimal secretion[66,69]. In contrast, the depressed corneal sensitivity caused by contact lens wear may decrease tear production, although this has not yet been demonstrated[29,70].

2.1.4 Tear film formation

The pre-ocular tear film is spread over the ocular surface by blinking. The sequential operation of the orbicularis and levator muscles of the lids spreads the tear fluid and reconstructs the tear film structure disturbed by evaporation or environmental contamination during the inter-blink period[71,72].

The movement of the lids leads to significant pressure on the bulbar surface at each blink, with retropulsion of the eye by 0.7–1 mm (up to 2 mm in forced blinking).

With a blink rate reportedly ranging from 3 to 15 times per minute, 3,000 –15,000 times per day[73], if not protected by an efficient visco-elastic tear film, the ocular surface epithelia would be damaged by the applied shear forces[72].

2.2 Tear film and dry eye

Dry eye is a multi-factorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance and tear film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface[2]. Objective findings are conjunctival and corneal staining[74-76], staining of the lid wiper area[77,78], conjunctival bulbar folds[79,80], ocular hyperaemia[13,81], inflammation of the lid margins and meibomian glands[62,76,82,83],

unstable tear film, reduction in tear film quality and reduced tear volume[76,84-89]. The main argument that is consistent in all forms of dry eye is tear film instability[28].

Based on data from large studies of dry eye to date, like the Women's Health Study (WHS)[90] and other studies[2,91,92], it has been stated that in the USA, about 3.23 million women and 1.68 million men, older than 49 years of age, suffer from dry eye. Tens of millions more have less severe symptoms which only become noticeable in association with some additional factors, such as low humidity or contact lens wear[2].

These different studies used a variety of methods (subjective and objective) to classifiy their patients, producing a prevalence range for dry eye of 5-30%. The higher estimates are derived from studies in which a less restrictive definition was used, and the lower estimates are derived from those studies in which a more restrictive definition was used. Looking more closely for differences between age groups, dry eye prevalence increases in subjects 40 years or older (18.1%) compared with those < 40 years (7.3%)[93]. Lipid anomaly dry eye might be the most prevalent sub-type (4.0%), followed by allergic/toxic dry eye (3.1%), primary epitheliopathies and lid surfacing/blinking anomalies (1.8%), and aqueous tear deficiency (1.7%)[93]. Dry eye patients suffer from many symptoms, but in particular, stinging, burning, itching, light sensitivity and blurry vision[94-98]. Dry eye limits a patient's quality of life as well as occupation options, for example computer work, dominant in many professions, is known to increase dry eye symptoms[59].

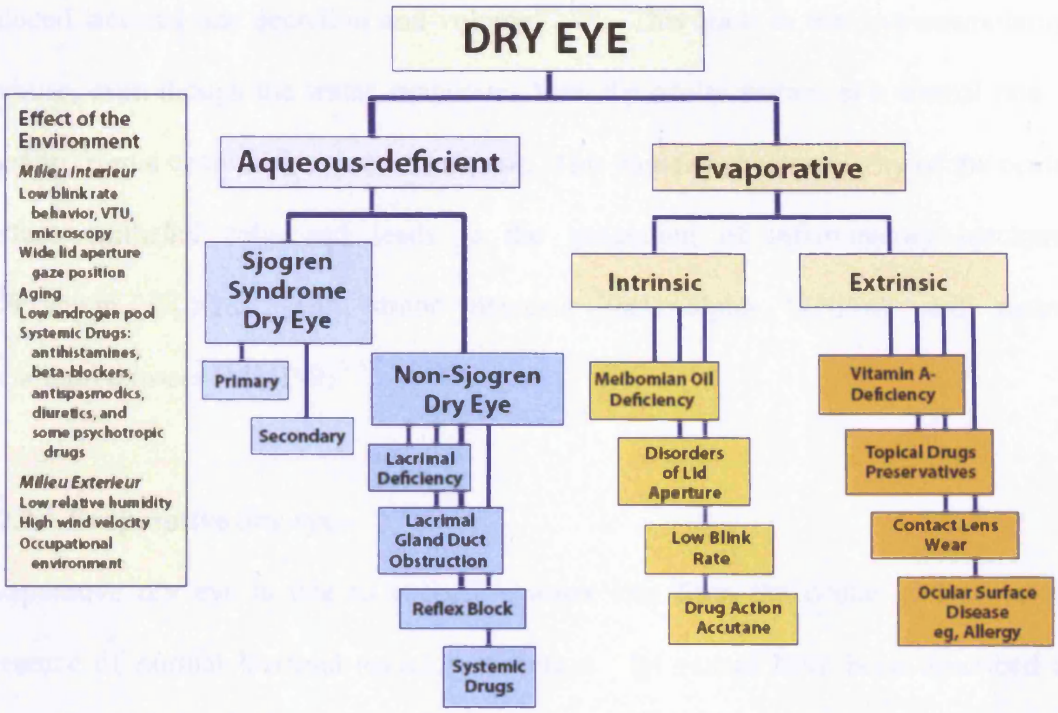


Figure 2.5: Schematic illustration of the relationship between dry eye and other forms of ocular surface disease[2].

2.2.1 Aqueous deficient dry eye

The two mayor classifications of dry eye are: aqueous tear deficient dry eye, and evaporative dry eye. From a practical perspective, the aqueous deficient dry eye is most often associated with reduced tear production, and the evaporative dry eye is most often caused by meibomian gland disease. The two types of dry eye frequently occur together[99,100].

Aqueous tear-deficient dry eye implies that dry eye is due to a failure of lacrimal tear secretion. Aqueous tear deficient dry eye is sub-divided into Sjögren's syndrome-related and non-Sjögren's syndrome tear deficiency, recognising the greater severity and associated systemic abnormalities of Sjögren's syndrome dry eye disease. In any form of dry eye due to lacrimal gland destruction or dysfunction, dryness results from

reduced lacrimal tear secretion and volume[101,102]. This leads to tear hyperosmolarity, because, even though the water evaporates from the ocular surface at a normal rate, it does so from a decreased aqueous tear pool. This causes hyperosmolarity of the ocular surface epithelial cells and leads to the generation of inflammatory cytokines (interleukin (IL)-1α; -1β; tumor necrosis factor-alpha (TNF-α) and matrix metalloproteinases (MMP-9)[103].

2.2.2 Evaporative dry eye

Evaporative dry eye is due to excessive water loss from the ocular surface in the presence of normal lacrimal secretory function. Its causes have been described as intrinsic or extrinsic. Intrinsic causes may be meibomian gland dysfunction, posterior blepharitis, low blink rate, lid disorders or meibomian gland obstruction and they are the most common cause of evaporative dry eye[28]. Extrinsic factors are disorders of the ocular surface (e.g. vitamin A deficiency, chronically applied topical anaesthetics and preservatives), ocular surface diseases (e.g. allergic conjunctivitis) and contact lens wear. However, the boundary between these two categories is blurred.

2.2.3 Hyperosmolarity

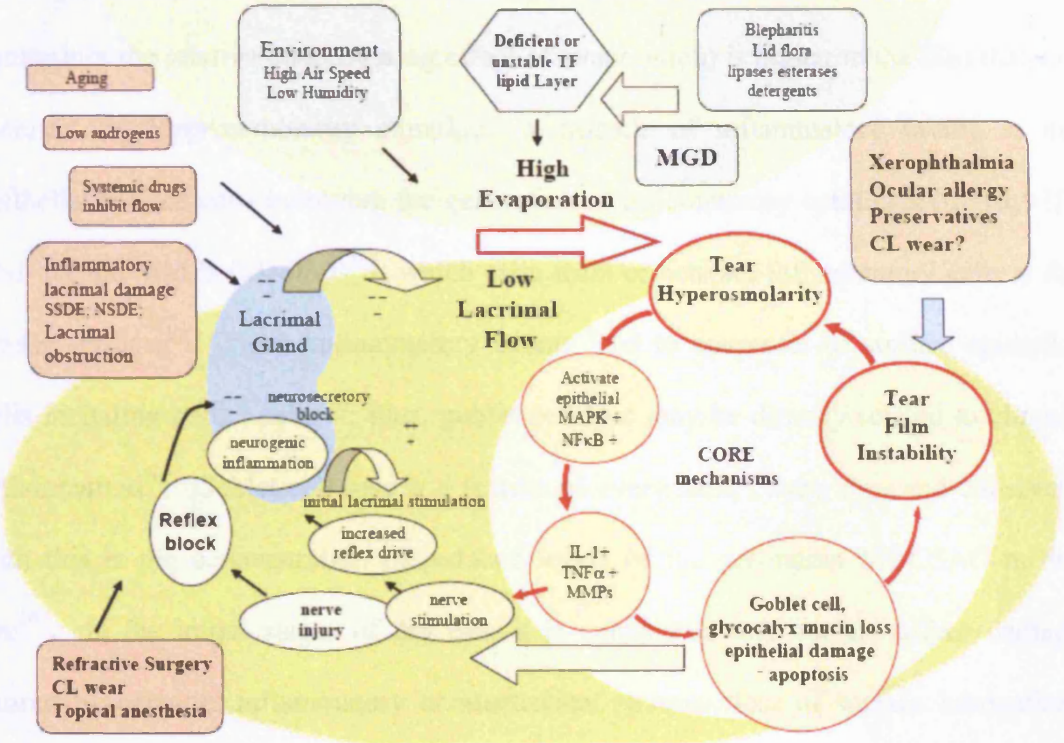


Figure 2.6 Mechanisms of dry eye (Report of the International Dry Eye Workshop[2].

Tear hyperosmolarity is a central mechanism causing ocular surface inflammation, damage and symptoms, and the initiation of compensatory events in dry eye. Tear hyperosmolarity results from water evaporation in situations of a low aqueous tear flow, or as a result of excessive evaporation, or a combination of these events. Nichols et al[7] reported the wide variation of tear film thinning rates in normal subjects, and that subjects with the fastest thinning rates would experience a greater tear film osmolarity than those with the slowest rates. Rapid thinning may be hypothesised as a risk factor for tear hyperosmolarity. Since the lacrimal fluid is secreted as a slightly hypotonic fluid, it will always be expected that tear osmolarity will be higher in the tear film than

in other tear compartments. Probably osmolarity is higher in the tear film itself than in the neighbouring menisci. One reason for this is that the ratio of area to volume (which determines the relative concentrating effect of evaporation) is higher in the film than the menisci[104]. Hyperosmolarity stimulates a cascade of inflammatory events in the epithelial surface cells involving the generation of inflammatory cytokines (IL-1α; -1β; TNF-α) and MMPs (MMP-9)[103], which arise from or activate inflammatory cells at the ocular surface[105]. These inflammatory events lead to apoptosis of surface epithelial cells including goblet cells[106]; thus, goblet cell loss may be directly related to chronic inflammation[76]. Goblet cell loss is a feature of every form of dry eye, and consistent with this is the demonstration of reduced levels of the gel-mucin MUC5AC in dry eye[107]. In the initial stages of dry eye, it is considered that ocular surface damage caused by osmotic, inflammatory or mechanical stresses (loss of surface lubrication) results in reflex stimulation of the lacrimal gland. Reflex trigeminal activity is thought to be responsible for an increased blink rate resulting in increased lacrimal secretion.

In lacrimal gland insufficiency, the reflex secretory response will be insufficient to compensate for the tear film hyperosmolarity and in the steady state, this form of dry eye will be characterised by a hyperosmolarity state with low tear volume and flow[2].

In evaporative dry eye (e.g. caused by MGD), it can be hypothesised that, since the lacrimal gland is healthy, lacrimal secretory compensation is at first able to compensate for tear hyperosmolarity. However, ultimately a hyperosmolar tear film would be produced with the characteristics of a larger tear volume and flow than normal. This possibility of a high tear volume dry eye is supported by the increased tear secretion (based on the Schirmer I test) noted in patients with MGD compared to normals[108]. However, these provocative hypotheses await experimental support. Knowledge is insufficient regarding the natural history of different forms of dry eye in relation to

ocular surface sensitivity. Most reports[109,110] suggest that corneal sensitivity is impaired in chronic dry eye disease, suggesting that an initial period of increased reflex sensory activity is followed by a chronic period of reduced sensory input. At this stage of dry eye, the reflex sensory drive for lacrimal secretion becomes reduced, which would reverse any compensatory drive that is postulated for the earlier phase of the disease. This would be expected to reduce the lacrimal secretory response, regardless of the aetiology of the dry eye, and would therefore worsen both aqueous deficient dry eye and evaporative dry eye by reinforcing the low volume state in aqueous deficient dry eye and converting a potentially high volume state in MGD-based evaporative dry eye to a normal or low volume state due to an added lacrimal deficiency[2].

2.2.4 Tear film instability

In some forms of dry eye, tear film instability may be the initiating event, unrelated to prior tear hyperosmolarity. Where tear film instability produces tear film break-up within the blink interval, it is assumed that this will give rise to local drying and hyperosmolarity of the exposed surface, to surface epithelial damage, and to a disturbance of the glycocalyx and goblet cell mucins[2]. This last consequence further reduces tear film stability as part of a vicious circle of events. An example of this clinical sequence, where tear film instability is due to a disturbance of ocular surface mucins, is allergic eye disease[111]. In seasonal allergic conjunctivitis, a disturbance of mucin expression at the surface of the eye is due to a hypersensitivity reaction, leading to the release of inflammatory mediators in response to allergen challenge. Other examples include the actions of topical agents, in particular preservatives, which excite the expression of inflammatory cell markers (e.g. human leukocyte antigen: HLA-DR, intercellular adhesion molecule: ICAM-1, which can be induced by interleukin-1 (IL-1)

and tumour necrosis factor-alpha (TNF-α) and is expressed by the vascular endothelium) at the ocular surface, causing epithelial cell damage, cell death by apoptosis, and a decrease in goblet cell density[2].

Use of preservative was associated with a lower expression of MUC5AC and the lowest MUC5AC levels were associated with inflammatory markers[112]. This negative correlation suggests inflammation as a basis for the decreased mucin expression, in addition to any direct effect of benzalkonium chloride on the goblet cells themselves.

Contact lens wear may also provide a route of entry into the dry eye mechanism, a route in addition to reduced corneal sensitivity. Contact lenses worsen the symptoms of dry eye patients. Even in an otherwise normal individual, the presence of a contact lens in the eye can produce the condition of dry eye. The contact lens induces tear film instability by disrupting normal tear physiology through thinning and break-up of the tear film, interrupting tear film reformation, rupturing the lipid layer with consequent increases in tear film evaporation, and by per-evaporation of fluid from the corneal tissue in soft contact lenses.

For a considerable time, contact lens wear has been recognised to cause changes to the ocular surface epithelia. Surface epithelial metaplasia and a reduced goblet cell density with hydrogel lens wear have been reported[113,114]. Other studies have shown an increase in goblet cell density evolving over a period of 6 months in subjects wearing polymacon, galyfilcon and silicone hydrogel lenses[115,116]. In another study combining impression cytology with flow cytometry, an increase in inflammatory markers at the ocular surface and a non-significant trend toward a decrease in the expression of mucin markers (MUC5AC) in patients with a history of chronic contact lens wear was found[117]. In summary, it appears that contact lens wear may activate pro-inflammatory markers and stimulate the ocular surface epithelia to a variable degree. It is not yet

possible to say whether these changes alone predispose individuals to the occurrence of dry eye with lens wear[2].

2.2.5 Summary of dry eye

Although it is a complicated condition, dry eye can very simply be focused on the presence of tear film hyperosmolarity, accompanied by symptoms. Hyperosmolarity can be induced by abnormal evaporation and/or reduced lacrimal flow or allergies, preservatives in medications or contact lens solutions, or by contact lenses themselves. Allergies, preservatives or contact lenses induce secondary hyperosmolarity due to tear film instability. The goal of the clinician and scientists is to find the initial cause of hyperosmolarity and the dry eye, which is a hard job to do, since, as can be seen from the core mechanism (Figure 2.6 and 2.7), many factors are associated and may have to be treated together.

2.3 Discomfort in contact lens wear

With the progressive development of contact lens design and materials over the last half-century, there has been an accompanying increase in the contact lens wearing population worldwide. Lenses are now available for the correction of ametropia, astigmatism and presbyopia; they can be worn for daily wear, extended wear or continuous wear; and in gas permeable, hydrogel or silicone hydrogel materials. Contact lens wear is now a very successful mode of refractive error correction. But there is still a shadow over this success – many contact lens wearers experience significant levels of discomfort when wearing their lenses, to the extent that discomfort is now the principal reason for wearers stopping or reducing their contact lens wear. Although the actual number of patients who have permanently abandoned contact lens wear is impossible to estimate, it is known that in the USA 73% stopped wear because of discomfort, in the UK 52% and in Germany 30%[6,8]. A larger number of wearers (approximately 30-50%) discontinue contact lens wear for a period, with at least half of these wearers doing so for two years or longer[1]. Putting these wearers together gives a very large number of failed or failing contact lens wearers - Morgan in 2001[118] reported that there were about 2.1 million contact lens drop-outs in the United Kingdom.

Dry eye symptoms are much more prevalent in patients who wear contact lenses than in the non-lens-wearing population[119,120]. It is reported that contact lens wearers are 12 times more likely to report symptoms of dry eye than clinical emmetropes and only 5 times more likely to report symptoms than spectacle wearers[7]. (The difference between emmetropes and spectacle wearers is presumably due to the increased number of presbyopes in the latter group). The implication from these data is that about 18 million

contact lens wearers in the USA will be experiencing symptoms of dryness[120]. Women were found to report dry eye more frequently than men, with 40% of men and 62% of women classified as having dry eye[2]. Dry eye is the most common reason for contact lens discontinuation, and the patient with pre-existing dry eye presents particular challenges to the contact lens fitter[6,11]. Comfort during contact lens wear strongly influences continuation of use: half the 2.1 million contact lens wearers drop-outs in the UK and three-quarters in the USA are caused by discomfort[6,118], 30% of new contact lens wearers in Germany stop wearing lenses after the first year[8].

2.3.1 Contact lens induced dry eye

Many soft contact lens wearers experience symptoms of dryness in contact lens wear, even if they are asymptomatic without lens wear[7,9-12]. This is named contact lens induced dry eye (CLIDE). CLIDE is a common source of ocular discomfort, and contact lens wear can increase symptoms in patients with pre-existing dry eye or even induce symptoms in otherwise asymptomatic patients. Dryness is the single most common reason for lens discontinuation[5].

The difference between asymptomatic and symptomatic contact lens wearers is evident in both subjective symptoms and objective signs. Symptomatic lens wearers report an increase in symptoms (e.g. discomfort or dryness) towards the end of the day, while asymptomatic lens wearers remain relatively constant[9,12,89]. Subjective dry eye symptoms are often worse than any objective clinical signs, making it difficult for the eye care practitioner to objectively treat and monitor the condition. Often, because many patients expect to experience dryness while wearing contact lenses, they may not report discomfort until they are extremely distressed, thus complicating evaluation and treatment. Contact lens tolerance represents a constant balance between motivation and

perceived annoyance. A highly motivated contact lens wearer is willing to tolerate many difficulties; however, when the level of discomfort/inconvenience becomes unacceptable, the patient may discontinue contact lens wear, frequently seeking alternative options or simply dropping out of the contact lens practice[11]. It is vital for the eye care provider, as well as the patient, to pre-empt dissatisfaction and establish successful management strategies for the patient with dry eye related to contact lens wear. The condition may be reported as dryness, irritation, burning, stinging, foreign body sensation, visual blurring, or general discomfort[9,11,80]. The causes of contact lens related symptoms and of lens intolerance are of personal and general economic importance. The causes of dryness during contact lens wear are complex and multi-factorial. Contact lens wear comfort depends on a number of factors, including the interaction between the tear film and the ocular surface. Poor tear film quality/stability, oxygen deprivation, lens deposits and adverse reactions to contact lens solutions all contribute to dry eye and lid disease. In addition, allergies, environmental factors, and medications can further hamper successful contact lens wear by the dry eye patient. Changes in the composition or quantity of the pre-ocular fluid as a result of excessive evaporation, hyperosmolarity, decreased tear clearance, or changes in the morphology of the ocular surface epithelia, might all influence the comfort of wearing contact lenses[104,121]. Changes in the quantity or quality of mucins, caused by hyperosmolarity and/or ocular inflammatory cytokines, likely induces ocular discomfort, since one of the functions ascribed to mucins[38,122] is lubrication of the ocular surface, which is pivotal in contact lens comfort. Surface mucins lubricate and anchor the tear film to surface epithelia. Further protection from friction is provided by shear thinning[31]. The ocular surface is also affected by water content, ionicity, oxygen permeability, and modulus of the lens, as well as by surface characteristics, such as protein, lipid and mucin

deposition, protein adsorption and wettability. The choice of contact lens cleaning solutions with regard to action, cytotoxicity and biocompatibility are as important as the choice of the lens itself. Principally contact lenses weaken or disturb the stability of the tear film and therefore can induce hyperosmolarity, which ultimately leads to epithelial damage. This progressively influences the still instable tear film in a negative way and the core mechanism is started (Figure 2.7).

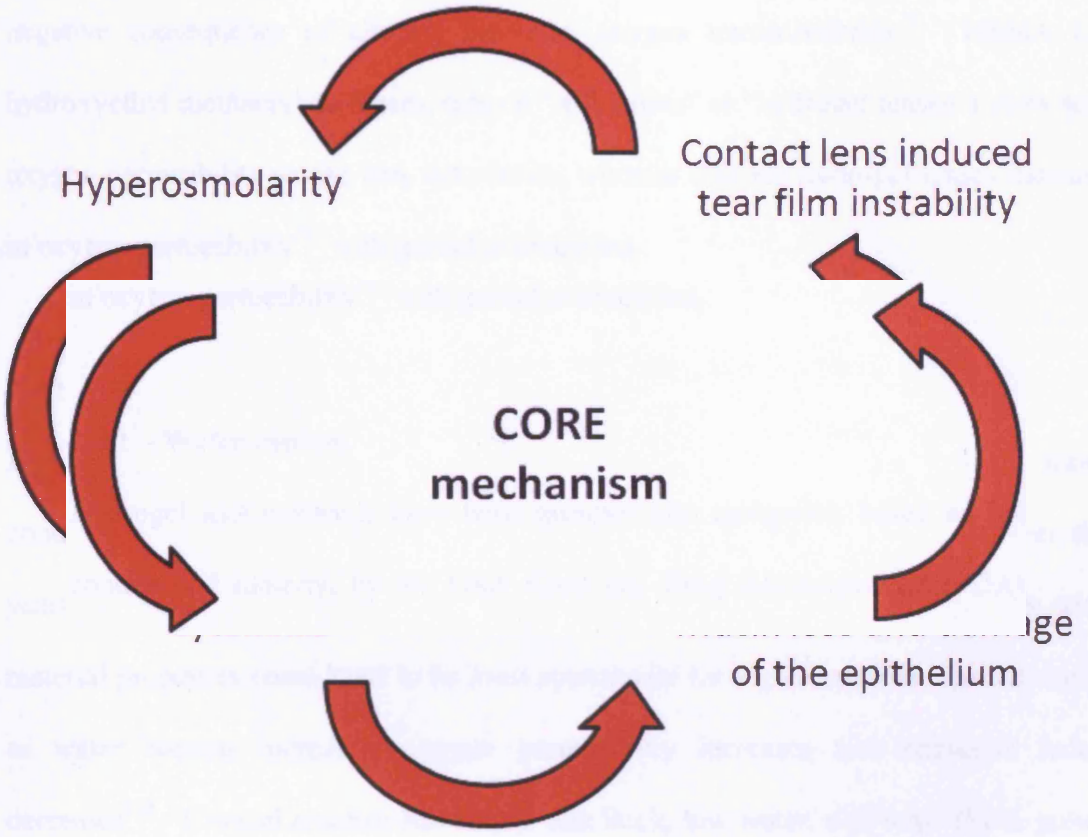


Figure 2.7: The core mechanism due to contact lens induced tear film instability.

2.4 Contact lens materials and properties

Many contact lens materials are available and depending on parameters like water content, ionicity, oxygen permeability, refractive index and modulus, they can be soft hydrogels, silicone hydrogels or rigid gas permeable. From the perspective of hydrogel lens wear comfort, these parameters (in particular water content, chemical composition and lens thickness) can affect the degree of dehydration of a contact lens. Dehydration affects the fit of a hydrogel lens by altering the lens parameters and, in addition, as the negative consequence of altering the lens' oxygen transmissibility[10]. HEMA (2-hydroxyethyl methacrylate lenses, termed "soft lenses" or "hydrogel lenses") show less oxygen permeability as the lens dehydrates, whereas silicone hydrogel lenses increase in oxygen permeability[123] with partial dehydration.

2.4.1 Water content

Hydrogel lens materials have been grouped into categories, based on polymer water content and ionicity, by the USA Food and Drug Administration (FDA). Over the years, many practitioners have used the FDA categories to select contact lenses with material properties considered to be most appropriate for a given patient. For example, as water content increases, oxygen permeability increases and refractive index decreases[124]. Clinical practice has shown that thick, low-water, non-ionic (FDA group I) lenses undergo less dehydration/deposition and, therefore, provide more hours of lens-wearing comfort[11]. Lenses with higher nominal water content have been associated with CLIDE[7]. Dehydration changes the flexibility of the contact lens, as well as oxygen transmission and lens fit, which can affect lens comfort, visual quality, and the ocular surface[125].

Contact lens materials go through phases of dehydration during lens wear. Lenses with higher ratios of free to bound water content show rapid initial dehydration[126], irrespective of the water content[11,127]. Low-water-content materials have approximately the same amount of bound water as materials with higher water content, but the higher water content lenses have more free water[127]. This was confirmed by Efron et al who reported that the bulk water content of hydrogel contact lenses changed significantly only within the first 5-10 minutes on the eye[128,129]. The amount of initial water fluctuation is related to the lens thickness, osmolarity of the storage solution and the temperature of the lens upon insertion[11]. After the initial change, bulk water content fluctuates very little throughout the wearing period. Tranoudis and Efron[130] reported a reduction in water content, oxygen transmissibility and lens diameter after a wearing period of 6 hours, as well as reduced lens movement on blink. Morgan and Efron[131] also demonstrated an "ageing effect," with water content gradually decreasing over a four week wearing period. Lens dehydration substantially reduces the oxygen transmission through HEMA-based materials, since hydrogel lenses depend on water for their oxygen transmissibility. This is important, since, ocular discomfort has been related to hypoxic conditions, even in non-lens wear[132]. However, Gispets et al[124] reported that water content of hydrogel lenses used on a daily wear basis do not influence either the objective or subjective tolerance of subjects with tear film deficiency. Therefore, it is difficult to attribute dry eye symptoms associated with lens dehydration to only one factor, such as lens fit, oxygen permeability or free lens water content.

The pre-lens tear film (PLTF) is prone to evaporation, rupture and dry spot formation. The PLTF is less than half the thickness of the normal pre-corneal tear film[15]. Even though the PLTF thickness on silicone hydrogel lenses is similar to that on hydrogel

lenses, the pre-lens break up time is shorter for silicone lenses[15]. Thinning of the PLTF depends on the quality and thickness of the lipid tear film layer[133], which is disrupted by a contact lens[11]. In PLTF rupture, an evaporative-dehydration process starts that draws water through the lens and out of the post-lens tear film, leading to corneal staining[134]. A reflective blink spreads a new PLTF on the contact lens surface and the soft contact lens partially rehydrates, which is repeated at each blink. In this way the contact lens reaches a steady state of hydration[15]. However, lens surface dehydration is more important than overall bulk dehydration[11], i.e. water lost through lens material dehydration is relatively minor compared to water evaporated from the anterior lens surface[133].

Hydrogels are made of complex polymeric matrices containing hydrophobic and hydrophilic ends. The hydrophobic ends are oriented towards the inside of the lens matrix, while the hydrophilic ends are on the lens surface. In drying of the contact lens surface, the hydrophilic ends turn inward toward the center of the material matrix to seek moisture and the hydrophobic ends move outward toward the dehydrated surface. This is called hydrophobisation, and it causes water to be repelled from the lens surface (Figure 2.8). Clinically, hydrophobisation of the lens or ocular surface is seen as decreased pre-lens tear break-up time (Figure 2.9).

There is some controversy as to how environmental factors affect water content and lens performance. Even though it is assumed that dehydration may be induced more by air flow than relative humidity[11], other studies were not able to correlate lens dehydration to changes in relative humidity and temperature[135,136]. A "glassy skin" forms at the air/membrane interface of the lens when the relative humidity is below 55-75%[137]. This glassy skin has not been well described, but may represent a transition from rubber-to-glass during drying. It is assumed that the glassy skin limits lens

dehydration in low-humidity environments[137], but despite the possible benefit of decreasing dehydration, the glassy skin can lead to discomfort[137]. The air/membrane interface also affects the stiffness and friction properties of the lens.

A dry ocular surface or surface of a contact lens has increased friction with the leading edge of the eyelid (lid wiper). Korb et al[77,78] showed a positive correlation between the presence of superior lid margin staining and symptoms of dry eye. However, dryness caused by lid friction may not be sufficient to adequately increase reflex tearing and relieve the symptoms of dryness[138]. It has been reported that lid wiper symptoms can decrease blink frequency and increase the number of incomplete blinks. Prolonged inter-blink intervals result in thinner tear films with reduced lubricating properties which, in turn, leads to increased friction[138].

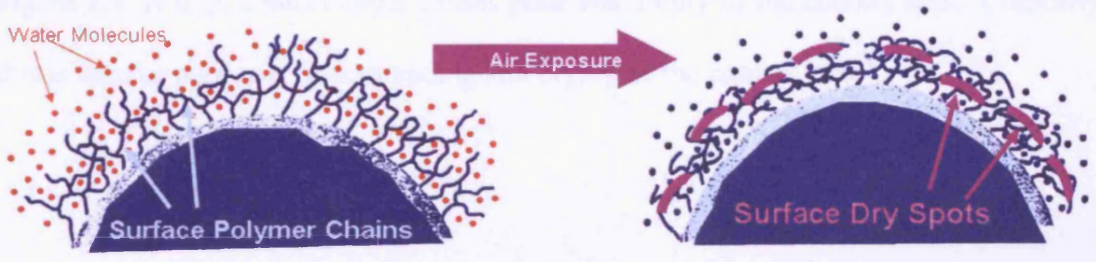


Figure 2.8: Upon exposure to air, the hydrophilic polymer chains on the surface seek the water-full inner matrix of the lens, causing hydrophobic areas. Left: Fully hydrated "conditioned" lens: mobile polymer chains allow faster rearrangement and good wettability when fully hydrated. Right: "Collapse" of surface structure: surface desiccation of lens causes polymer chains to entangle and form hydrophobic domains. Once hydrophobisation has occurred, the molecule can be flipped back to the desired orientation only by heat, alcohol, or surfactants (Alcon, 2007).

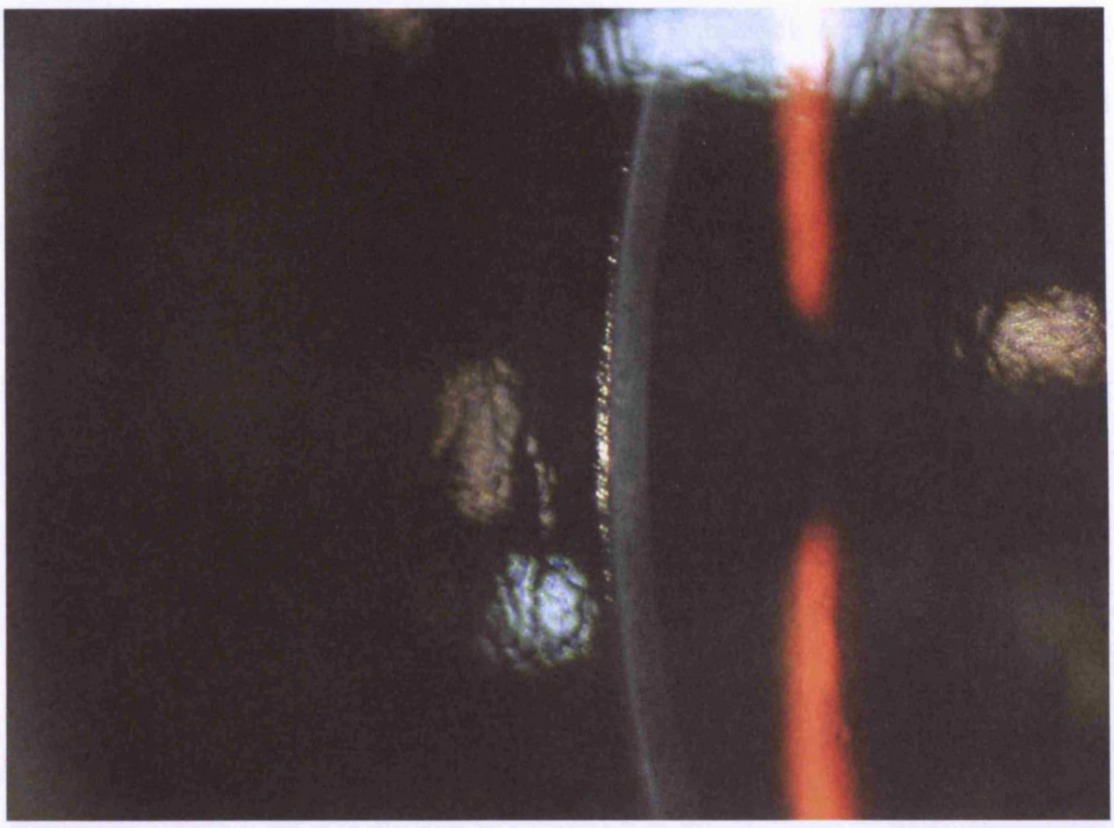


Figure 2.9: A high contact angle causes poor wettability of the contact lens. Clinically this is seen as poor tear film spreading and drying of the contact lens[11].

2.4.2 Ionic vs non-ionic materials

Ionicity is important in the interaction between the contact lens and the tear film. A bio-film forms around the lens when the lens encounters the lipoproteinemic environment of the ocular surface. This bio-film, consisting of different natural proteins, allows adequate wettability[137,139,140]. Theoretically, non-ionic materials do not attract protein to the surface as much as ionic materials and would show reduced PLTF stability, but it has been demonstrated that both ionic and non-ionic materials can attract sufficient proteins to the surface[139]. A related concern is that ionic materials attract too much protein. Indeed, the amount of protein deposits after one month of lens wear is

positively correlated with degree of ionicity[141]. Ionic materials continue to accumulate more protein, showing more at three months than at one month[141]. Even though this bio-film can, at first, improve the lens wettability, later it can lead to discomfort and giant papillary conjunctivitis (Figures 2.10). This problem can best be addressed by careful cleaning or replacement of the lens, as done by disposable four-week soft contact lenses in clinical practice. Although ionic materials are more likely to deposit increased amounts of protein, non-ionic polymers will deposit more lipid. Regular replacement, in addition to appropiate lens hygiene, will result in improved clinical performance[142,143].

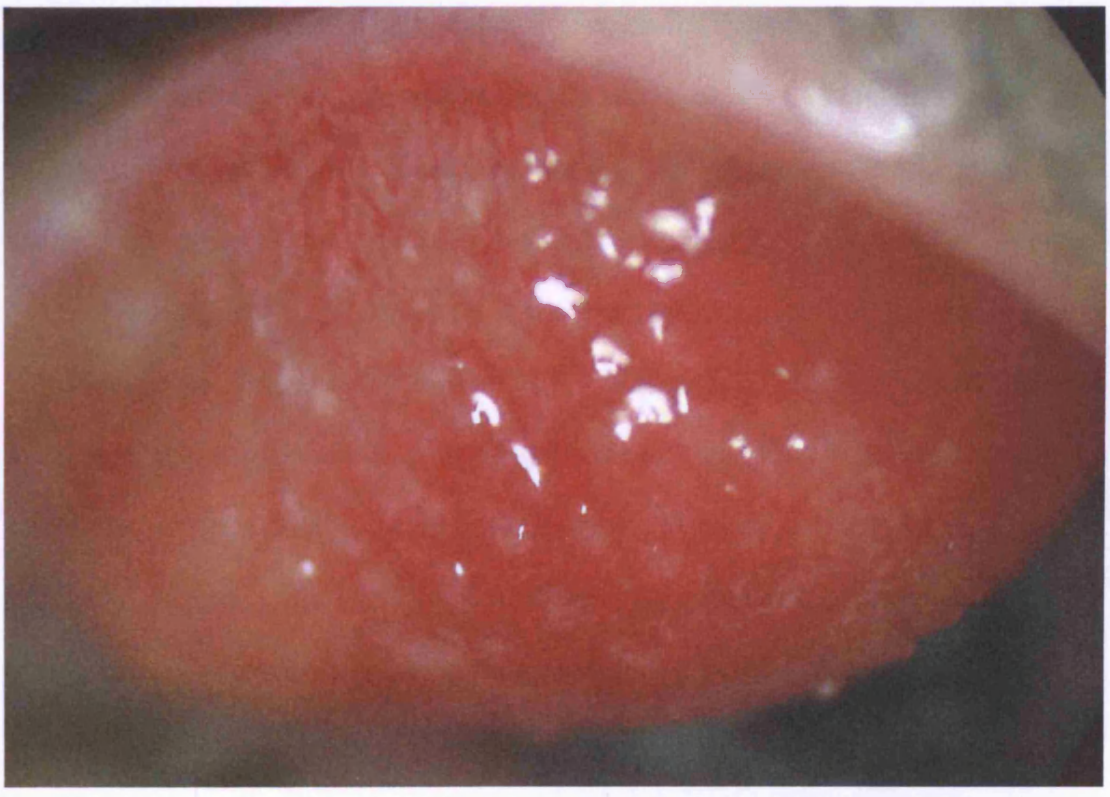


Figure 2.10: Giant papillary conjunctivitis results from antigenic response of protein denaturation and bio-film build up[11].

2.4.3 Oxygen permeability

The need to maintain a good supply of oxygen to the cornea during contact lens wear is very well known[144]. A sufficient level of available oxygen is fundamental for healthy eyes and, therefore, successful contact lenses wear[125]. In the absence of a contact lens, the oxygen required for the aerobic metabolism of the cornea comes primarily from the atmosphere. The peripheral cornea will also receive some oxygen supply from the limbal vasculature, while the posterior cornea will be supplied oxygen from the tear film[144]. Extended wear modalities of low oxygen transmission (Dk) lenses create a state of hypoxia in the cornea[145,146]. This can result in corneal swelling, limbal hyperaemia, bulbar hyperaemia, neovascularisation, refractive changes, epithelial microcysts, bacterial binding to epithelial cells and central epithelial thinning[147]. Many studies have observed the effects of oxygen availability on ocular comfort. In those studies which limited oxygen availability while controlling for humidity, 100% of subjects experienced various signs of corneal oxygen deficiency and 70-80% reported increased levels of discomfort[132,148,149]. Low Dk soft contact lens wearers also experience up to a 50% increase in corneal touch thresholds[146]. High Dk soft and rigid gas permeable lenses are not associated with decreased corneal sensation[146]. Decreased corneal sensitivity interferes with the normal neural feedback loop[150], which, in turn, decreases blink rate, increases tear osmolarity, and increases inflammatory components on the ocular surface, eventually producing the sensation of dry eye[11]. Even though a decrease in corneal sensation may reduce lens awareness, long-term hypo-aesthesia could affect the lacrimal system, leading to significant symptoms of dry eye.

For many years, it was thought that soft contact lens dehydration was the primary driver of discomfort in contact lens wear. However, although it may play a role in discomfort, research studies have been unable to prove that theory[124,125]. Conversely, the extensive

historic literature and current clinical research have clearly established that clinical signs of hypoxia are improved concurrently with improvements in patient symptoms of comfort and dryness with silicone hydrogel lenses[125]. Several studies have reported decreased symptoms of dryness and discomfort with silicone hydrogel lenses[151-155].

2.4.4 Modulus

The modulus of the contact lens is the material's resistance to deformation under tension: the higher the modulus, the stiffer the lens. A contact lens is subject to external forces when on the eye by the lids, during handling and also during the manufacturing process. The success of a contact lens material and the impact of these external forces are governed by the mechanical properties of the material[156]. In general, higher-water-content contact lenses are lower modulus lenses. Silicone hydrogel materials vary in modulus, with Senofilcon A (Acuvue Oasys, Vistakon, Jacksonville, FL, USA) being the lowest and Lotrafilcon A (Focus Night &Day, CIBA Vision, Duluth, GA, USA) being the highest[157,158]. The optimal contact lens fit distributes the pressure of the lens evenly across the eye's surface and minimises mechanical interaction to ocular tissue. Higher modulus-lenses are known to have gap formation at the edge of the lens when contact lens-cornea alignment is not achieved, resulting in significant foreign body sensation. In addition to gap formation, high modulus lenses are associated with general lens awareness, mechanically induced giant papillary conjunctivitis (GPC), conjunctival flaps[159] and superior epithelial arcuate defects[11]. A high degree of flexibility can also be a disadvantage when trying to achieve optimum vision[156]. However, the increasing use of higher modulus silicone hydrogels lenses, e.g. for extended wear, may result in an increase of these ocular complications presenting at aftercare[144]. Many questions remain to be answered regarding lens modulus and its

relationship to dry eye, e.g. how do modulus complications affect goblet cell density and, in the long term, the dry eye cycle[11]? Although the further development of silicone hydrogels to allow reduced modulus while still maintaining excellent oxygen permeability or a greater spectrum of lens parameters, may be a benefit.

2.4.5 Wettability

Surface wettability, objectively measured as contact angle (Figure 2.11) or clinically evaluated by the pre-lens break-up time, shows how the tear film spreads across the material during a blink. A lower contact angle (<90°) indicates an increased wettability of the material[11]. Surfactant wetting agents affect the wettability of a lens by reducing the contact angle. The substantivity, which means how long the surfactant stays on the lens, determines the longevity of this effect. The surface wettability of contact lenses is important for stable vision and biocompatibility[144]. Symptoms of dryness are closely related to the surface wettability of a contact lens[160]. Increasing wettability of the lens surface provides the lens wear comfort throughout the entire wearing time, since wettability affects the interaction of the lid with the lens and the deposition of the lens[160]. Hydrogel lenses are known to have a better wettability than silicone hydrogel lenses[161]. However, symptoms of dryness and discomfort in symptomatic hydrogel contact lens wearers have been reported to be reduced in silicone hydrogel lenses[151-155,162]. Prospective clinical trials showed statistically superior performance in both symptoms and ocular signs[151,152]. On the other hand, Fonn and Dumbleton[163] compared a silicone hydrogel lens (Lotrafilcon A) to three different standard hydrogel lenses in a 7-hour non-dispensing study. They found no difference in symptoms of dryness between silicone hydrogel and hydrogel lenses. However, this study was restricted to short-term subjective differences and may not have been able to elicit differences

between lens materials, since the refitted lens wearers would have needed a longer period to get adapted to the different modulus of a silicone hydrogel contact lens. Materials like Omafilcon A (Proclear, Coopervision, Fairport, NY, USA) and Hioxiflicon A (Extreme H2O, Hydrogel Vision Corporation, Sarasota, FL, USA) were invented to improve the symptoms of CLIDE by providing surface chemistries that allow better wettability through the use of phosphorylcholine. Phosphorylcholine is suggested to actively bind water molecules and so maintain better lens hydration[11]. Omafilcon A has a lower dehydration ratio than etafilcon A, although both have similar water content[11]. Hioxifilcon A binds water by strong hydrogen bonds between the water and its hydrophilic branch groups. In head-to-head studies, both Omafilcon A and Hioxifilcon A provided similar increased comfort to dry eye patients who wore contact lense[164].

Biocompatibility represents a relatively new concept in contact lenses. The aim is to use biomimetic materials which are less likely to disturb the normal ocular surface. Goda and Ishihara[165] have proposed improving silicone hydrogel lenses by coating them with biomimetic phospholipid polymers, such as 2-methacryloyloxyethyl phosphorylcholine. The combination of high oxygen permeability provided by silicone-containing lenses and biomimetic material used to increase hydrophilic properties and decrease protein/lipid build-up would potentially result in a particularly favorable lens material[11]. First-generation silicone hydrogel lenses Balafilcon A (Purevison, Bausch & Lomb, Rochester, NY, USA), Lotrafilcon A and Lotrafilcon B (O2Optix; CIBA Vision, Duluth, GA, USA) require surface treatments to keep the lens wettable. In the case of Balafilcon A, plasma oxidation converts surface silicone (trimethylsilyl [TRIS] molecules) to islands of glassy silicate, which are not hydrophobic. Lotrafilcon A and B are treated with a chemically uniform, dense, high- refractive index plasma coating[11].

Second-generation silicone hydrogel materials Galyfilcon A (Acuvue Advance; Vistakon, Jacksonville, FL, USA) and Senofilcon A are not surface treated. These lenses contain a long-chain, high-molecular-weight, flexible, humectants molecule, polyvinyl pyrolidone (PVP), which functions as an internal wetting agent[157,158]. PVP produces a hydrophilic layer on the outside lens surface by sequestering the silicone within the center of the lens. Comfilcon A (Biofinity, Coopervision, Rochester, NY, USA) is the newest, third-generation silicone hydrogel lens. This lens contains no TRIS or PVP-based chemistry. The material is inherently wettable and does not require surface treatment. *In-vivo* wettability may be different from *ex-vivo* wettability because of the different contact angles created by standard tear film components and biofilm, regardless of the material[140]. However, wettability is a more complex issue than just the surface contact angle since, for new lens materials, it can be influenced by leaching of solution from lenses. Lid interaction and tear film stability also need to be further studied with regard to *in-vivo* wettability.

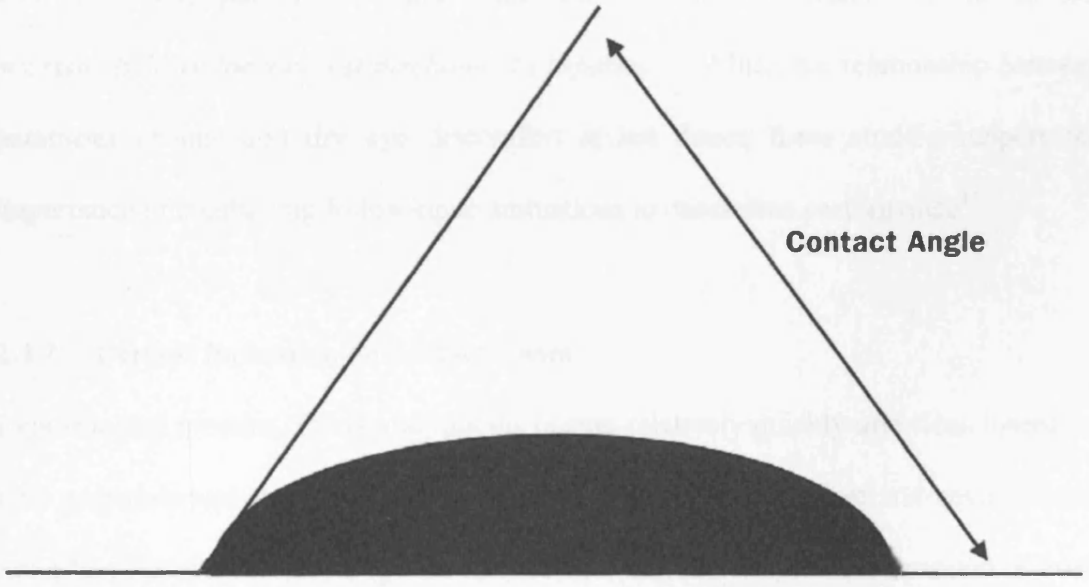


Figure 2.11: Contact angle of a liquid droplet on a surface.

2.4.6 Evaporation and parameters

The evaporation rate of the pre-lens tear film with contact lens wear is about 35% higher than that for the tear film without a contact lens[11]. All contact lens materials have similarly increased rates of evaporation, independent of water content or material[166-169]. Thinning of the tear film interferes with the continuity of the lipid layer on the pre-lens tear film, as well as with the spread of mucins across the cornea.

Due to disruption of the lipid layer, the tear film becomes unstable and the PLTF break-up time decreases. Tear film thinning due to increased evaporation can result in increased tear osmolality, which may increase symptoms of dry eye[2,7]. Tranoudis and Efron[130] showed that oxygen transmissibility, total lens diameter, water content and back optic zone radius of all the lens types they studied were reduced after 6 hours of wear. A further study by Tranoudis and Efron[170] looked at the tensile properties of soft contact lens materials before fitting and 6 hours after CL wear[170]. They concluded that "*hydrogel materials with high stiffness and strength displayed less tendency to change their geometric parameters*" and "*materials with a high water content do not necessarily have the weakest mechanical properties*". While, the relationship between parameter change and dry eye discomfort is not direct, these studies support the importance of conducting follow-up examinations to check lens performance[11].

2.4.7 Deposit formation on contact lenses

Deposition of proteins, lipids and mucins occurs relatively quickly after lens insertion. Lens materials and surface characteristics, as well as surfactant use and environment, can affect lens deposition. Deposits may come from the tears, the environment or even handling of the contact lens. Lens deposits can decrease the PLTF break-up time, and consequently can lead to symptoms of dryness.

2.4.7.1 Adsorption of protein

In lens dehydration, the internal hydrophobic regions of tear proteins (including lactoferrin, albumin and lysozymes) bind to the hydrophobic regions of the material. The water content and the surface charge (ionicity) influences the rate of deposit absorption and adsorption to the contact lens.

Lysozyme accounts for 90% of the total lens protein deposits. In its natural state, lysozyme is a bacteriolytic enzyme that plays an important role in the eye's defense against pathogens. Unfortunately, these proteins get denaturised if deposited on the contact lens surface, which is a likely cause of immunological responses[171]. Lysozyme is a very small, positively charged protein that becomes adsorbed in negatively charged materials with relatively large pore size. Increased lysozyme deposition has been measured on conventional hydrogel materials, particularly FDA group IV materials. It has been reported that hydroxyethyl methacrylate/glycerol methacrylate (HEMA/ GMA) lenses absorb the least amount of protein of the pHEMA lenses, which indicates that carboxymethylation (increasing the negative charge) is probably a more significant factor than high water content in protein spoliation[172]. However, increasing charge density often leads to an increase in effective pore size, which may promote diffusive penetration of lysozyme[173]. Denatured protein is related to contact lens complications, such as GPC and inflammation, both of which are associated with symptoms of dry eye[11].

Silicone hydrogel lenses show reduced protein deposition, but they have a greater percentage of denatured lysozyme. The difference in deposition can be attributed to the hydrophobic nature and small pore size of silicone hydrogel materials[171].

2.4.7.2 Lipid deposition

More than 45 different lipids are secreted from the meibomian glands to create the lipid layer, however the composition varies between individuals[174]. The composition of the meibomian oil is suggested to be related to diet (including large amounts of protein, alcohol and fat), systemic medications (including diuretics, anticholinergics or sympathomimetic drugs), age, gender and environment, as well as to contact lens wear wear[174-176]. Since lipids attach to the hydrophobic areas of the lens surface, some patients who previously wore hydrogel lenses may experience more lipid deposition with silicone hydrogel lenses. Indeed, silicone lenses have more interaction with the hydrophobic lipid layer than hydrogel lenses[174]. Interestingly, Lorentz et al[161] showed that in silicone hydrogels lipid deposition influences the lens surface wetting: surface treated silicone hydrogel lenses show improved wettability after several days of exposure to lipids, while non-surface treated lenses show a constant contact angle. Rubbing and rinsing the lenses has been shown to be effective in removing lipid and protein from the lens surface, since lipids are not soluble in water-based cleaners[11].

2.4.8 Contact lens solutions

Contact lens solutions remove proteins by using negatively charged molecules which pull the positively charged proteins from the lens surface. Alternatively, protection of a lens from deposition can be made by enhancing the surface wettability.

Surfactant wetting agents have both a hydrophilic and a hydrophobic end to their structures. In surfactant cleaning products, the hydrophobic end clusters around debris to form micelles. The hydrophilic ends are then able to react with water, and the micelles can be washed off the lens surface. Surfactant wetting agents also improve lens hydrophilicity - the hydrophobic end of the agent interacts with the dry

hydrophobic lens surface, exposing the hydrophilic end of the lens polymer at the lens surface. The newest generations of contact lens care products address surface wettability through the addition of increased surfactants or humectants.

To increase lens wettability and to prevent the lens from sticking to the package or itself, surfactant agents are added to the contact lens packaging solution. Removing the lens from the packaging and soaking it in a contact lens solution may further increase the initial lens wettability[11]. However, solution-related cytotoxic biguanide (poly-hexylmethyl biguanide or PHMB) disinfecting agents are related to contact lens dryness[177](Figure 2.12). Significantly more corneal staining has been shown with PHMB than with hydrogen peroxide and polyquaternarium-based solutions. Andrasko et al[178] reported that PHMB solutions cause different cytotoxicity in all lenses and therefore not all solutions are compatible with all contact lens materials. Garofalo et al[179] reported increased corneal staining with the combination of FDA group II lenses and biguanide-based systems. As a result, peroxide based systems are in revival, since they are preservative free, produce less solution related dry eye symptoms, are not toxic and are compatible with all soft contact lens materials[180-182].

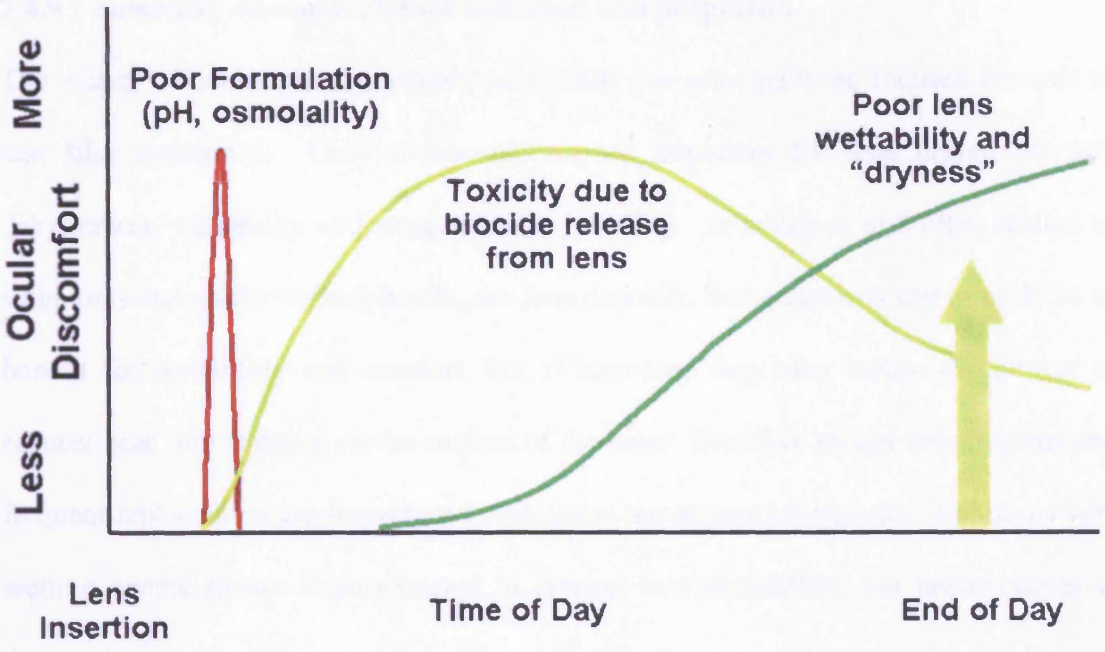


Figure 2.12: Factors affecting discomfort with some chemical disinfection systems. The time of day that staining is present provides a clue to determining the cause of contact lens related ocular discomfort (Alcon, 2007).

2.4.9 Summary of contact lenses materials and properties

The effects of contact lens materials on CLIDE can principally be focused towards to tear film instability. Only a few factors are important for lens design: surface dehydration, wettability and oxygen transmissibility. In addition, and often related to symptoms and ocular surface health, are lens deposits. Some deposits can initially be of benefit for wettability and comfort, but if increased they also induce discomfort or reduced tear film stability on the surface of the lens. Therefore proper lens hygiene and frequent replacement are important to minimise the impact of deposits. Solutions with wetting agents shows improvements to contact lens wettability, but preservatives in these solutions should be avoided. Since oxygen plays a major role in the comfort and health of the ocular surface, silicone hydrogel lenses should be preferred, however further advancements have to be done to improve the surface characterstics since wettability is definitely poorer compared to common mid-water hydrogels. Due to the modulus factor in silicone hydrogels, accurate fitting and serious aftercare is important. "One size fits all" may be not adequate in those stiffer lenses. However the next generation silicone hydrogels are very promising, being softer and more wettable.

2.5 Evaluation of the tear film and ocular surface

There is a range of tests used to evaluate the quality and quantity of the tear film, and the state of the ocular surface. Some are generally used in normal contact lens practice, but others require laboratory equipment or specialised instruments.

2.5.1 Tear turn over rate

Tear turnover rate (TTR) and tear volume can be assessed using fluorophotometry. This test determines the tear production, primarily from the lacrimal gland, and tear loss via drainage and evaporation. The FM-2 Fluorotron Master (OcuMetrics Inc, Mountain View, CA, USA) provides a reliable and objective non-touch technique of measuring the reduction in concentration of fluorescein in the tear film over a fixed period of time. 1uL of 0.1% sodium fluorescein is instilled into the eye at the lateral upper bulbar conjunctiva and allowed to mix with the tear film. The Fluorotron measures the level of fluorescence in the tear film every minute for 20 minutes after instillation. The TTR is then calculated from the regression line of tear film fluorescence. In normal eyes the TTR is around 3.4 µl/min, but is reduced for dry eye (2.48 µl/min). The normal turnover rate of the tear film is about 16% of the whole tear volume per minute[183].

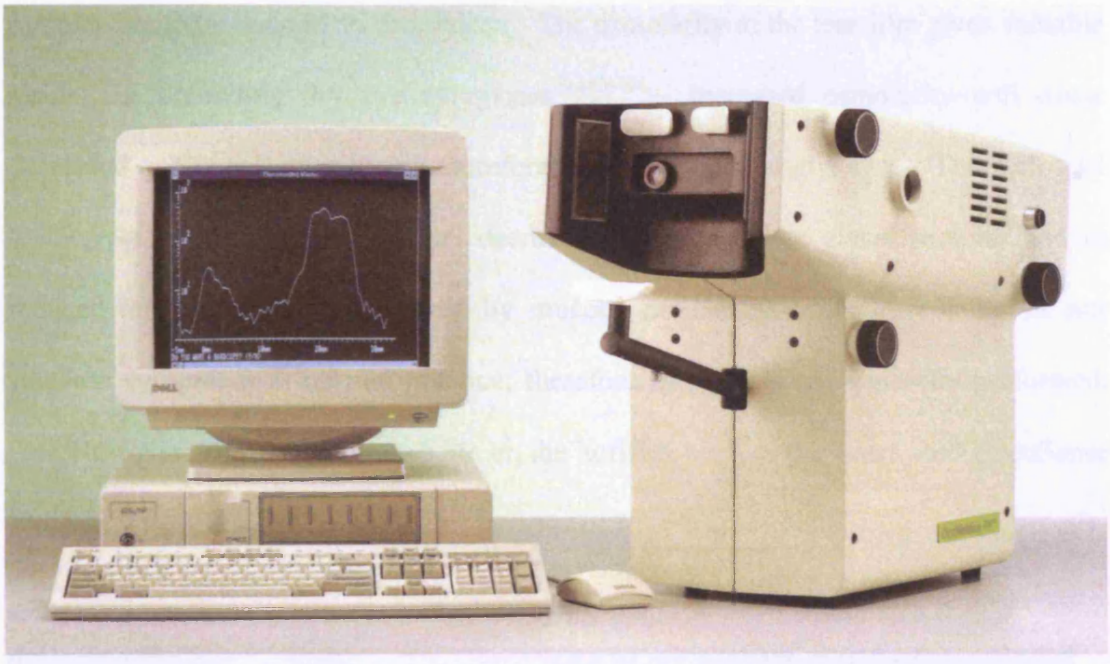


Figure 2.13: The FM-2 Fluorotron Master (OcuMetrics Inc, Mountain View, CA, USA).

2.5.2 Osmolarity

A loss of water from the tear film leads to an increase in its osmolarity. Osmolarity in the tear meniscus above the normal limit of 316 mOsm/L has been considered as the threshold level for dry eye[184]. Tear film osmolarity increases when water is lost from the tear film, while solutes, such as sodium and potassium, are not. This loss of water and increase in osmolarity may result from any condition that either decreases tear production or increases tear evaporation. Studies of pre-clinical models of lacrimal gland disease and meibomian gland dysfunction show that the ocular surface changes in dry eye disease are dependent upon, and are proportional to, increases in tear film osmolarity[76,185,186].

This test requires the collection of a tear specimen by dipping the end of a micro-litre glass capillary tube into the lower tear meniscus. The analysis of osmolarity of the

samples has to be done in an osmometer. The osmolarity of the tear film gives valuable results for predicting dry eye symptoms[76,185-195]. Increased osmolarity will cause decreased goblet cell density and therefore decreased corneal glycogen. This will lead to increased corneal desquamation, decreased corneal surface glycol proteins and to reduced tear break-up time caused by mucous deficiency. An osmometer is not standard equipment in normal practice; therefore this test is not generally performed, yet. However, affordable systems are on the horizon, such as the TearLab™ (OcuSense Inc, San Diego, CA, USA).

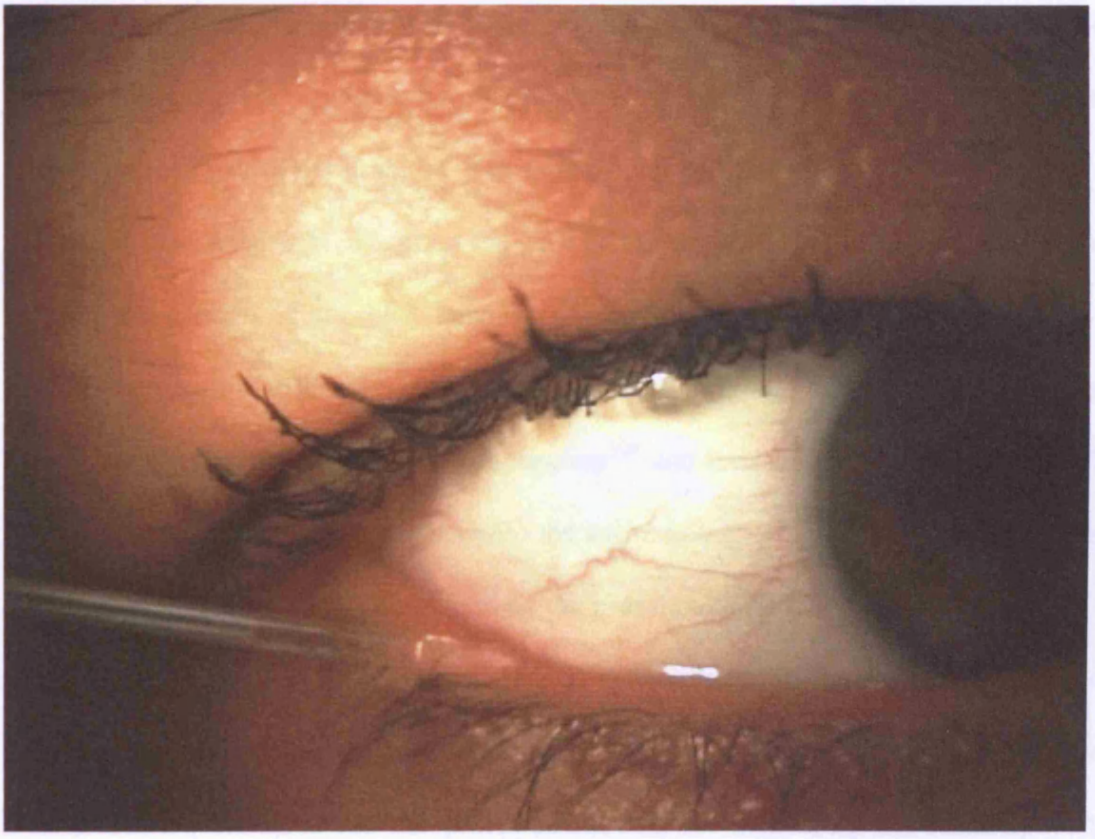


Figure 2.14: Tear samples taken by dipping a micro-litre glass capillary into the reservoir of the lower tear meniscus.

2.5.3 Tear film break-up time

The structural integrity of the tear film can be assessed by measuring the elapsed time from the execution of a normal blink until the tear film breaks up; this is known as the tear break-up time (BUT). The precise mechanism that leads to tear film break-up is not known. The most popular theory is that advanced by Holly et al[196]. According to this theory, tear break-up occurs when lipid, which is hydrophobic in nature, migrates down to the mucous layer and compromises the hydrophilicity of the epithelial surface. Tears recede from this region of poor wettability and a dry spot forms. As the tears continue to recede, further inter-mixing of lipid and mucous occurs at the receding edge and the field of hydrophobicity increases, which thus increases the dry area - and the process continues. Alternative theories propose that tear break-up is caused by rupture of the mucous layer or disturbance of the superficial epithelial glycocalyx[17,197].

The mechanism of tear break-up on the surface of contact lenses must be different from that on the surface of the eye, because of the absence of properly formed lipid or mucous layers on the lens surface. Rapid pre-lens break-up times[198] suggest that tear thinning occurs as a result of both evaporation[199] and lateral surface tension forces that draw tear fluid from the lens surface into the surrounding tear meniscus at the lens edge. Tear break-up is likely to be expedited by the presence of surface deposits.

The classic method used to measure BUT is to instil sodium fluorescein stain into the tear film and then to observe the break-up of the fluorescein pattern using a slit-lamp. Non-invasive techniques are the preferred method for measuring the break-up of the tear film, since too much fluorescein is assumed to destabilise the tear film[200,201]. An illuminated black and white grid, in a hemispheric dome, is projected onto the cornea, and the reflection observed using a microscope. Any break-up or thinning of the tear film will distort the reflected grid image. The time taken for the reflected grid to begin

breaking up is recorded as the non-invasive break-up time (NIBUT). Tolerant contact lens wearers shows a median break-up time of approximately 20 secs and intolerant wearers 13 secs[89]. Other investigators assumed that NIBUT or BUT are poorly related to patient syndromes[13]. However, NIBUT is recommended by the International Dry Eye Workshop (DEWS), who define the threshold as <10secs[2].

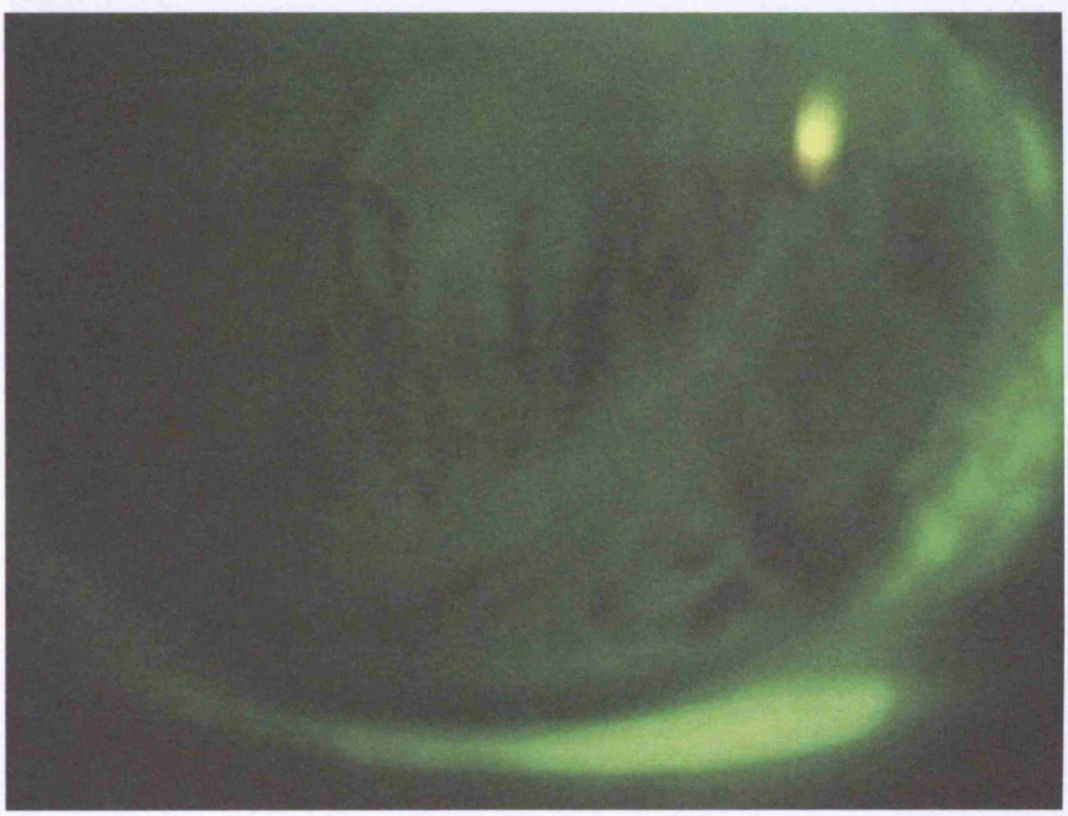


Figure 2.15: Break-up of the tear film, stained by fluorescein.

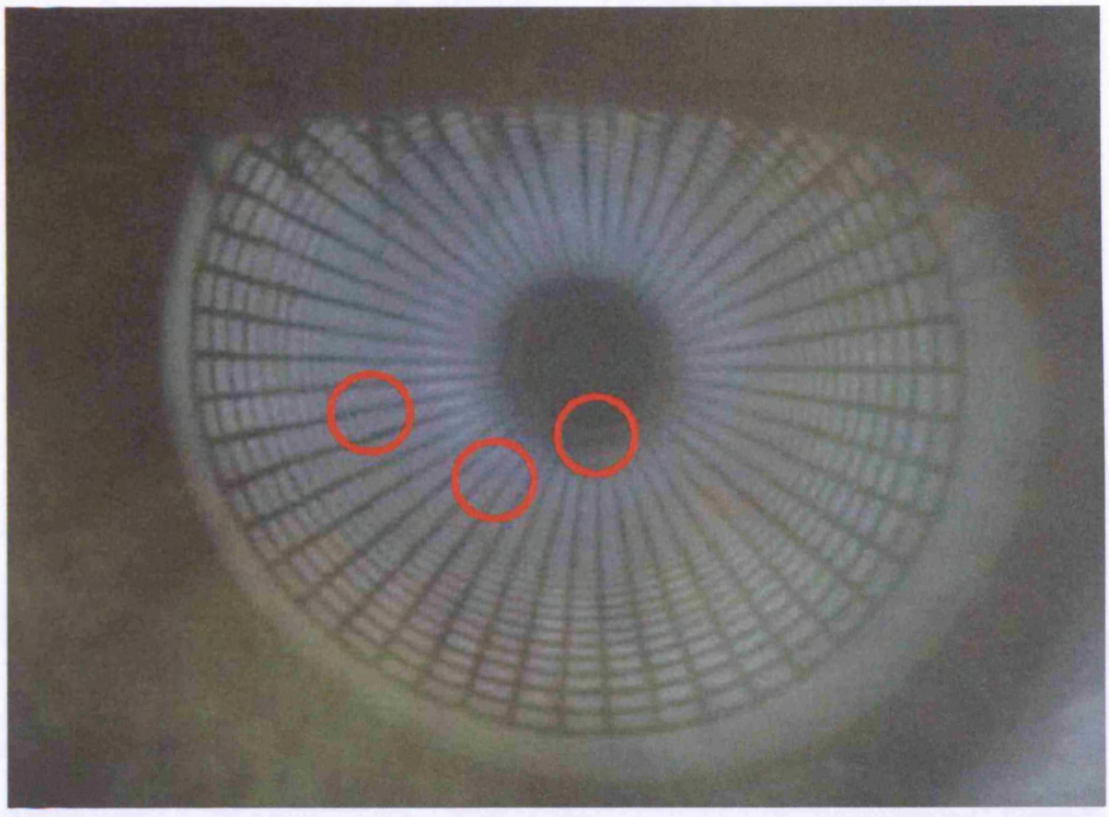


Figure 2.16: Non-invasive break-up of the tear film, evaluated by Tearscope Plus™ (Keeler Ltd, Windsor, UK) with fine grid insert.

2.5.4 Lipid layer interference pattern

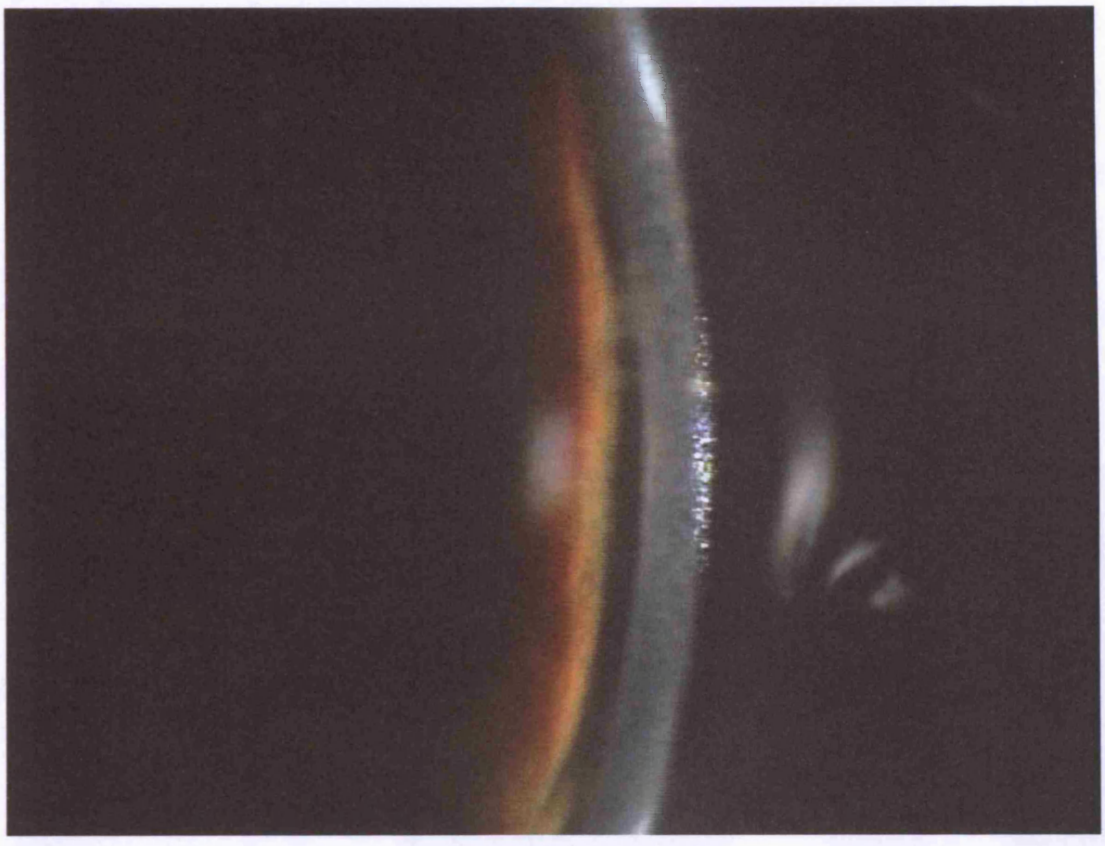


Figure 2.17: Pre-corneal tear film observed by slit-lamp microscope in optical section.

The component layers of the tear film are extremely thin and so the refractive index differences between the air-lipid and lipid-aqueous boundaries cause destructive interference within the lipid layer. This results in the appearance of coloured fringes, from which the thickness of the lipid layer can be inferred. The fringes can be observed using a wide-field, cold cathode light source, which is available as a handheld instrument known as the Tearscope Plus™ (Keeler Ltd, Windsor, UK)[202]. The insufficient refractive index difference between the aqueous-mucous and mucous-epithelium interfaces means that the aqueous layer of the pre-corneal tear film cannot be

observed using this technique. The coloured fringe patterns, coupled with the general morphological appearance and dynamic characteristics of the lipid layer when viewed in specular reflection led Guillon[202] to devise a six-category lipid layer classification scheme, sub-divided in dark and light eyes[17] (Figure 2.18), while Korb et al[58] used an 11 grade scale by evaluating the dominant colour of the interference pattern (Table 2.1). A lipid layer thickness of less than 30nm may represent a contact lens contra-indication[17,202].

Figure 2.18: Tearscope Plus™ (Keeler Ltd, Windsor, UK).

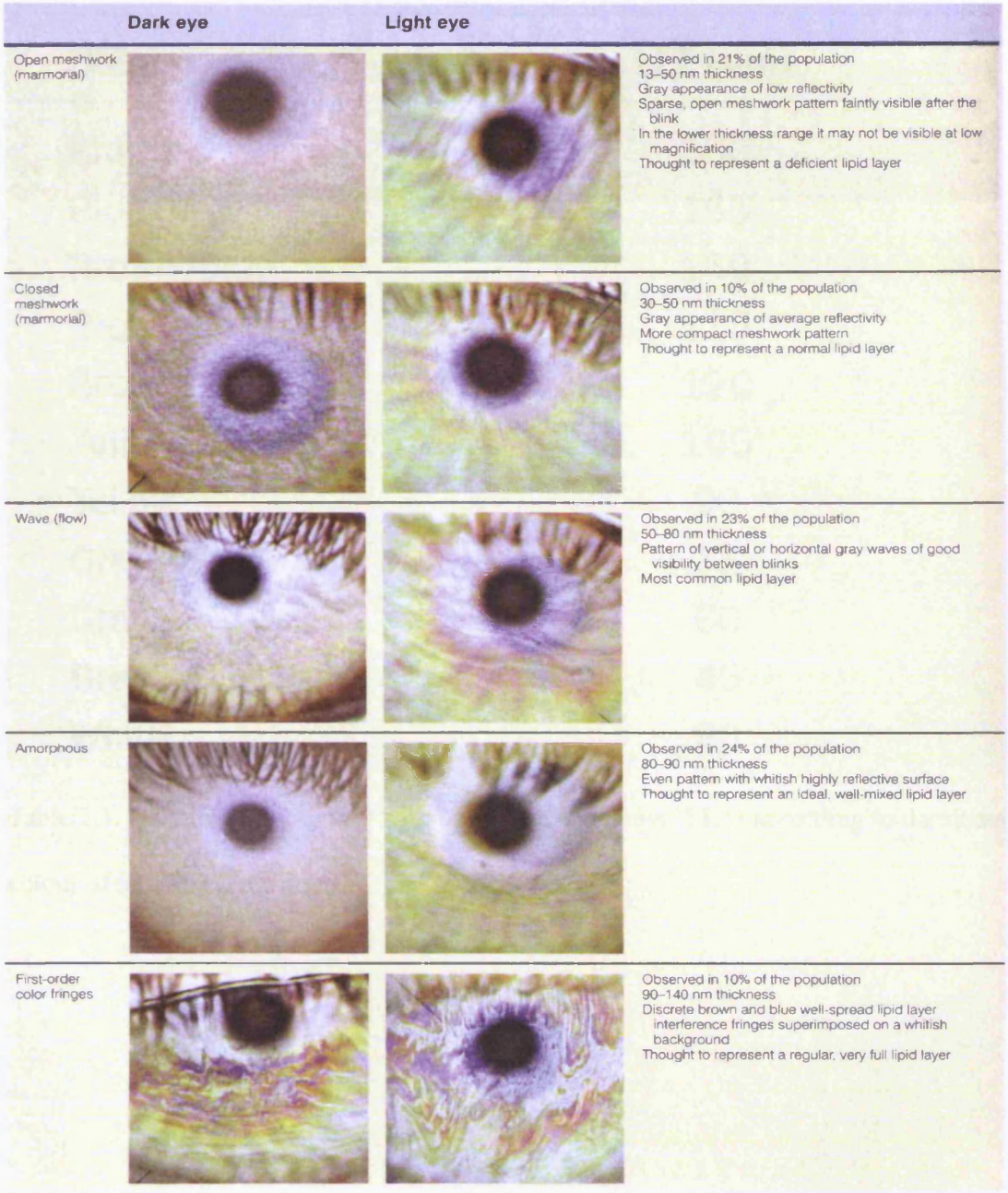


Figure 2.19: Comparison of different coloured fringes: Open meshwork, assumed to represent a deficient of lipid versus "amorphous" which is suggested to be a well-mixed, ideal lipid layer. *(Not shown: sixth category: second-order colour fringes; observed in 5% of the population; thickness 140-180 nm. Thought to represent an abnormal lipid layer)*[17].

Color	LLT (nm)
Blue	180
Blue/brown	165
Brown/blue	150
Brown	135
Brown/yellow	120
Yellow/brown	105
Yellow	90
Grey/yellow	75
Grey	60
Grey/white	45
White	30

Table 2.1: Quantification of tear film lipid layer thickness (LLT) according to dominant colour of interference pattern[58].

2.5.5 Tear meniscus height

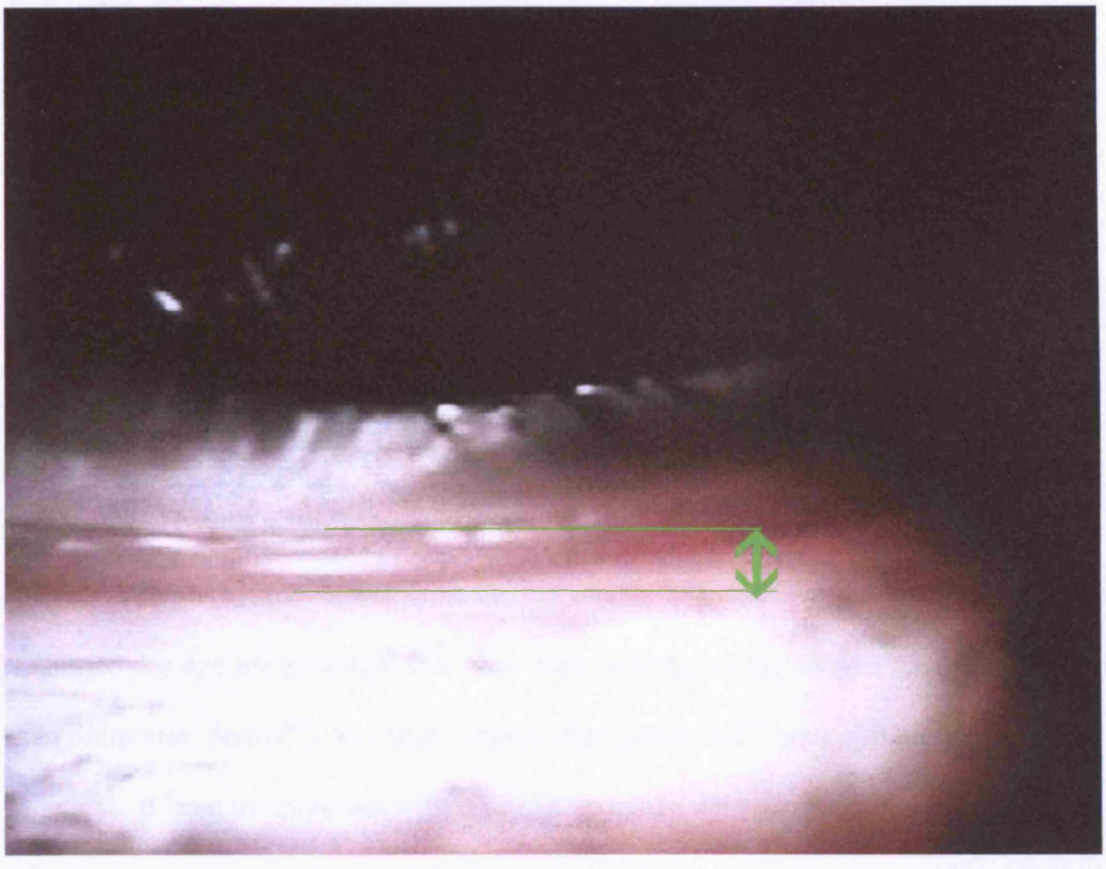


Figure 2.20: Tear meniscus of the lower lid, observed with a slit lamp microscope in 12x magnification. The horizontal green lines indicate the upper and lower edges of the tear meniscus. *Tear meniscus height is classified as good: > 0.2 mm; normal: = 0.2 mm; poor < 0.2 mm*[203].

The quantity of the tear film can be evaluated by observation of the tear meniscus height. Tear volume is assessed by observing the lower tear prism, with the slit lamp microscope, and then measuring its height using a reticule set perpendicularly from the centre of the lower lid to the top of the tear meniscus. Many studies demonstrate the good correlation between tear meniscus height (TMH) and symptoms of dryness[89], and

with cotton thread test results, break-up time, non-invasive tear break-up time (NIBUT) and scores for ocular surface staining and osmolarity[204-206]. In addition, it is important to observe if the tear meniscus is interrupted, which could indicate dry eye[207]. A TMH of less than 0.2mm indicates dry eye[203].

2.5.6 Schirmer test

Tear volume can be measured using the Schirmer test, which involves placing one end of a filter paper strip into the lower fornix and measuring the length of paper that becomes wet over a given time period. The greater the length of wetting, the greater is the tear volume (assuming that there has been no reflex stimulation). Different cut-off values to dry eye are reported: less than 3mm wetting of the strip after 5mins[188], less than 5mm after 5mins[186], less than 5.5mm after 5mins and[208] and less than 10mm after 5mins[209]. It is an invasive test and, if anaesthetics are not used (Schirmer II), the reflex stimulation can be very large. It is also influenced by lipid deficiency, which can lead to increased wetting of the strip[76]. Although there are many papers that demonstrate a good correlation between the Schirmer test and dry eye, these papers mostly report on the sicca syndrome and not on mild to moderate dry eye. In general, this test measures the reflectory secretion and differentiates between aqueous deficiency and non-aqueous deficiency[66,210,211].

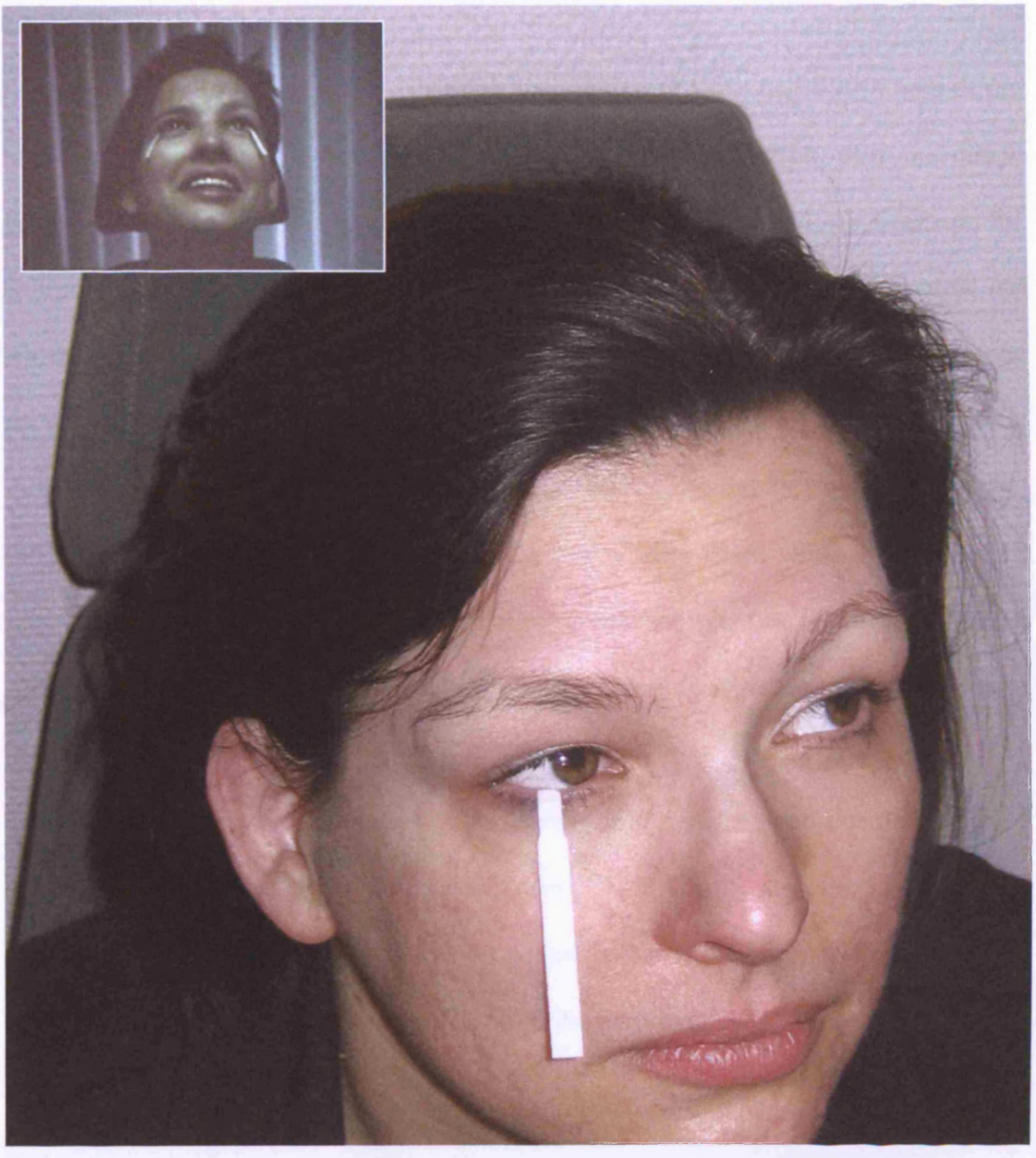


Figure 2.21: A subject undergoing the Schirmer test.

2.5.7 Phenol red thread test

For the phenol red thread test, as adapted by Hamano et al[212], fine cotton threads are impregnated with the pH-reactive dye phenolsulfophthalein, which turns the thread yellow in air. The thread is looped over the lower lid margin in a manner similar to the Schirmer test. As a result of a tear-induced shift in pH, the yellow thread turns red when it is wetted by the tears. The further the passage of redness down the thread, the greater the tear volume. If there is less than 10mm wetting in 15 secs then dry eye is assumed[212]. Hamano et al[212] applied this test to 1600 asymptomatic rigid (PMMA) and soft (HEMA) lens wearers, and observed a mean wetting length of 16.9 mm over 15 secs. This result was no different from that of normal subjects (no lenses worn), which suggests that, from a clinical perspective, contact lenses do not alter tear production in normal subjects.

The ability of the phenol red thread test to differentiate between aqueous deficient and non-aqueous deficient dry eye is not confirmed. The difficulty arises from the dispute over what it is measuring – the general opinion is that it measures tear volume, with some reflectory secretion, which is different to the Schirmer test[213-215].

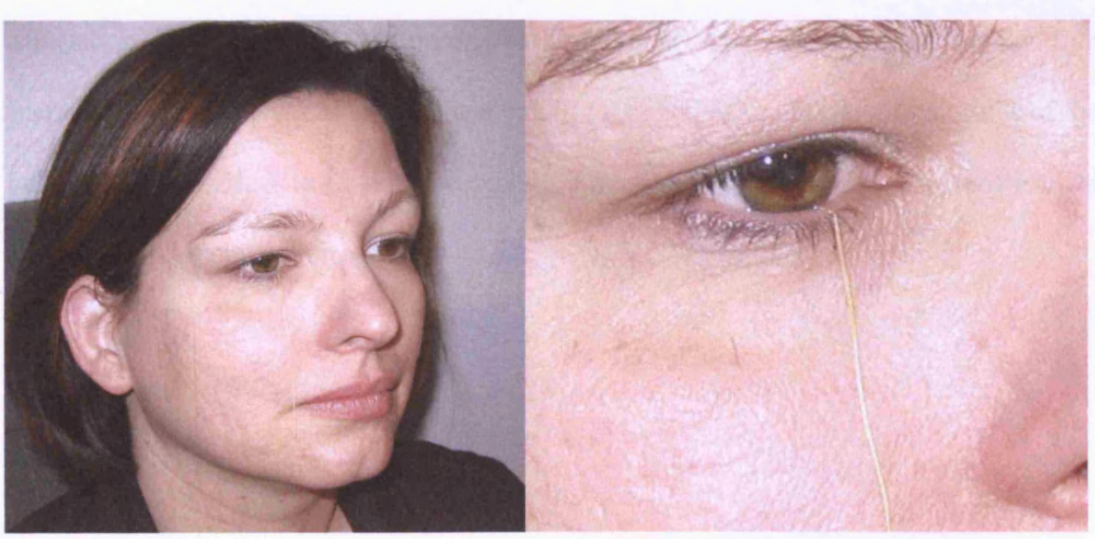


Figure 2.22: A subject undergoing the phenol red thread test.

2.5.8 Staining

Fluorescein staining of the cornea indicates the presence of disrupted and/or missing superficial cells. Staining with rose Bengal or lissamine green reveals the presence of devitalised or dead superficial cells. Fluorescein stain is effective for both conjunctival and corneal staining, while rose Bengal or lissmanine green are used mainly for conjunctival staining[216-218]. The principal disadvantage of rose Bengal is that it stings: at 1% concentration, stinging is mild, at 2% concentration, stinging is moderate to severe, and evaluation using 3% is not recommended[219]. Lissamine green does not sting at 1% concentration, but can be mild to moderate at 2% concentration, and at 3% concentration results in severe stinging[219]. The best staining effects of the conjunctiva are produced by a combination of 1% lissamine green or 1% rose Bengal plus 2% fluorescein[219]. Corneal staining of more than 0.5, evaluated by the Cornea and Contact Lens Research Unit (CCLRU) grading scale, is reported to be unusual[220].

Staining can have mechanical, metabolic, toxic, allergic or infectious causes. Even though staining is common in dry eye patients, its relation to dry eye symptoms is poor[13], as well as its repeatability[14,221]. In those areas of the tear film that are most affected by evaporation (tear thinning/break-up), the hyperosmolarity of the tear film increases dramatically, but while these values (600-700 μOsm/l) cause symptoms of the cell being stressed, it does not necessarily induce staining[222-225] and does not lead to apoptosis of the epithelium cells.

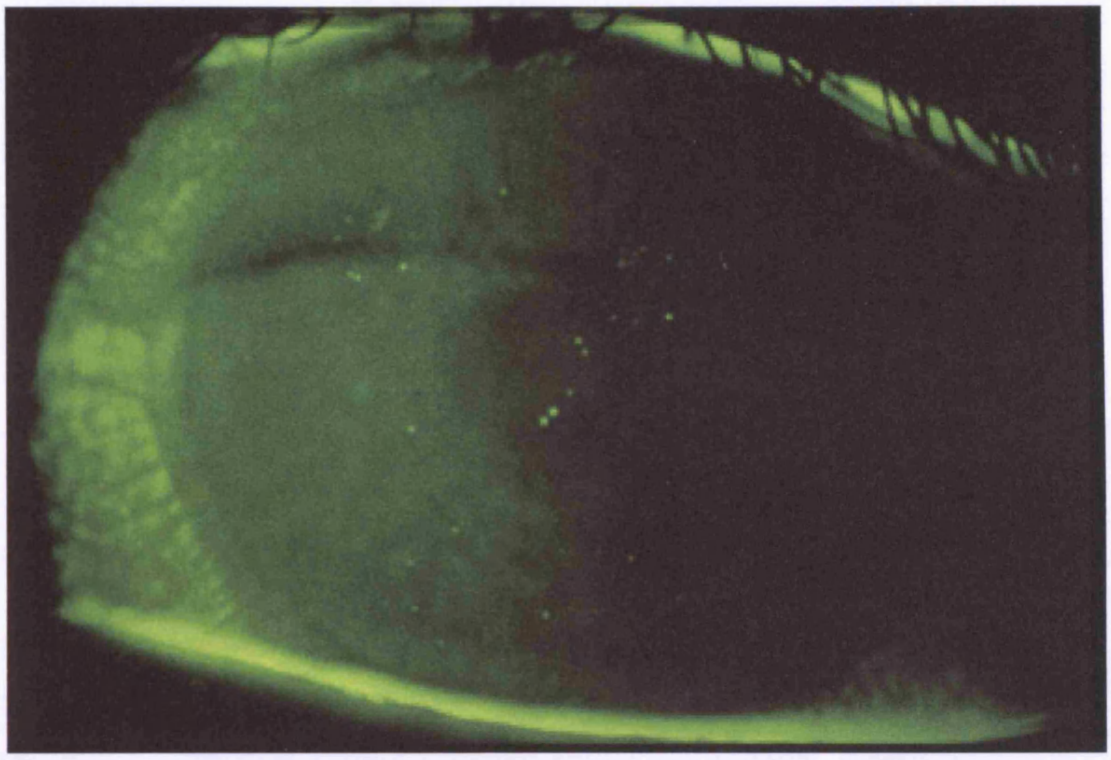


Figure 2.23: Corneal staining with sodium fluorescein.

2.5.9 Ocular redness

Bulbar conjunctival hyperaemia and limbal hyperaemia of the anterior eye is a common sign associated with an unhappy eye. Ocular hyperaemia is an important sign in contact lens practice, since a wide range of ocular diseases can cause increased hyperaemia of the bulbar conjunctiva and limbus[84,151,226-232]. Relations between ocular hyperaemia and symptoms of dryness in contact lens wear are disputed[13,162,231]. The redness observed is the result of an increase in the volume of blood in the anterior scleral, bulbar conjunctival and limbal vessels and occurs in response to inflammation, irritation and systemic disease[84,151,226-232]. Bulbar hyperaemia is more typically caused by general ocular and systemic factors, while limbal hyperaemia is associated with corneal 'stress' (e.g. keratitis, infiltrates, staining, abrasion, hypoxia)[17,226,228,233-238].

Hyperaemia is assessed by evaluating the ocular surface with the slit-lamp microscope at 10 to 12x magnification and diffuse illumination. The relationship between conjunctival bulbar hyperaemia and limbal hyperaemia, as well as the normal values of limbal hyperaemia in healthy eyes, are unknown.

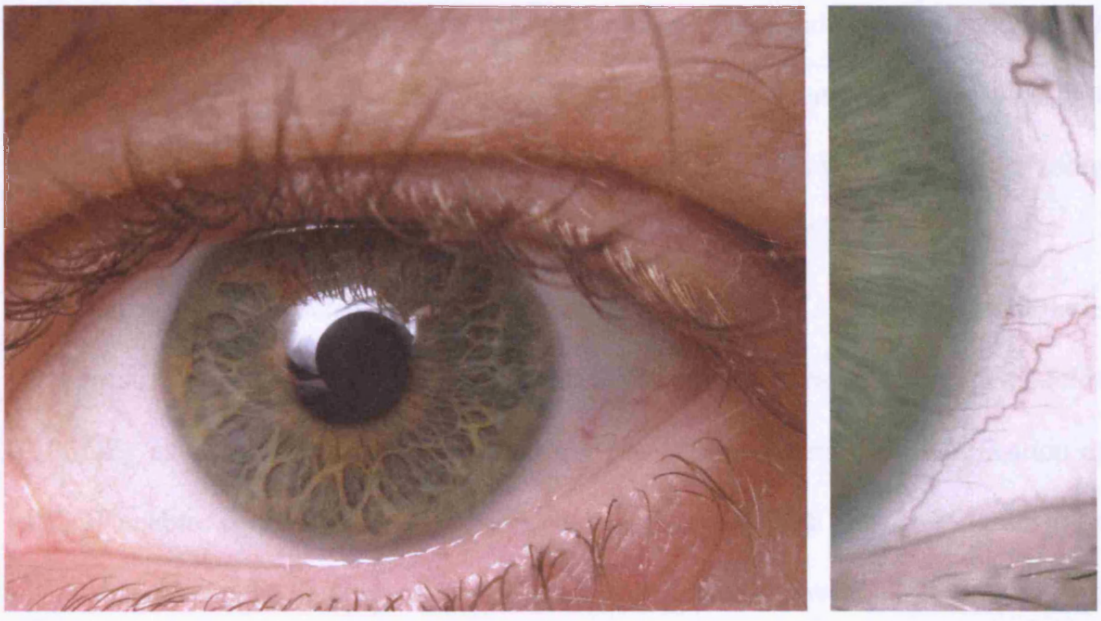


Figure 2.24: Bulbar redness and limbal redness.

2.5.10 Lid parallel conjunctival folds (LIPCOF)

LIPCOF are sub-clinical folds in the lateral, lower quadrant of the bulbar conjunctiva, parallel to the lower lid margin[79,80,239] (Figures 2.25 to 2.29), easily observable by slit-lamp. Several causes of bulbar conjunctival folds are hypothesised: conjunctival 'looseness' as a result of inflammatory processes, a decrease of elastic fibres, aging, and lymphatic dilation by mechanical forces between the lower lid and conjunctiva that gradually interfere with lymphatic flow[207,240-244]. Bulbar conjunctival folds were first described by Hughes[245] and named conjunctivochalasis (CCH). Different grading scales and observation techniques in CCH are reported[243,244,246,247].

Sub-clinical conjunctivochalasis was investigated by Höh et al[79]. They found that these folds could occur at any age, and so described them as lid-parallel conjunctival folds (LIPCOF), as distinct from CCH. They reported that assessment and classification of LIPCOF enables better diagnosis of dry eye syndromes. LIPCOF are also reported to be a valuable diagnostic instrument for predicting contact lens wear discomfort[80]. To avoid confusion LIPCOF refers only to sub-clinical conjunctival folds at a defined location, observed without fluorescein instillation and used as a test for predicting dry eye in non- and contact lens wearers. LIPCOF are evaluated using a slit-lamp microscope with 18 to 24x magnification, and with a vertical slit of 2-3 mm diameter. It is recommended to keep the angle between the microscope and the illumination to between 20°-30°. The area of observation is perpendicular from the temporal limbus down to the bulbar conjunctival above the lower lid (Figure 2.25). In some cases it is helpful to swing the slit-lamp ±10° and/or turn the slit parallel to the lid for indirect illumination.

LIPCOF are classified using a four-grade scale (0-1-2-3) (Figure 2.26)[80]. Care has to be taken to differentiate between parallel, permanent conjunctival folds (LIPCOF) and

disrupted micro-folds or conjunctival flaps[159,248,249]. Patients with LIPCOF degree ≥2 are more likely to have dry eye symptoms[80,250].

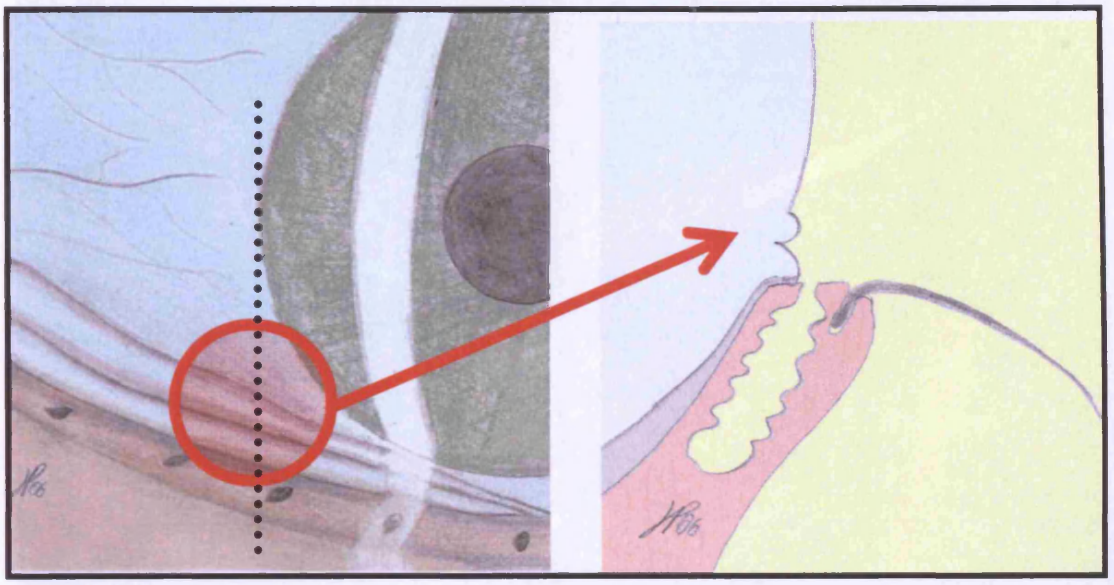


Figure 2.25: Sketch of LIPCOF and area of observation, perpendicular from the temporal limbus down to the bulbar conjunctival above the lower lid.

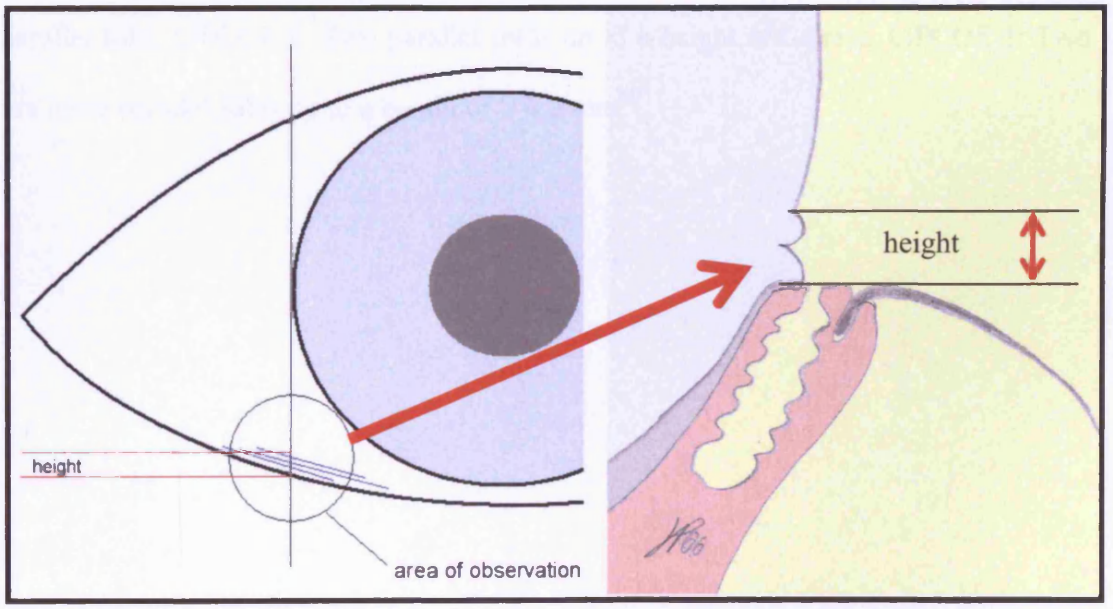


Figure 2.26: Sketch of LIPCOF and definition of height of the folds, measured by use of a reticule.

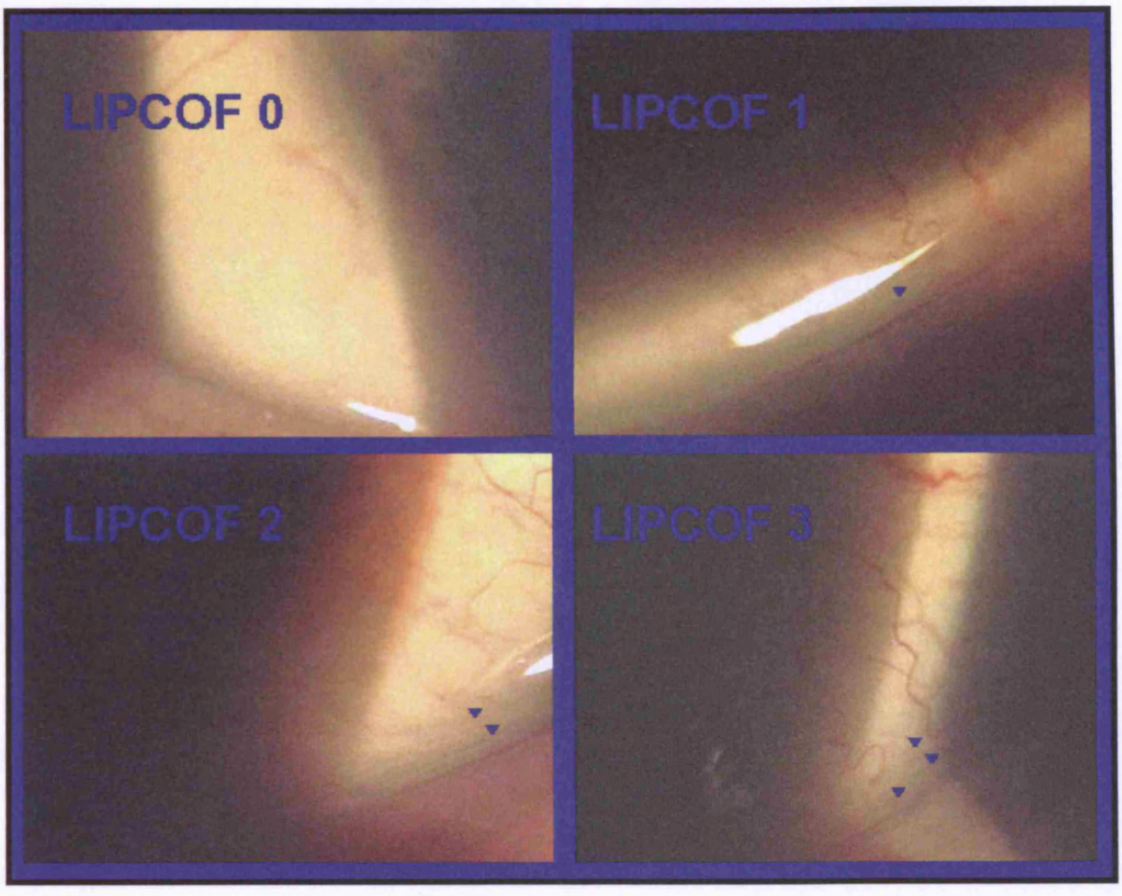


Figure 2.27: Clinical grading scale for LIPCOF: LIPCOF 0: non fold; LIPCOF 1: one parallel fold; LIPCOF 2: Two parallel folds up to a height of 0.2mm; LIPCOF 3: Two ore more parallel folds up to a height of > 0.2 mm[80].

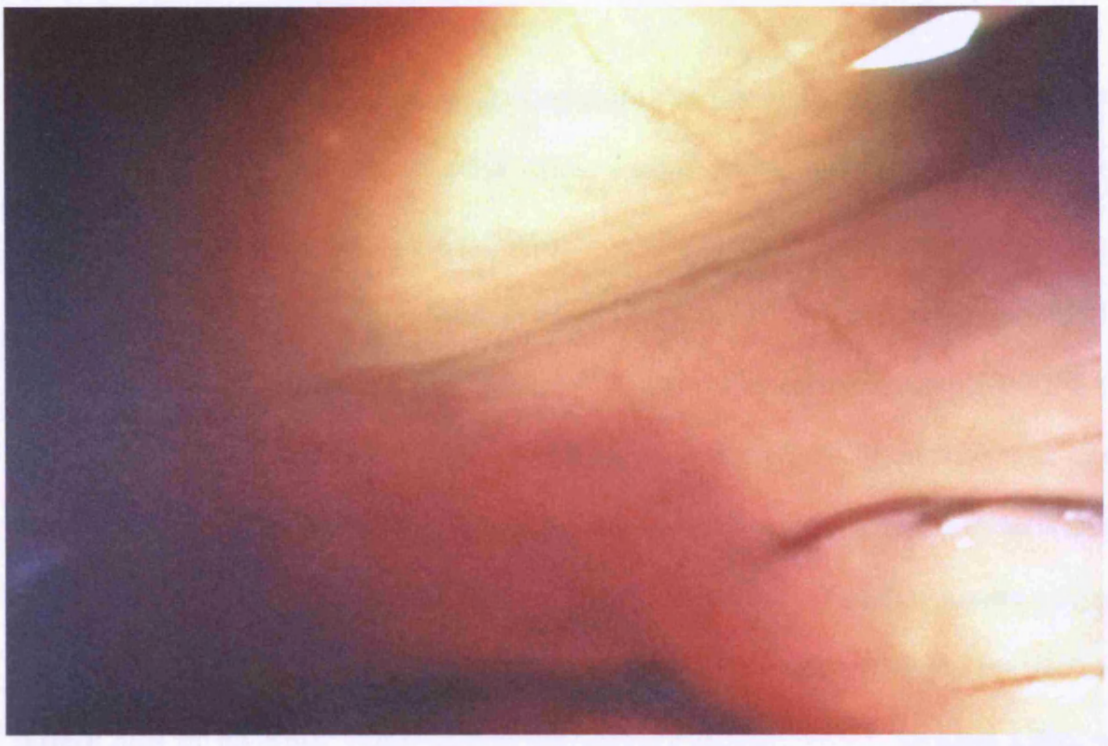


Figure 2.28: LIPCOF degree 2.

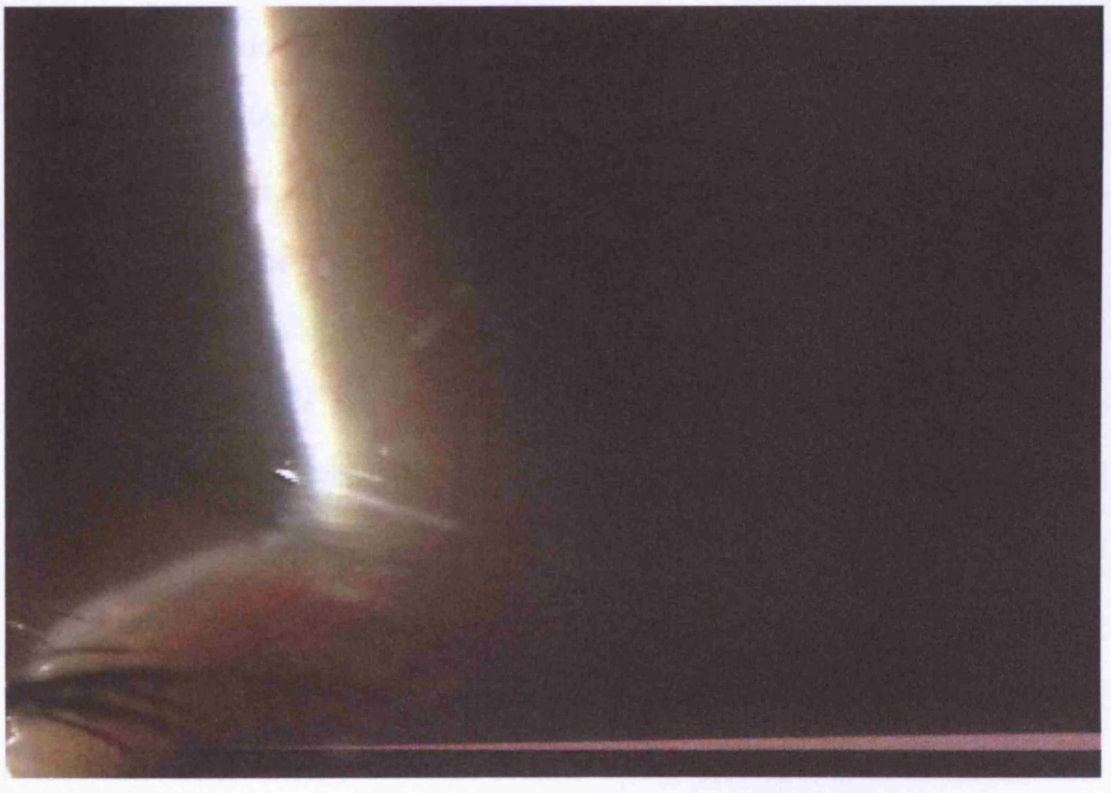


Figure 2.29: LIPCOF degree 3.

2.5.11 Lid wiper epitheliopathy (LWE)

Only a small portion of the marginal conjunctiva of the upper lid acts as a wiping surface to spread the tear film over the ocular surface or over the surface of a contact lens[77]. This is because the palpebral surface of the upper lid arches away from the ocular surface, and so creates a space (Kessing's space[251]). This contacting surface at the lid margin has been termed the 'lid wiper'[77]. Lid wiper epitheliopathy (LWE) is a clinically observable alteration of the epithelium of the lid wiper. In patients with dry eye, the thickness of the tear film is insufficient to separate the corneal and lid wiper tissue surfaces. Due to this, when blinking, the lid wiper is subjected to trauma during the entire lid movement via the friction produced by the continual rubbing of the narrow surface area of lid wiper tissue against the corneal surface[77,78,252]. To stain LWE, a combination of fluorescein and rose Bengal or lissamine green is used[219,253,254]. When using fluorescein, observation and diagnosis of LWE is enhanced one minute after instillation of two drops of fluorescein, separated by five minutes[255]. LWE is classified using a four-grade scale after lifting the patient's upper lid and measuring the length and sagittal width of staining[77]. The individual grades for each of these two characteristics are averaged for a final grade of staining.

Care has to be taken to differentiate the staining associated with Marx's line from staining of the lid wiper[77,256]. This distinction can be readily achieved by following the description of Norn[257], who noted that: "*The line runs along the lid margin in relation to the base of the tear meniscus just behind the orifices of the meibomian glands. It forms an imprint, as it were, of the course of the streaming lacrimation.*" According to Korb et al[77], more than 80% of contact lens wearers who suffer from dry eye display fluorescein and/or rose Bengal staining of the lid wiper (lid wiper epitheliopathy),

versus only 13% of asymptomatic lens wearers. Cut-off values between asymptomatic and symptomatic patients are unknown.

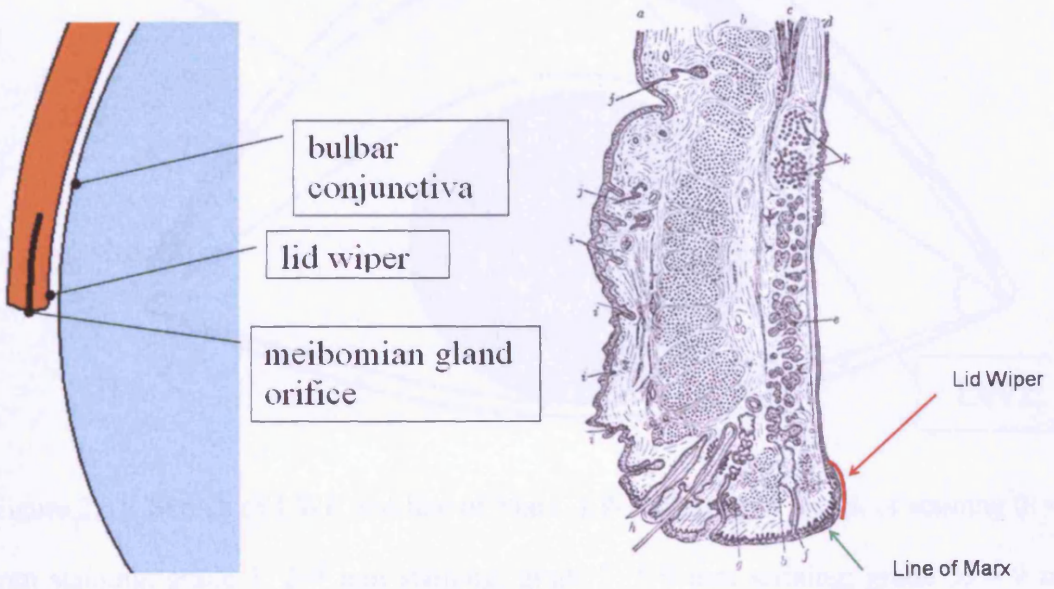


Figure 2.30: Sketch of the upper lid in a side view and definition of the location of the lid-wiper and line of Marx[77].

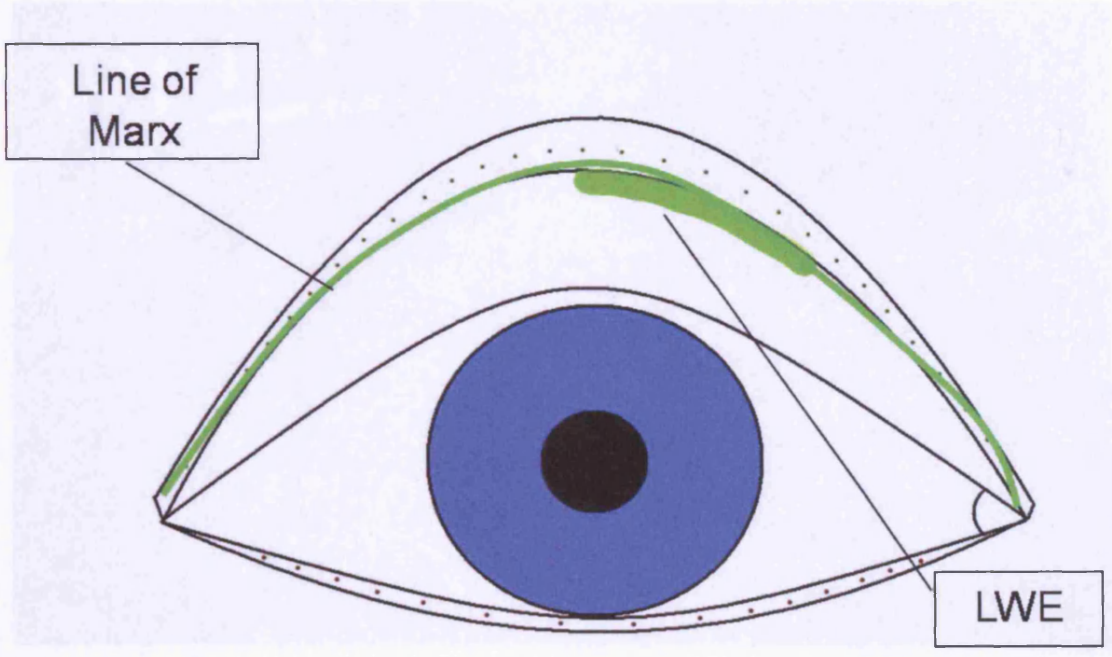


Figure 2.31: Sketch of LWE and line of Marx. LWE horizontal length of staining 0: < 2 mm staining; grade 1: 2-4 mm staining; grade 2: 5-9 mm staining; grade 3: > 9 mm staining[77]. The average sagittal width of staining was graded as 0: <25%, grade 1: 25%-50% (mild), grade 2: 50%-75% (moderate), or grade 3: >75 (severe) of the width of wiper. The sagittal width of the lid wiper extends from just proximal to the line of Marx to the sub-tarsal fold. The individual grades for each of these two characteristics are averaged for a final grade for staining.

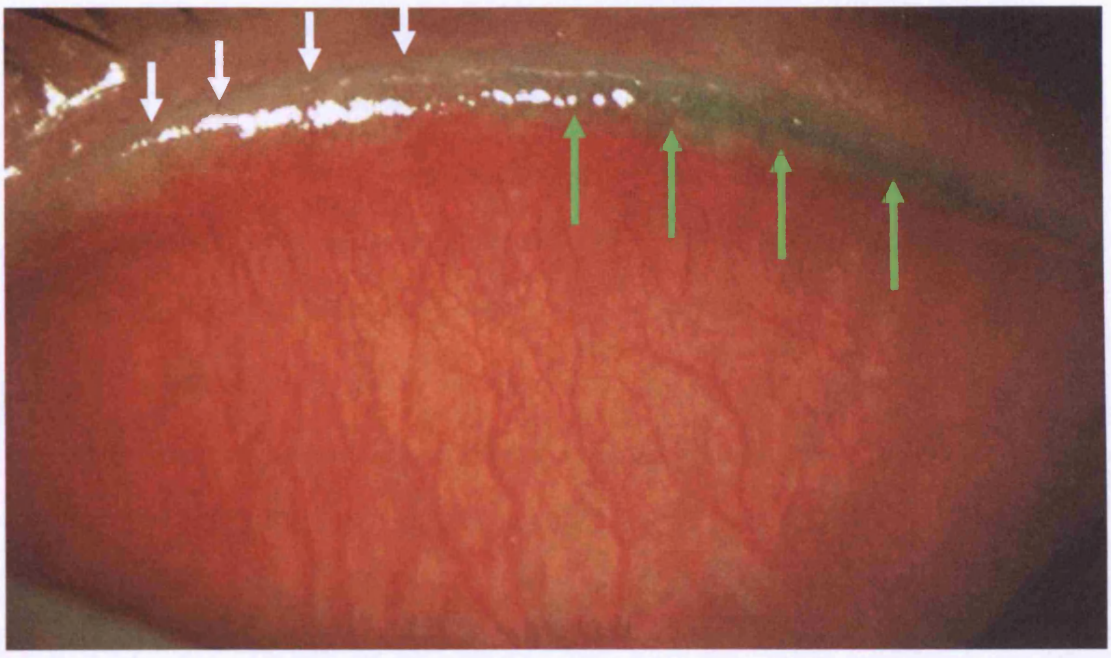


Figure 2.32: LWE Grade 3 (green arrows). Note that Marx's line follows the path of the meibomian orifices and the mucocutaneous junction (white arrows).

2.5.12 Grading scales

Ocular signs like hyperaemia or staining have to be estimated and recorded by the observer when assessing each patient. This can be done more carefully by use of grading scales or digital imaging.

Grading scales are frequently used to assess the severity or degree of change on the ocular surface. These scales are of verbal description, photographs, or paintings that illustrate an increasing level of the appearances of the ocular surface, like hyperaemia, staining, or palpebral roughness, and they have been particularly used in clinical studies[233,258-264]. Even though digital imaging provides a permanent record of the appearance of the eye, where this is not available to the clinician, verbal [265,266] or pictorial [260,264,267] grading scales are used to record ocular status and allow comparison

across time. It is important that the clinician knows which grade signifies normality in order to determine what is abnormal, and for the grading scale to be reliable and repeatable. Many grading scales are available, following the pioneering work of McMonnies and Chapman-Davies in the late 1980*s*, who developed a pictorial scale for ocular hyperaemia (Figure 2.33). After irritating the patients' eyes, the decrease of hyperaemia was photographed and combined to produce a 6-grade scale[259,260]. Nowadays there are two grading scales dominant in clinical use, the Efron grading scale and the Cornea and Contact Lens Research Unit (CCLRU) grading scale. The Efron scale is composed of paintings (Figure 2.34), while the CCLRU scale is made of photographs (Figure 2.35). Both are five-grade scales, although the CCLRU scale does not show grade 0 (normal). Interpolating the scales into decimal intervals increases their sensitivity[268,269].

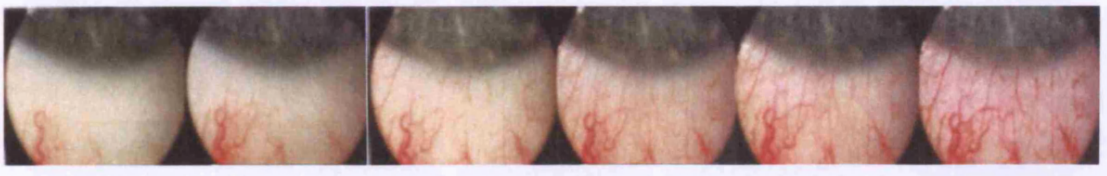


Figure 2.33: Grading scale devised by McMonnies and Chapman-Davies, 1987[259,260].

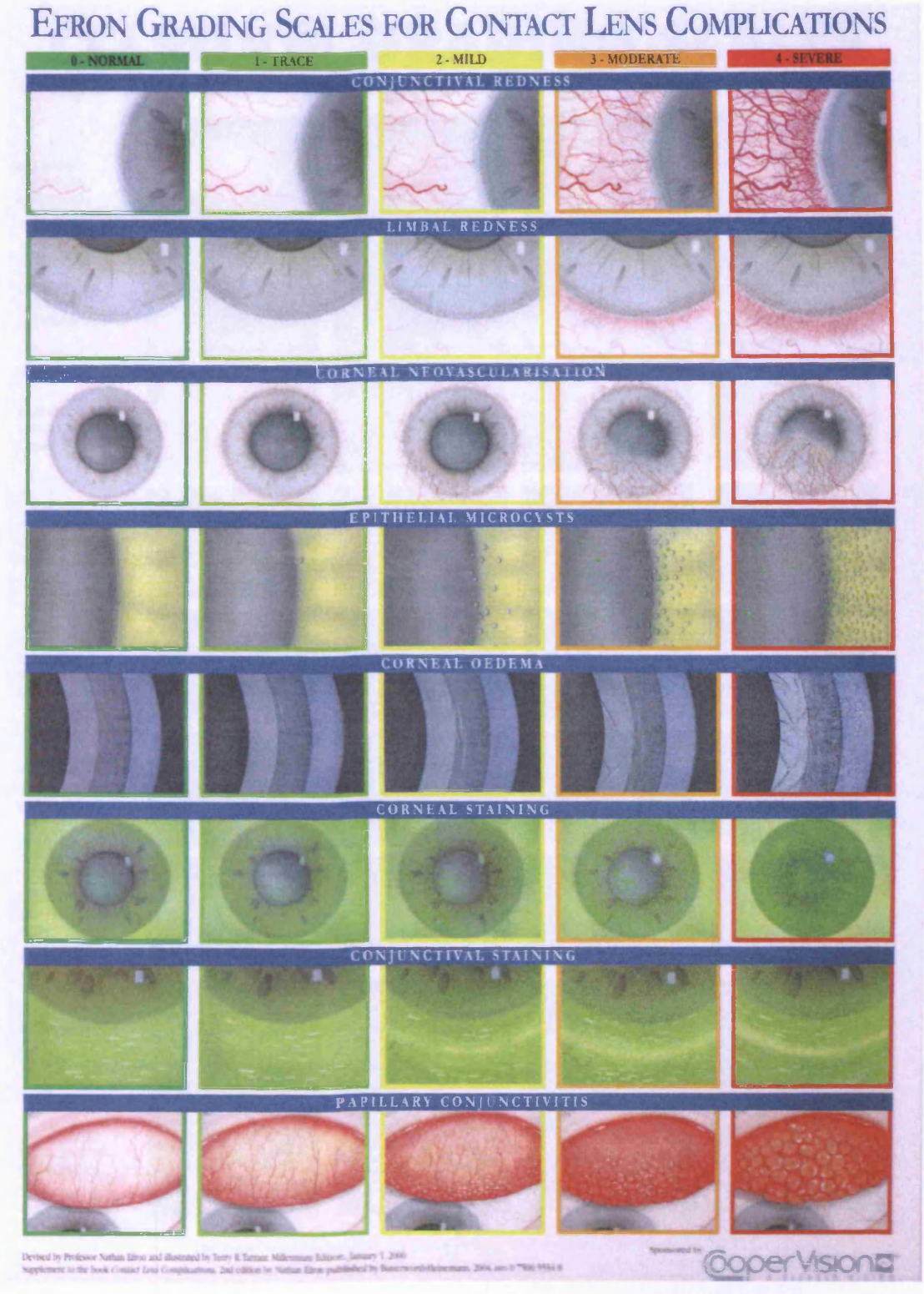


Figure 2.34: Efron grading scale[17].

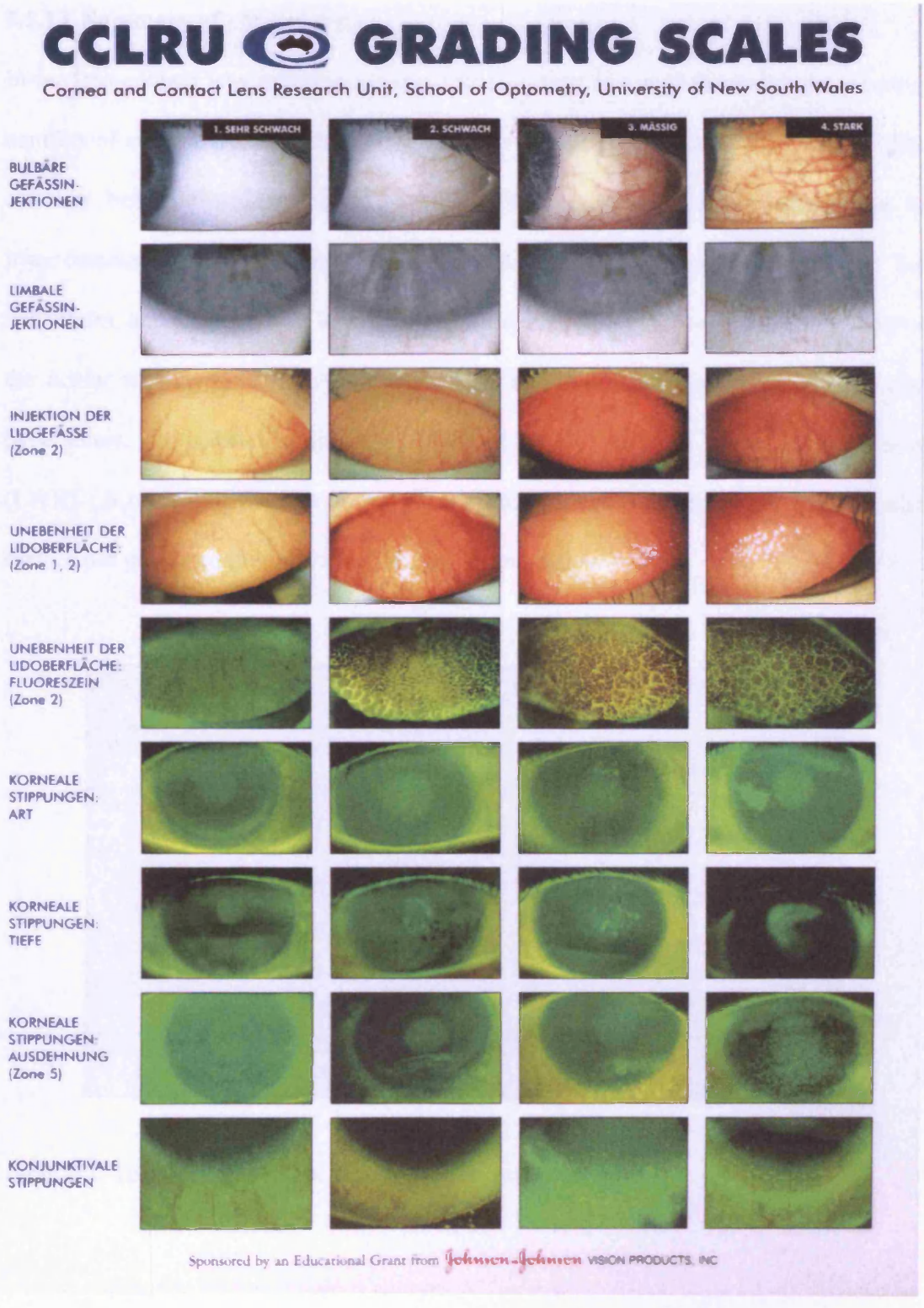


Figure 2.35: CCLRU grading scale (courtesy of University of New South Wales, Sydney, Australia[267]).

2.5.13 Summary of clinical tests

In modern contact lens practice, several tear film tests are used to predict the wearing comfort of contact lenses, although non-invasive tests are preferred[17,270,271]. Since dry eye has been defined as being linked to damage of the ocular surface due to hyperosmolarity, accompanied by inflammatory processes and damages of the epithelium, and since contact lens wear causes instability of the tear film, assessment of the ocular surface is vital. Appropriate tests include ocular surface staining, ocular hyperaemia, lid parallel conjunctival folds (LIPCOF) and lid wiper epitheliopathy (LWE). A review of the literature on tear film tests and objective signs of the ocular surface has produced the following table of relationships:

Correlations	NIBUT	Lipid Layer	Tear Volume
Staining	No[83,89,272]vs[273]	Yes[83,274]	Yes[83,204,275]
Ocular Redness	No[89,272]vs[273]	Yes[59,83]	Yes[83,275]
MGD	Yes[276-278]vs[279]	Yes[58,277,280]vs[279]	No[17,281]
LIPCOF	Yes[79,80,250]	No[79,80,250]	Yes[79,80,250]
LWE	Yes[78,282]	Yes[283,284]	Unknown

Table 2.2: Tear film test and ocular surface correlations.

However, the ability of these tests to predict contact lens wearing comfort/discomfort, alone is poor[89,272]. In response, recent papers have presented the importance of using a combination of several tests[89,285]. The most promising combination of tests appears to be composed from ocular hyperaemia, tear meniscus height, non-invasive break-up time, lid parallel conjunctival folds and lid wiper epitheliopathy.

2.6 Dry eye questionnaires

Clinical tear film tests are semi-objective (objective for the patient, not to the observer), while questionnaires reflect the subjective response of the patient. Although the results can then vary, depending on the patient's feelings, surroundings and sensitivity, a questionnaire is a useful instrument in dry eye management[97,98,119,285-289]. Many different dry eye questionnaires have been developed, about fourteen are cited in the 2007 Report of the International Dry Eye Workshop (DEWS)[2]. The most known and important are the McMonnies Dry Eye Index, Ocular Comfort Index (OCI), Ocular Surface Disease Index (OSDI) and Contact Lens Dry Eye Questionnaire (CLDEQ)[76,96,119,285,289-294].

2.6.1 McMonnies Dry Eye Index

The McMonnies Dry Eye Index, one of the first and most popular questionnaires, was designed by Charles McMonnies to assist in the diagnosis of mild and moderate dry eye syndrome. Epidemiological risk factors like gender, age, systemic health, the use of various medications, the frequency of symptoms of ocular irritation, and sensitivity to environmental triggers like alcohol or cigarette smoke are considered. Responses are assigned empirically established weights and summed, with higher values indicating a greater likelihood of dry eye syndrome being present[96,119,286,289,293]. Dry eye is assumed for a score of more than 14.5[98,293].

2.6.2 Ocular Surface Disease Index

The Ocular Surface Disease Index (OSDI) (Figure 2.36 and 2.37) is a 12-item, 5-category likert scale design that sequentially probes the symptoms of ocular irritation and the functional consequences and environmental triggers of dry eye symptoms. The

OSDI investigates the responses from different symptom categories like sensory, functional consequences and environmental triggers. Therefore the OSDI attempts to reflect the complex gamut of dry eye symptoms in one index[96,292]. In distinction to the McMonnies questionnaire, the OSDI attempts to estimate disease severity. Advocates of this questionnaire suggest that the OSDI score is proportional to symptom intensity. Schiffman et al[96] defined a mean score of 4.5 ± 6.6 as normal, 18.1 ±17.1 as mild-moderate and 36.3 ±23.1 as severe dry eye, a cut-off value for all dry eye patients of 6.0 and severe dry eye patients of 15.0.

2.6.3 Ocular Comfort Index

The OCI contains 8 items (1 positive and 7 negative) that specifically examine the discomfort of ocular surface disease. Each of these questions has two parts, which enquire separately about the frequency and severity of symptoms, the components of symptom intensity. To maximise the applicability of the OCI, only questions are used which are not specific to any one aetiology. Numerous items were included to reduce measurement error, which is inversely proportional to the square-root of the number of questions. A major advantage of the OCI is the way that it analyses the data. It converts the ordinal raw data counts into more useful abstract, equal-interval, additive measures, using methods introduced by Thurstone in the 1920s and later developed by Georg Rasch in the 1960s[294]. The OCI is suggested as an improved OSDI[294], what has to be confirmed in further studies. Unfortunately it is not tested in contact lens wearers and is supposed only to measure symptoms, not to diagnose[294]. Thresholds between symptomatic and asymptomatic patients are unknown.

2.6.4 Contact Lens Dry Eye Questionnaire

The Contact Lens Dry Eye Questionnaire (CLDEQ) (Figure 2.38-2.42) was developed to examine the symptoms distribution among contact lens wearers[119]. This questionnaire focuses on ocular surface symptoms rather than presumed risk factors for dry eye syndrome. The CLDEQ consists of 36 questions specific to symptoms of contact lens related dry eye. The constructs for these symptoms were derived from literature and clinical knowledge of dry eye symptoms among contact lens wearer and patients with dry eye. There are nine symptom sub-scales: discomfort, dryness, visual changes, soreness and irritation, grittiness and scratchiness, foreign body sensation, burning, photophobia, and itching. Each sub-scale asks about the frequency of the symptom, which is followed by three questions concerning the intensity of the symptom at different times of day, to examine diurnal fluctuations in symptoms[4,119,288]. In a recent study, dryness and discomfort were found to be the most frequently reported dry eye symptoms of contact lens wearers, reported significantly more frequently than non-contact lens wearers[5]. The scoring algorithm of the CLDEQ score groups the subjects in two categories: asymptomatic and symptomatic contact lens wearers.

2.6.5 Summary of dry eye questionnaires

In contact lens practice the correct assessment of patients symptoms is fundamental. Therefore the following important recommendations for the use of a dry eye questionnaire can be stated:

- The questionnaire has to be appropriate for both current contact lens wearers and for naïve contact lens wearers.
- The questionnaire has to be understandable by patients as well as practicable in normal contact lens practice (length and type of questions).
- The results of the questionnaire should present a high degree of prediction for the severity of the patient's symptoms in contact lens wear.
- The questionnaire has to have been validated with the appropriate population.
- The questionnaire has to be available and appropriate for normal practitioners.

Although the McMonnies questionnaire demonstrates good prediction of dry eye, it is long and includes some questions which are difficult for the patients to deal with (e.g. items are included asking for dryness in other mucous membranes such as mouth, nose and vagina). The OSDI and CLDEQ showed in many studies their ability to evaluate dry eye in contact lens practice. Since the OCI is an improved questionnaire of the OSDI this one might be interesting too. Unfortunately the OCI does not diagnose, but just measures symptoms and was not validated for contact lens wearers[294]. As a result, the OSDI is preferred over the OCI. Therefore the CLDEQ and OSDI questionnaires should be the preferred questionnaires in further experiments. These questionnaires demonstrated a good description of dryness and are understandable to patients.

Which questionnaire should be used depends on the patient pool and study protocol. Even though the OSDI does not cover the important questions of change in symptoms during the day, which the CLDEQ does[119], it has been successfully used in several contact lens studies[96,295].

Therefore the OSDI is suggested as the preferred questionnaire for naïve contact lens wearers and current lens wearers, while the CLDEQ is usable in experienced lens wearers only. Since the CLDEQ only diagnoses subjects (dry eye or normal), it should be used for grouping subjects, while the OSDI is able to evaluate dry eye symptoms in non-lens wearers as well as contact lens wearers and can monitor symptoms in prospective studies. However, as "dryness" and "discomfort" are the most important symptoms of unsatisfied lens wearers[5], these questions of the CLDEQ could be considered in measuring and monitoring symptoms of contact lens wearers too.

Ocular Surface Disease Index® (OSDI®)[2]

Ask your patients the following 12 questions, and circle the number in the box that best represents each answer. Then, fill in boxes A, B, C, D, and E according to the instructions beside each.

Have you experienced any of the following *during the last week*?	All of the time	Most of the time	Half of the time	Some of the time	None of the time
1. Eyes that are sensitive to light?	4	3	2	1	0
2. Eyes that feel gritty?	4	3	2	1	0
3. Painful or sore eyes?	4	3	2	1	0
4. Blurred vision?	4	3	2	1	0
5. Poor vision?	4	3	2	1	0

Subtotal score for answers 1 to 5 (A)

Have problems with your eyes limited you in performing any of the following *during the last week*?	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
6. Reading?	4	3	2	1	0	N/A
7. Driving at night?	4	3	2	1	0	N/A
8. Working with a computer or bank machine (ATM)?	4	3	2	1	0	N/A
9. Watching TV?	4	3	2	1	0	N/A

Subtotal score for answers 6 to 9 (B)

Have your eyes felt uncomfortable in any of the following situations *during the last week*?	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
10. Windy conditions?	4	3	2	1	0	N/A
11. Places or areas with low humidity (very dry)?	4	3	2	1	0	N/A
12. Areas that are air conditioned?	4	3	2	1	0	N/A

Subtotal score for answers 10 to 12 (C)

Add subtotals A, B, and C to obtain D
(D = sum of scores for all questions answered) (D)

Total number of questions answered
(do not include questions answered N/A) (E)

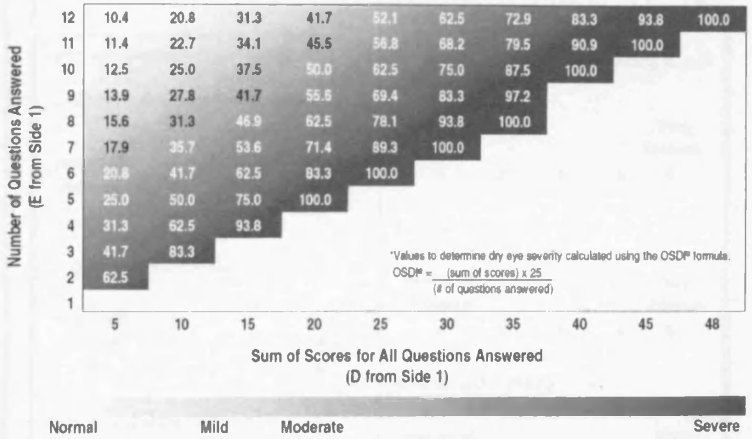
Please turn over the questionnaire to calculate the patient's final OSDI® score.

Evaluating the OSDI® Score[1]

The OSDI® is assessed on a scale of 0 to 100, with higher scores representing greater disability. The index demonstrates sensitivity and specificity in distinguishing between normal subjects and patients with dry eye disease. The OSDI® is a valid and reliable instrument for measuring dry eye disease (normal, mild to moderate, and severe) and effect on vision-related function.

Assessing Your Patient's Dry Eye Disease[1, 2]

Use your answers D and E from side 1 to compare the sum of scores for all questions answered (D) and the number of questions answered (E) with the chart below.* Find where your patient's score would fall. Match the corresponding shade of red to the key below to determine whether your patient's score indicates normal, mild, moderate, or severe dry eye disease.



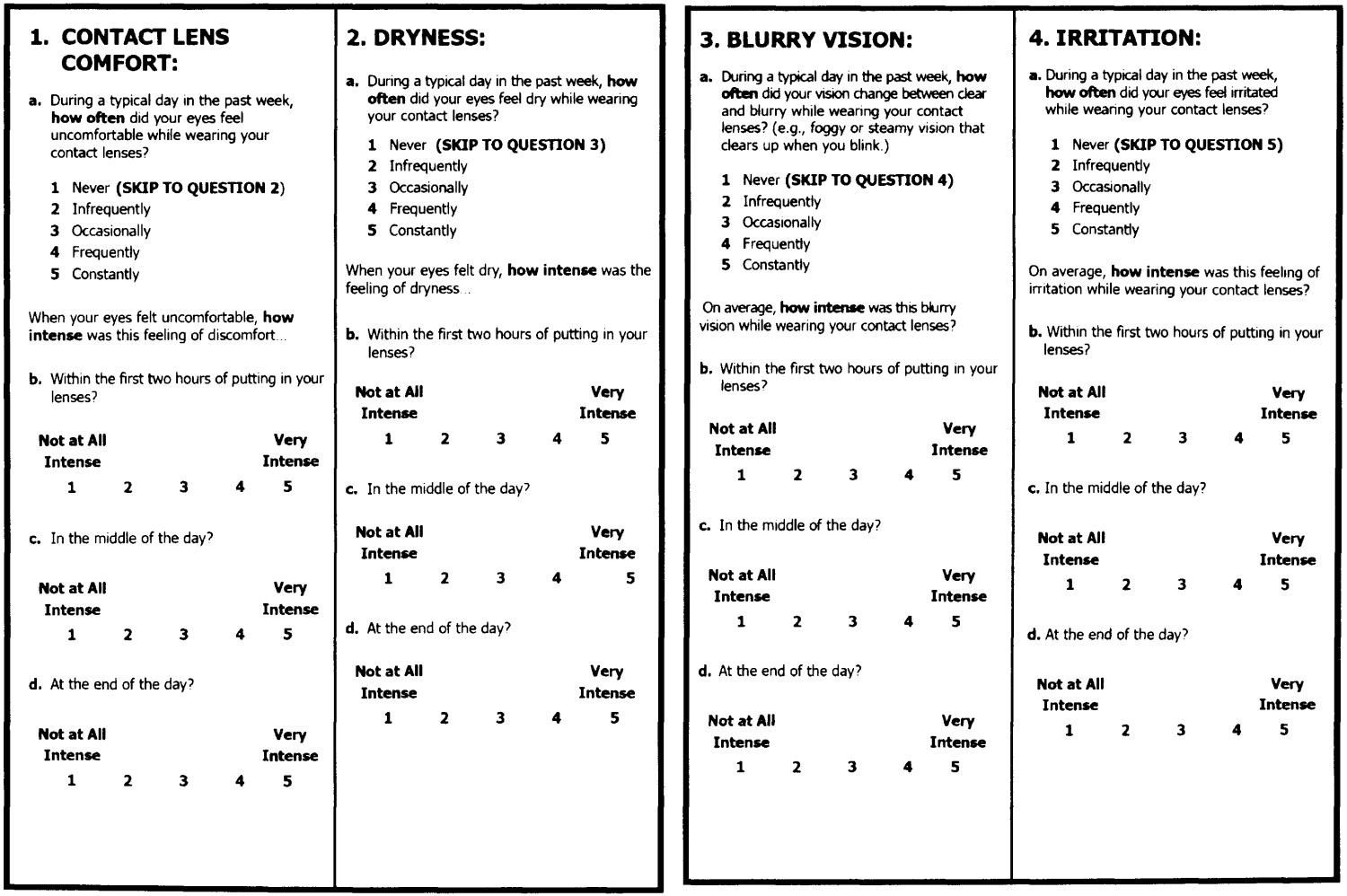
Patient's Name: _____ Date: _____

How long has the patient experienced dry eye disease? _____

Eye Care Professional's Comments: _____

1. Data on file, Allergan, Inc.
2. Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the Ocular Surface Disease Index. *Arch Ophthalmol.* 2000;118:615-621

Figure 2.36 and 2.37: Ocular Surface Disease Index (Allergan, 1995).



1. CONTACT LENS COMFORT:

a. During a typical day in the past week, **how often** did your eyes feel uncomfortable while wearing your contact lenses?

1 Never **(SKIP TO QUESTION 2)**
2 Infrequently
3 Occasionally
4 Frequently
5 Constantly

When your eyes felt uncomfortable, **how intense** was this feeling of discomfort...

b. Within the first two hours of putting in your lenses?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

c. In the middle of the day?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

d. At the end of the day?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

2. DRYNESS:

a. During a typical day in the past week, **how often** did your eyes feel dry while wearing your contact lenses?

1 Never **(SKIP TO QUESTION 3)**
2 Infrequently
3 Occasionally
4 Frequently
5 Constantly

When your eyes felt dry, **how intense** was the feeling of dryness ..

b. Within the first two hours of putting in your lenses?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

c. In the middle of the day?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

d. At the end of the day?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

3. BLURRY VISION:

a. During a typical day in the past week, **how often** did your vision change between clear and blurry while wearing your contact lenses? (e.g., foggy or steamy vision that clears up when you blink.)

1 Never **(SKIP TO QUESTION 4)**
2 Infrequently
3 Occasionally
4 Frequently
5 Constantly

On average, **how intense** was this blurry vision while wearing your contact lenses?

b. Within the first two hours of putting in your lenses?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

c. In the middle of the day?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

d. At the end of the day?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

4. IRRITATION:

a. During a typical day in the past week, **how often** did your eyes feel irritated while wearing your contact lenses?

1 Never **(SKIP TO QUESTION 5)**
2 Infrequently
3 Occasionally
4 Frequently
5 Constantly

On average, **how intense** was this feeling of irritation while wearing your contact lenses?

b. Within the first two hours of putting in your lenses?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

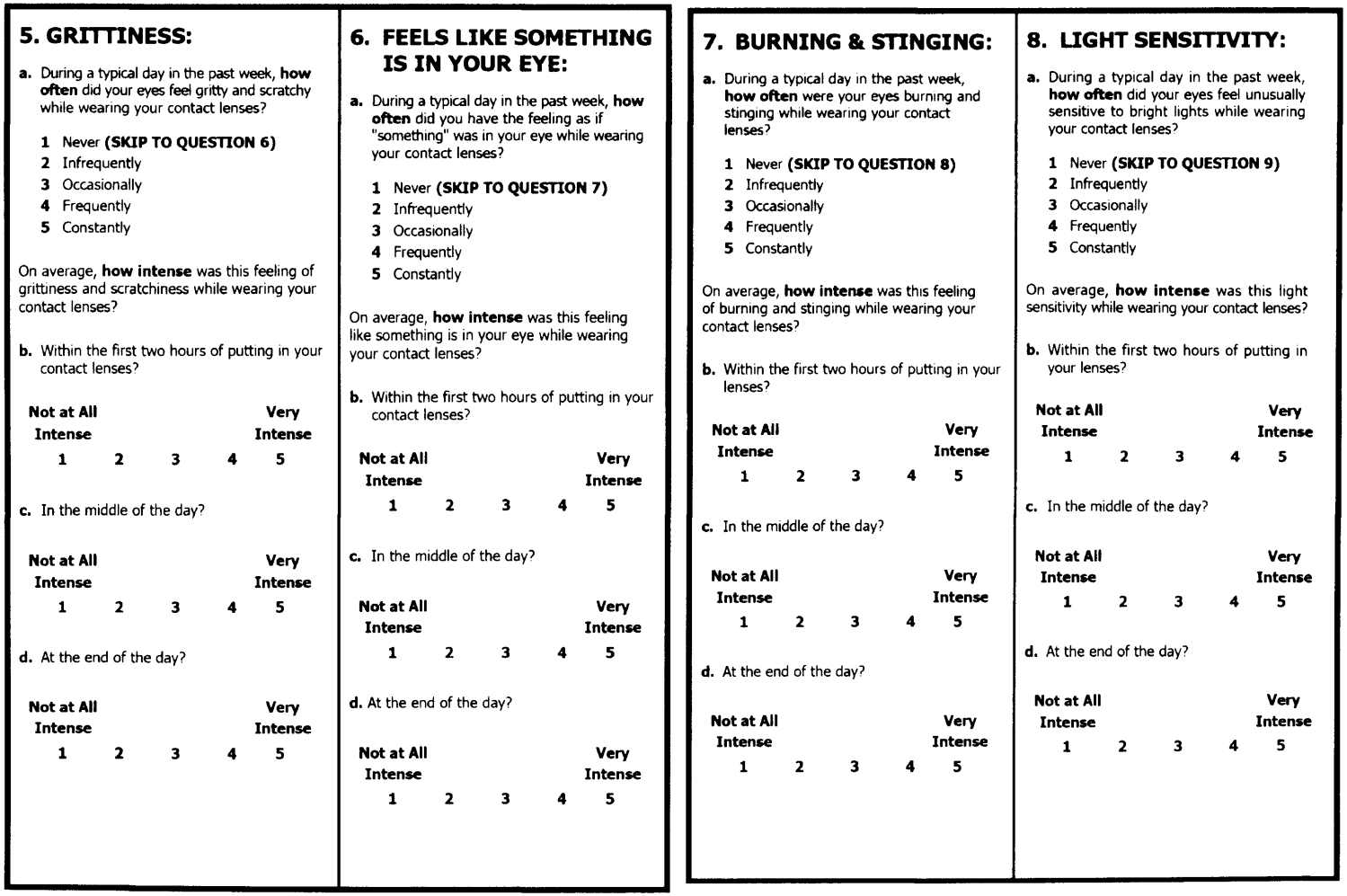
c. In the middle of the day?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

d. At the end of the day?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

Figure 2.38 and 2.39: Contact Lens Dry Eye Questionnaire[119].



5. GRITTINESS:

a. During a typical day in the past week, **how often** did your eyes feel gritty and scratchy while wearing your contact lenses?

1 Never **(SKIP TO QUESTION 6)**
2 Infrequently
3 Occasionally
4 Frequently
5 Constantly

On average, **how intense** was this feeling of grittiness and scratchiness while wearing your contact lenses?

b. Within the first two hours of putting in your contact lenses?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

c. In the middle of the day?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

d. At the end of the day?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

6. FEELS LIKE SOMETHING IS IN YOUR EYE:

a. During a typical day in the past week, **how often** did you have the feeling as if "something" was in your eye while wearing your contact lenses?

1 Never **(SKIP TO QUESTION 7)**
2 Infrequently
3 Occasionally
4 Frequently
5 Constantly

On average, **how intense** was this feeling like something is in your eye while wearing your contact lenses?

b. Within the first two hours of putting in your contact lenses?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

c. In the middle of the day?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

d. At the end of the day?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

7. BURNING & STINGING:

a. During a typical day in the past week, **how often** were your eyes burning and stinging while wearing your contact lenses?

1 Never **(SKIP TO QUESTION 8)**
2 Infrequently
3 Occasionally
4 Frequently
5 Constantly

On average, **how intense** was this feeling of burning and stinging while wearing your contact lenses?

b. Within the first two hours of putting in your lenses?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

c. In the middle of the day?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

d. At the end of the day?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

8. LIGHT SENSITIVITY:

a. During a typical day in the past week, **how often** did your eyes feel unusually sensitive to bright lights while wearing your contact lenses?

1 Never **(SKIP TO QUESTION 9)**
2 Infrequently
3 Occasionally
4 Frequently
5 Constantly

On average, **how intense** was this light sensitivity while wearing your contact lenses?

b. Within the first two hours of putting in your lenses?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

c. In the middle of the day?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

d. At the end of the day?

Not at All Intense **1** **2** **3** **4** **5** **Very Intense**

Figure 2.39 and 2.40: Contact Lens Dry Eye Questionnaire[119].

9. ITCHING:

a. During a typical day in the past week, **how often** did your eyes itch while wearing your contact lenses?

1 Never (**SKIP TO QUESTION 10**)
2 Infrequently
3 Occasionally
4 Frequently
5 Constantly

On average, **how intense** was this feeling of itchiness while wearing your contact lenses?

b. Within the first two hours of putting in your lenses?

Not at All Intense				Very Intense
1	2	3	4	5

c. In the middle of the day?

Not at All Intense				Very Intense
1	2	3	4	5

d. At the end of the day?

Not at All Intense				Very Intense
1	2	3	4	5

10. DO YOU THINK YOU HAVE DRY EYES WHILE WEARING YOUR CONTACT LENSES?

1 Yes

2 No

3 Unsure

END OF SURVEY

Thank you for your time in completing this survey.
We appreciate your accuracy and thoroughness.

Figure 2.41: Contact Lens Dry Eye Questionnaire, page 5[119].

Scoring Algorithm for the CLDEQ Long-Form

Step 1	Code each of the intensity questions zero if the frequency of that symptom is reported as "never". Otherwise, the scoring remains 1-5.
Step 2	Calculate the average intensity for **each** of the 9 symptoms.**
Step 3	Score each symptom using the table below.

A	Contact Lens Comfort (Q1)	=	Frequency response*average intensity*	0.03
	Dryness (Q2)	=	Frequency response*average intensity*	0.18
	Blurry Vision (Q3)	=	Frequency response*average intensity*	0.01
	Grittiness (Q5)	=	Frequency response*average intensity*	0.02
	Burning (Q7)	=	Frequency response*average intensity*	0.07
	Itching (Q9)	=	Frequency response*average intensity*	0.09
B	Irritation (Q4)	=	Frequency response*average intensity*	0.07
	Foreign Body Sensation (Q6)	=	Frequency response*average intensity*	0.09
	Photophobia (Q8)	=	Frequency response*average intensity*	0.10

Step 4	Sum the first 6 symptoms (TOTAL A). Sum the last 3 symptoms (TOTAL B).
Step 5	Calculate score = TOTAL A – TOTAL B.

If subject thinks he/she has dry eye and score > -0.13 then diagnose dry eye

If subject thinks he/she does not have dry eye and score > 1.27 then diagnose dry eye

If subject is unsure about whether he/she has dry eye and score > 1.44 then diagnose dry eye

** Score cannot be calculated unless **ALL** frequency and intensity questions are answered.

Figure 2.42: Contact Lens Dry Eye Questionnaire scoring algorithm (Nichols JJ, 2002).

3. Review Conclusions and Plan

Dry eye is a multi-factorial disease that results in symptoms and tear film instability with potential damage to the ocular surface and is accompanied by inflammation of the ocular surface[2]. Therefore it is vital to assess which test best relates to damage or irritation of the ocular surface and which abnormalities are related to symptoms.

3.1 Tear film instability

Tear film stability mainly depends on the quality and balance of the lipid layer[28,169], and mucin layer[296].

The lipid layer can be assessed by classifying the lipid interference pattern[202,297] by Tearscope Plus™ (Keeler Ltd, Windsor, UK). This test needs practice by the clinician to obtain the best results since visible differences between grades are minimal and the colour of the patient's iris can influence the appearances of the interference fringes.

In contrast, assessing mucin quality in the tear film is not possible in clinical practice without the use of sophisticated research instrumentation. So if there was a clinical test available that can provide indirect information on this tear film parameter, clinicians may be able to apply it when investigating the source of contact lens wear discomfort in their patients.

Tear-film break-up time, best observed non-invasively, reflects the balance and quality of both the lipid layer and aqueous-mucin layer[200,201,298]. Thus it is a useful test for dry eye diagnoses[89,298].

Assessment of tear film volume is essential for understanding tear film formation and stability[205,299] and since the assessment of tear meniscus height shows adequate correspondence with dry eye symptoms[204,300], use of this test is appropriate.

3.2 Ocular surface

The ocular surface can provide much information, however not all of the signs observed are related to only dry eye. For example, moderate to severe hyperaemia of the ocular surface can occur in response to inflammation, but also in irritation and systemic disease[84,151,226-232]. Nevertheless, assessment of ocular hyperaemia is important in contact lens wear, since reduced oxygen transmission of some contact lens materials can induce hyperaemia and can result in discomfort[125].

Ocular surface staining is seen in dry eyes and is related to the lipid layer and tear film volume (Table 2.2), but this sign is not well related to symptoms and is poorly repeatable[14,224]. Hyperosmolarity induces symptoms, but not necessarily corneal staining in dry eye patients[224].

LWE and LIPCOF are reported to be predictive in dry eye symptoms[77-80]. LWE and LIPCOF are assumed to be related to friction in blinking and tear film stability[78,244,283]. LIPCOF are inversely related to tear meniscus height and NIBUT in contact lens wearers[80]. The relationship between LWE, tear film quality and LIPCOF is unknown, nor is their relationship to mucins, which is important since mucins are essential in tear film spreading and viscosity of the tear film[31,301].

3.3 Symptoms

Symptoms in dry eye are essential for analysing the predictability of objective tests in symptomatic dry eye patients. In CLIDE, symptoms are best assessed by use of dry eye questionnaires, e.g. OSDI and CLDEQ[97,98,119,285-289]. The OSDI is able to monitor symptoms over time, and the CLDEQ is useful for grouping subjects in clinical trials into symptomatic and asymptomatic cohorts.

3.4 Summary

The literature review has shown that although there are a wide range of tear film tests, their ability to predict contact lens comfort is poor[89,272]. However, there are some interesting correlations between the tear film and the ocular surface, which suggests that a combination of some tests may improve the prediction of contact lens induced dry eye (CLIDE).

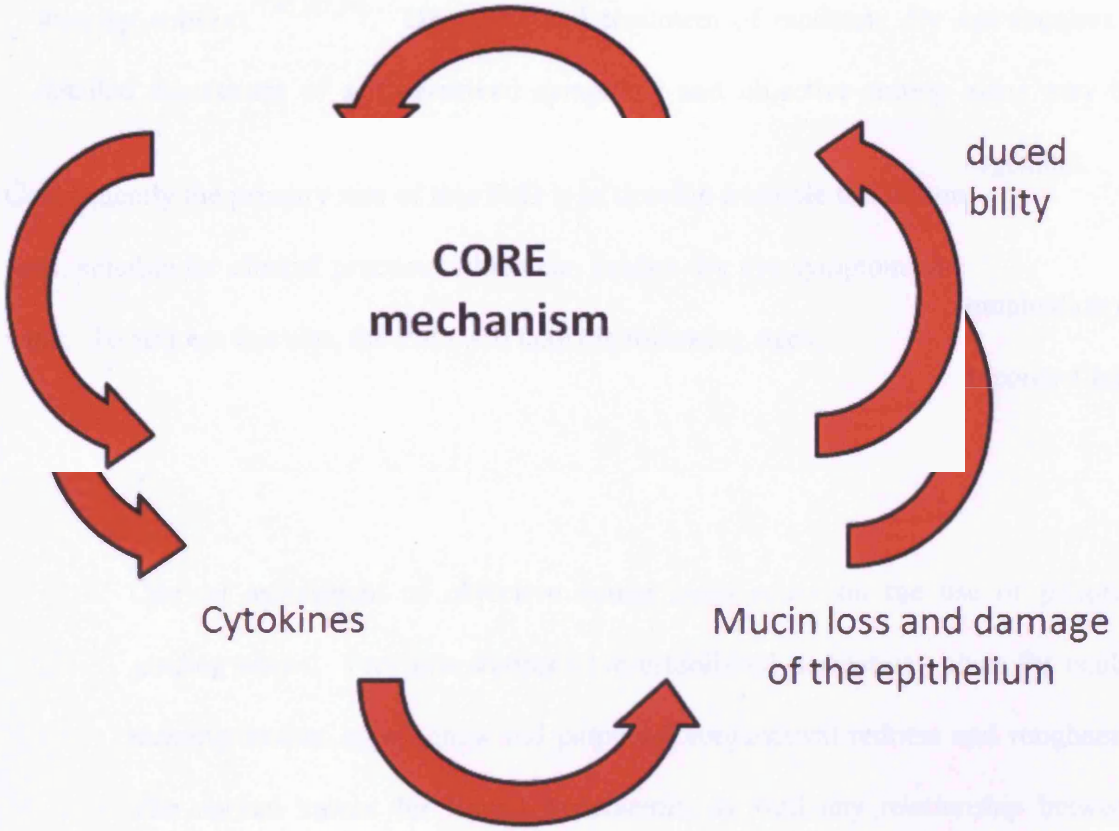


Figure 3.1: The core mechanism due contact lens induced tear film instability.

The core mechanism for dry eye formation proposed by DEWS can be used as a guide towards deciding on suitability of these clinical tests in developing the predictive test. The complexity and/or expense of assessing tear osmolarity and tear film inflammatory cytokines content excludes further investigation of these two components of the mechanism from analyses. However, NIBUT, tear volume, ocular hyperaemia, LWE and LIPCOF could be usefully combined in a defined set of tests to forecast contact lens wearing comfort[77,80,287,302]. Diagnosis and treatment of moderate dry eye requires a detailed assessment of self-perceived symptoms and objective testing alone may be insufficient[289,302], therefore questionnaires are also essential in dry eye management.

Consequently the primary aim of this PhD is to develop a simple test or combination of tests, suitable for clinical practice, which can predict dry eye symptoms in contact lens wear. To address this aim, the PhD will take the following steps:

1. Clinical assessment of objective ocular signs relies on the use of pictorial grading scales. Previous studies have established normative values for ocular staining, bulbar hyperaemia and palpebral conjunctival redness and roughness. The normal values for limbal hyperaemia, as well any relationship between limbal and bulbar conjunctival hyperaemia, are unknown. Chapter 4 investigates limbal and bulbar hyperaemia in normal eyes.
2. For the five clinical tests proposed as being the most suitable for a predictive tests, further investigation is necessary to understand how the tests relate to each other, and of what part of the core mechanism they inform. Chapters 5 and 6 evaluate these clinical tests in symptomatic and asymptomatic contact lens wearers.

3. Chapter 7 reports on how these tests perform in non-contact lens wearers, as it is also important to assess influence of "normality" on the performance of these tests.
4. Finally, in Chapter 8 a prospective longitudinal study evaluates which test or combination of tests can best predict symptoms in later contact lens wear for a group of naïve soft contact lens wearers.

4. Limbal and Bulbar Hyperaemia in Normal Eyes

4.1 Introduction

This study will help to determine the prevalence of hyperaemia in a healthy non-contact lens wearing population, as well as the 'normal' grade of hyperaemia. Determining the 'normal' amount of limbal hyperaemia will assist practitioners in detecting abnormality by use of this grade as a baseline. The results of the study will be useful for analysis of the results in later experiments, e.g. in which areas does redness increase and is there a correlation between the bulbar and limbal redness changes in symptomatic contact lens wearers; do the areas of increased redness change between quadrants; how much does redness increase in contact lens wearers compared to 'normal' eyes?

Moderate to severe hyperaemia of the anterior eye is a common sign associated with an unhealthy eye. The redness observed is the result of an increase in the volume of blood in the anterior scleral, bulbar conjunctival and limbal vessels and occurs in response to inflammation, irritation and systemic disease[84,151,226-232]. A review of the literature suggests that bulbar hyperaemia is more typically caused by general ocular and systemic factors, while limbal hyperaemia is associated with corneal 'stress' (e.g. keratitis, infiltrates, staining, abrasion, hypoxia)[17,226,228,233-238]. Many studies have observed the effects of hypoxia on ocular comfort, visible in increased limbal hyperaemia. Seventy to eighty percent of patients with corneal oxygen deficiency report increased levels of discomfort[132,148,149]. Even though limbal hyperaemia is an important indicator of corneal stress, particularly in contact lens wear [151,226,227,230,231,233,235,238] and normal levels of some appearances evaluated by the Cornea and Contact Lens Research Unit (CCLRU) grading scale (Figure 4.1) are published[220,232,303], the expected clinical appearance of the normal limbal vasculature is unknown.

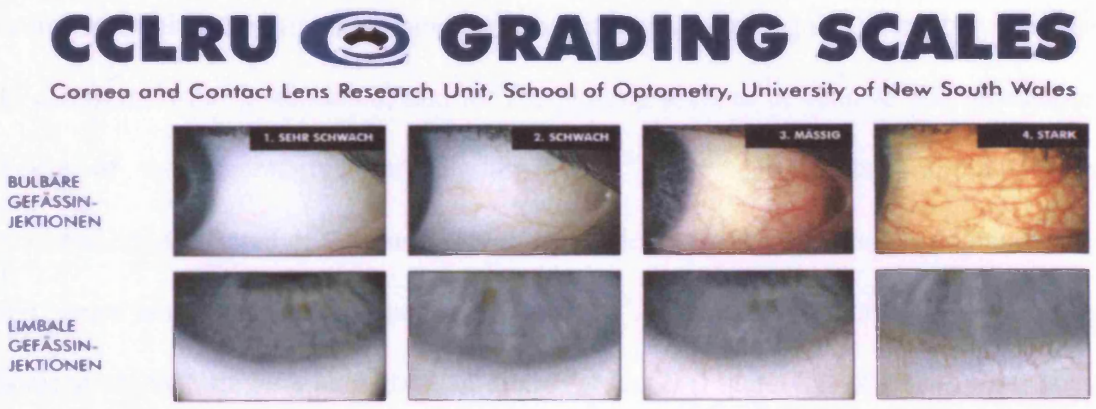


Figure 4.1: CCLRU Grading Scale: Bulbar redness and limbal redness[267].

Furthermore, although there is an obvious anatomical link between limbal redness and bulbar redness, the literature review suggests that there may be some dissociation depending on the cause of the hyperaemia. Some studies have assessed both bulbar and limbal redness with contact lens wear[151,226,227,230,231,233,235,238], but not for healthy, non-contact-lens-wearing normal subjects. Also, to our knowledge, no previous studies have considered the relationship between limbal and bulbar hyperaemia.

As hyperaemia is an important clinical sign of ocular disease, inflammation and contact lens wearing comfort, grading scales are frequently used to assess the severity or degree of change in bulbar and limbal redness[125,151,226,227,230,231,233,235,238,259,260,263,264,304]. These scales have utilised verbal description, photographs, or paintings that illustrate an increasing level of hyperaemia, and they have been particularly used in clinical studies[233,258-264]. With the introduction of digital imaging into clinical ophthalmic practice, it is possible to obtain a permanent record of the appearance of the eye. However, where this is not available to the clinician, verbal [265,266] or pictorial [260,264,267] grading scales may be used to record ocular status and allow comparison across time. It

is important that the clinician knows which grade or grades signify normality, in order to determine what is abnormal, and for the grading scale to be reliable and repeatable. Some of the commonly used grading scales[264,267,305] imply that normality and abnormality are found at the same grading scale level for each clinical appearance (i.e. the scales are aligned), as proposed by Woods[266]. For example, the CCLRU grading scale states that, in general, a grade of slight (grade 2) (Figure 4.1) or less is considered within normal limits. However, previous studies[220,232,260,303] have shown that the normal ocular appearance is not necessarily the lowest level on a grading scale, nor is the grading scale level the same for each clinical appearance. As shown in Figure 4.2, corneal fluorescein staining[220] was typically less than palpebral roughness[303] which was typically less than bulbar conjunctival hyperaemia[306]. Also, Efron et al[221] showed, in a comparison of four different grading scales, grading cross-comparison between grading scales could not be made.

This study continues on from previous reports[220,303,306] on the normal clinical grading scores for limbal and bulbar hyperaemia, their relationship, and the inter- and intra-observer agreement for such grading.

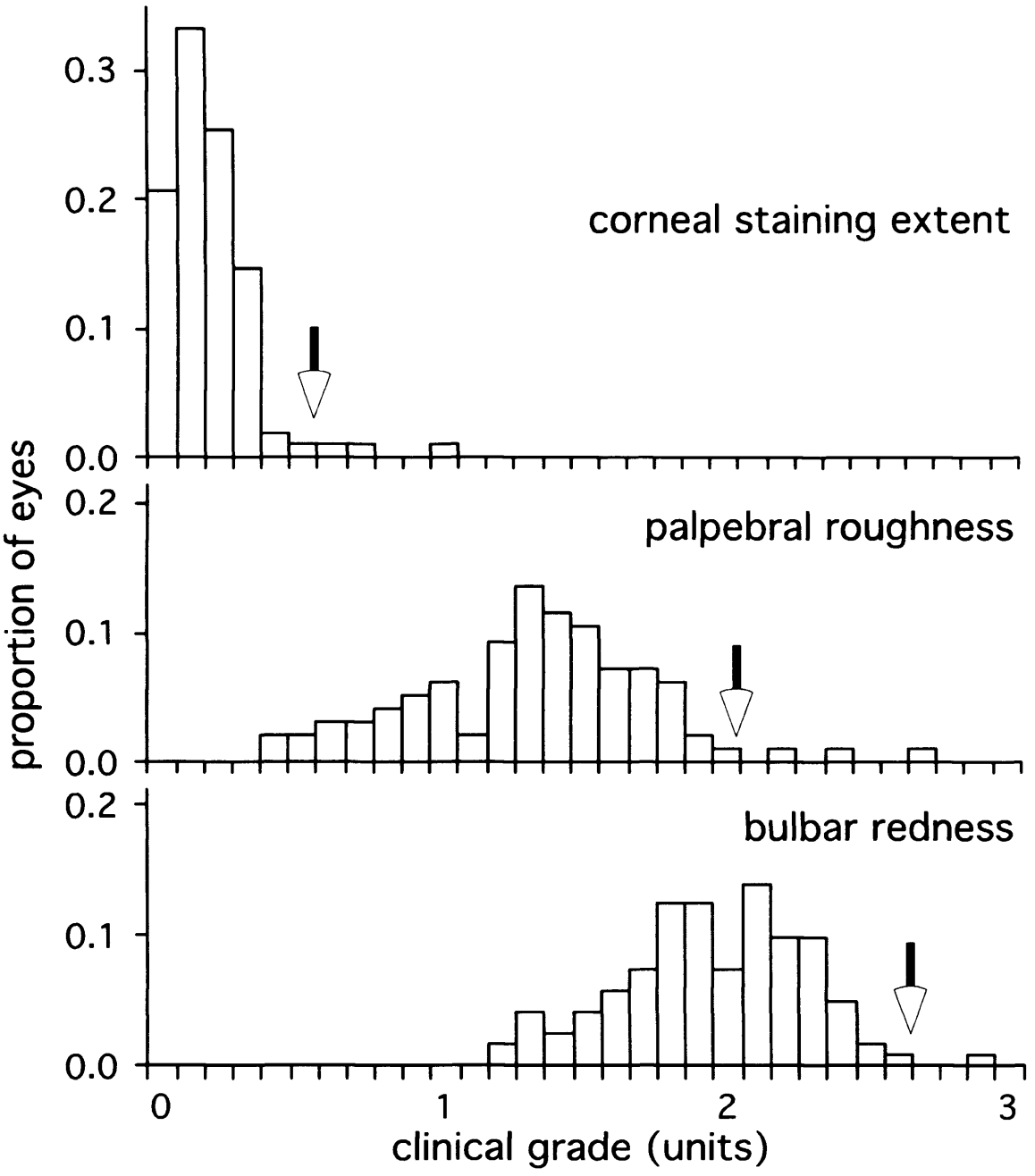


Figure 4.2: The typical grade of a normal eye of corneal staining (top panel, n=102,[220]), palpebral roughness (middle panel, n=96,[303]) and bulbar redness (bottom panel, n=121,[232]) is shown. For each of these studies, the average of scored zones is shown. None of these studies of real eyes scored overall appearance. The arrows mark the upper 95% confidence limits, above which an eye may be considered to have an unusually high score.

4.2 Methods

4.2.1 Subjects

One hundred and twenty subjects (male = 57, female = 63, median age = 45 years, range = 18-78; Figure 4.3) were selected from volunteers attending the optometry practice of Horst Riede GmbH, Weinheim, Germany.

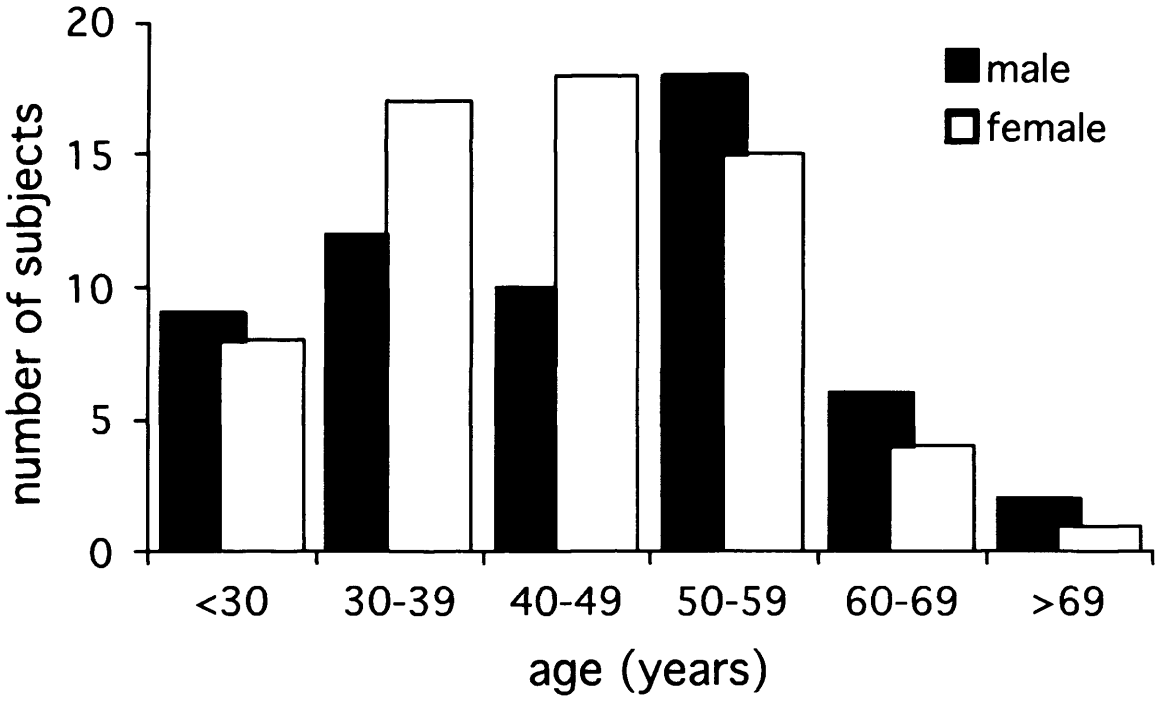


Figure 4.3: Frequency of age (median age = 45 years) and gender (male = 57, female = 63) among the 120 subjects.

4.2.2 Inclusion and exclusion criteria

All procedures obtained the approval of the Ethics Committee of the Cardiff University and were conducted in accordance with the requirements of the Declaration of Helsinki. Since McMonnies and Ho[307] described how conjunctival hyperaemia can vary with factors such as lack of sleep, eyestrain, wind, dust, smog, smoke and alcohol, subjects

were screened for these factors. All subjects had no current ocular disease, systemic disease, medication or allergy known to affect ocular hyperaemia. Contact lens wearers were included, if they had not worn contact lenses during the previous two weeks. Two weeks has been considered sufficient time for any contact lens related ocular hyperaemia to have resolved[308].

The time of observation was restricted to office-hours (10:00am to 6:00pm), since ocular redness is reported to be relatively constant in that period[309].

To assess inter-observer agreement, the first twenty subjects (subject numbers 1 to 20) (male = 6, female = 14, median age = 41 years) and the last twenty subjects (subject numbers 101 to 120) (male = 7, female = 13, median age = 43 years) were assessed by both observers involved in the study. Intra-observer agreement was assessed in a further twenty subjects (male = 12, female = 8, median age = 54 years). The time between observations (observer 1 to 2; 1 to 1; 2 to 2, respectively) of the inter- and intra-observer experiments was restricted to a maximum of 15min.

4.2.3 Grading

Bulbar and limbal hyperaemia were assessed by two trained observers (optometrists – Heiko Pult and Thomas Heinz) using the CCLRU grading scale[267,305], interpolated to 0.1 unit increments. The observers were instructed that, if they considered the ocular hyperaemia was less than the Grade 1, they should attempt to grade between the pictured Grade 1 and an imagined perfectly white eye, which would represent Grade 0. The ability to extrapolate CCLRU grading scales has been demonstrated in previous studies[220,303] (see Figure 4.2). These photographic scales were developed by the CCLRU at the University of New South Wales (Sydney, Australia) and each of the ten anterior-eye-appearance scales comprises four photographs that increase in the

appearance of severity, and are labelled: 1 Very Slight; 2 Slight; 3 Moderate; 4 Severe (Figure 4.1). Bulbar and limbal hyperaemia were graded using the bulbar and limbal redness scales, respectively. Since the CCLRU grading scale was designed for use with a slit-lamp bio-microscope[310], the right eye only of each subject was examined using a slit-lamp bio-microscope (x12 magnification). To provide consistent and even illumination over the eye, the slit-lamp diffuser was used, the beam-width was full and the brightness was set to maximum. Bulbar and limbal overall scores were evaluated by the observer making a judgement of the overall redness appearance. Then, the subject's position of gaze was directed to allow grading of four quadrants: superior, nasal, inferior and temporal. Bulbar and limbal quadrant-average scores were calculated from the average of the scores of the four quadrants.

4.3 Statistical analyses

Since the interpolated grading scales approximate an interval scale[311], and Barbeito and Simpson [312] have argued that parametrical statistical tests can be applied to such data, we conducted both parametric and non-parametric tests (where there was an equivalent), but only report the parametric tests as the outcomes were similar. Differences between means were examined by t-test and ANOVA, relations were analyzed by Pearson Correlation. Inter-observer agreement and intra-observer agreement was defined as the agreement coefficient of Bland and Altman[313]. The agreement coefficient is 1.96 times the standard deviation of the inter-observer difference scores (i.e. score from Observer 1 minus score from Observer 2). Differences of the agreement coefficients between first and last inter-observer group was evaluated by O'Brien's test for homogeneity of variance. The data were analysed

by use of WinSTAT 2005.1-Software (R Fitch Software, Bad Krozingen, Germany) and JMP IN 5.1.2 (SAS Institute, Belmont, Canada).

4.4 Results

4.4.1 Prevalence study

The distributions of overall and quadrant-average limbal and bulbar redness scores for the 120 subjects are shown in Figure 4.4. For bulbar redness, the quadrant-average grade (1.82 ± 0.39) (mean units ± sd) was significantly less than the overall grade (2.02 ± 0.49) (post-hoc t-test: $t=8.05$, $p<0.001$), whereas, for limbal redness, quadrant-average (1.61 ± 0.40) and overall (1.62 ± 0.46) grades were similar ($t=0.88$, $p=0.38$) (Figure 4.4). There were significant differences between quadrants for both limbal (repeated measures ANOVA, $F=19.7$, $p<0.0001$) and bulbar ($F=49.0$, $p<0.0001$) redness. As shown in Figure 4.5, the nasal and temporal quadrants were redder than the superior and inferior quadrants for both limbal and bulbar redness ($t\geq3.44$, $p<0.001$). Significant correlations were found between bulbar and limbal redness scores in all quadrants (Pearson $r\geq0.43$ $p<0.0001$). For each of those correlations, as illustrated in Figure 4.6, on average, limbal and bulbar redness were similar for low grades, but as bulbar redness increased, limbal redness increased more slowly. Bulbar redness was significantly higher than limbal redness in all four quadrants and for the overall scores ($t\geq4.21$, $p<0.001$) (Figure 4.4). Females had slightly lower limbal redness quadrant scores than males (mixed-model ANOVA, $F=3.78$, $p=0.054$) and there were small, but statistically significant, decreases in superior, inferior and overall limbal redness with age (multiple regression analysis, $F\geq3.96$, $p<0.05$). There were no significant effects of

age or gender on bulbar redness. The power calculation of the completed prevalence study resulted in a power of 1.00.

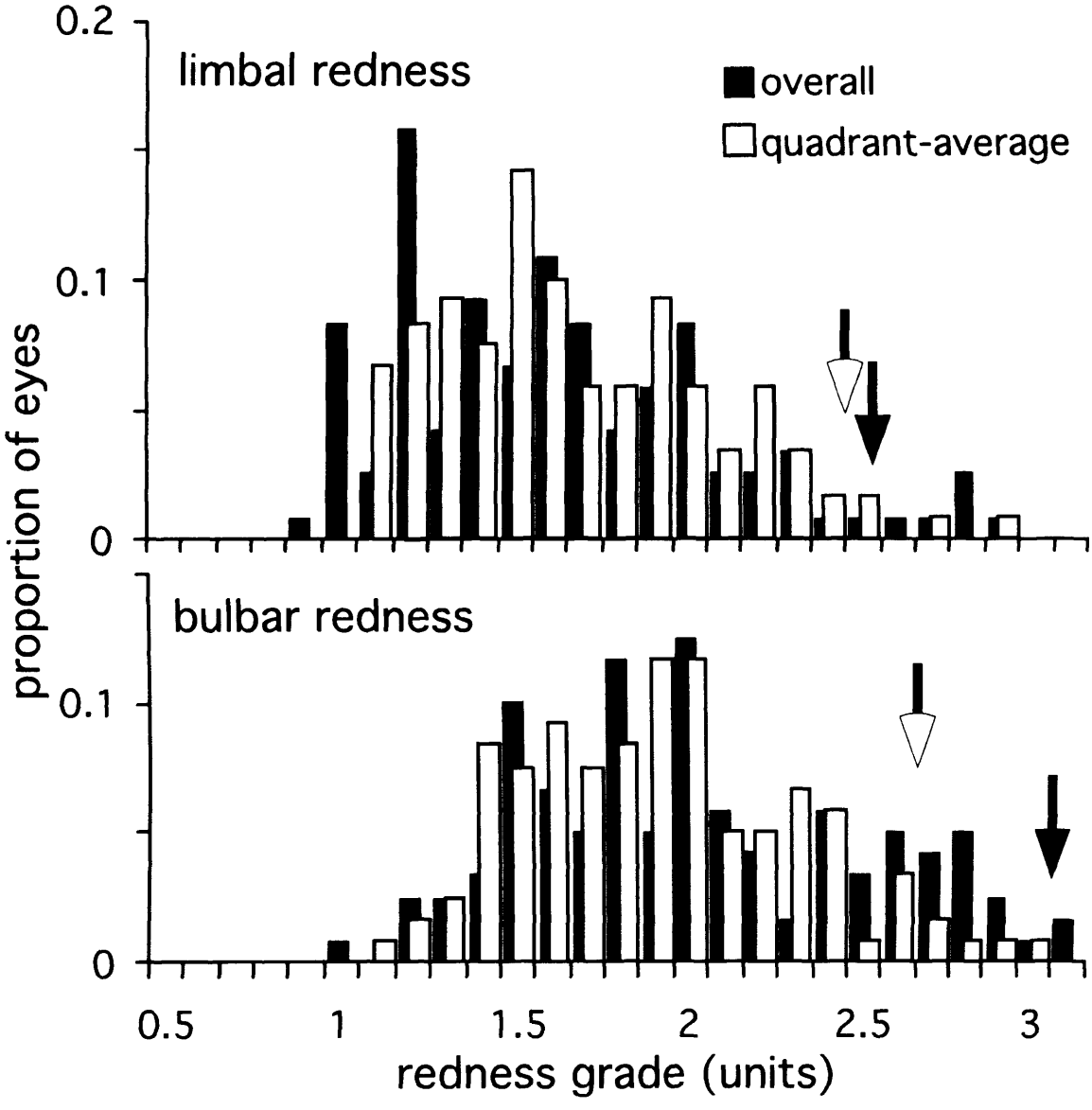


Figure 4.4: Distribution of overall and quadrant-average scores for limbal and bulbar redness (n=120). The arrows show the 95% confidence limits, above which a redness score may be considered as unusual.

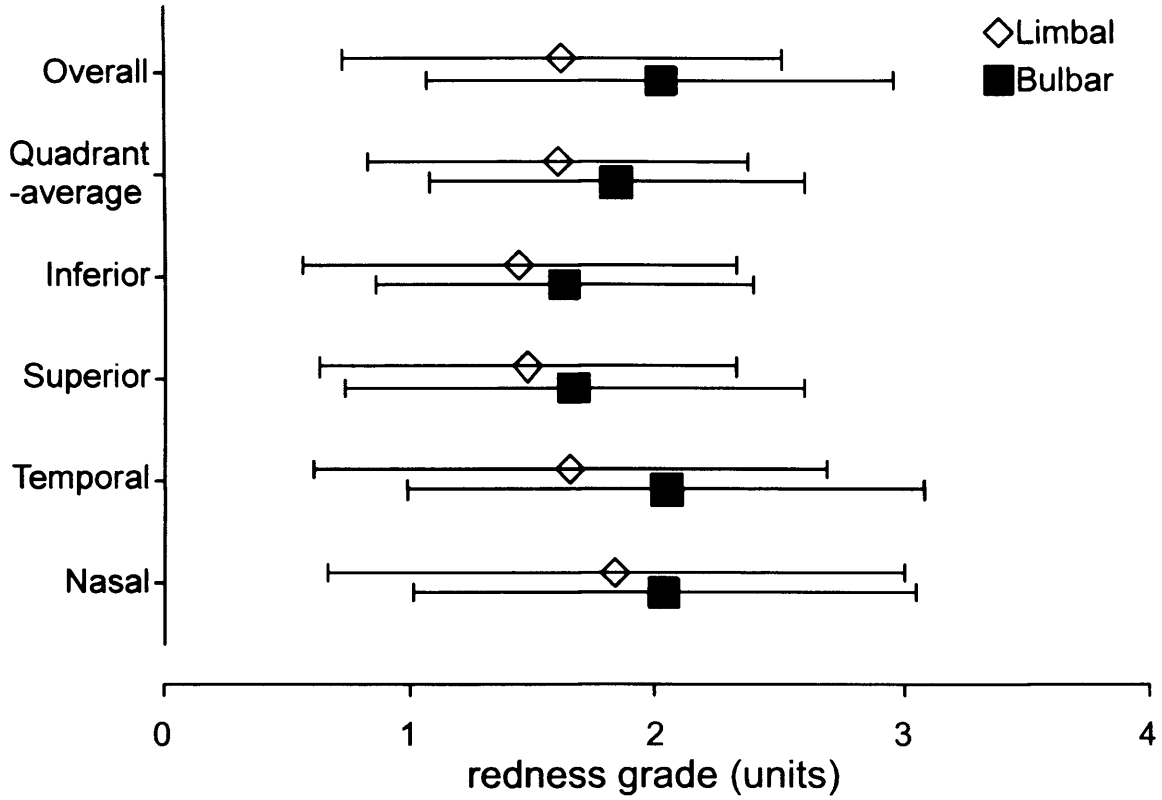


Figure 4.5: Mean and 95% confidence limit (error bars) of limbal and bulbar redness for overall, the quadrant-average and each quadrant.

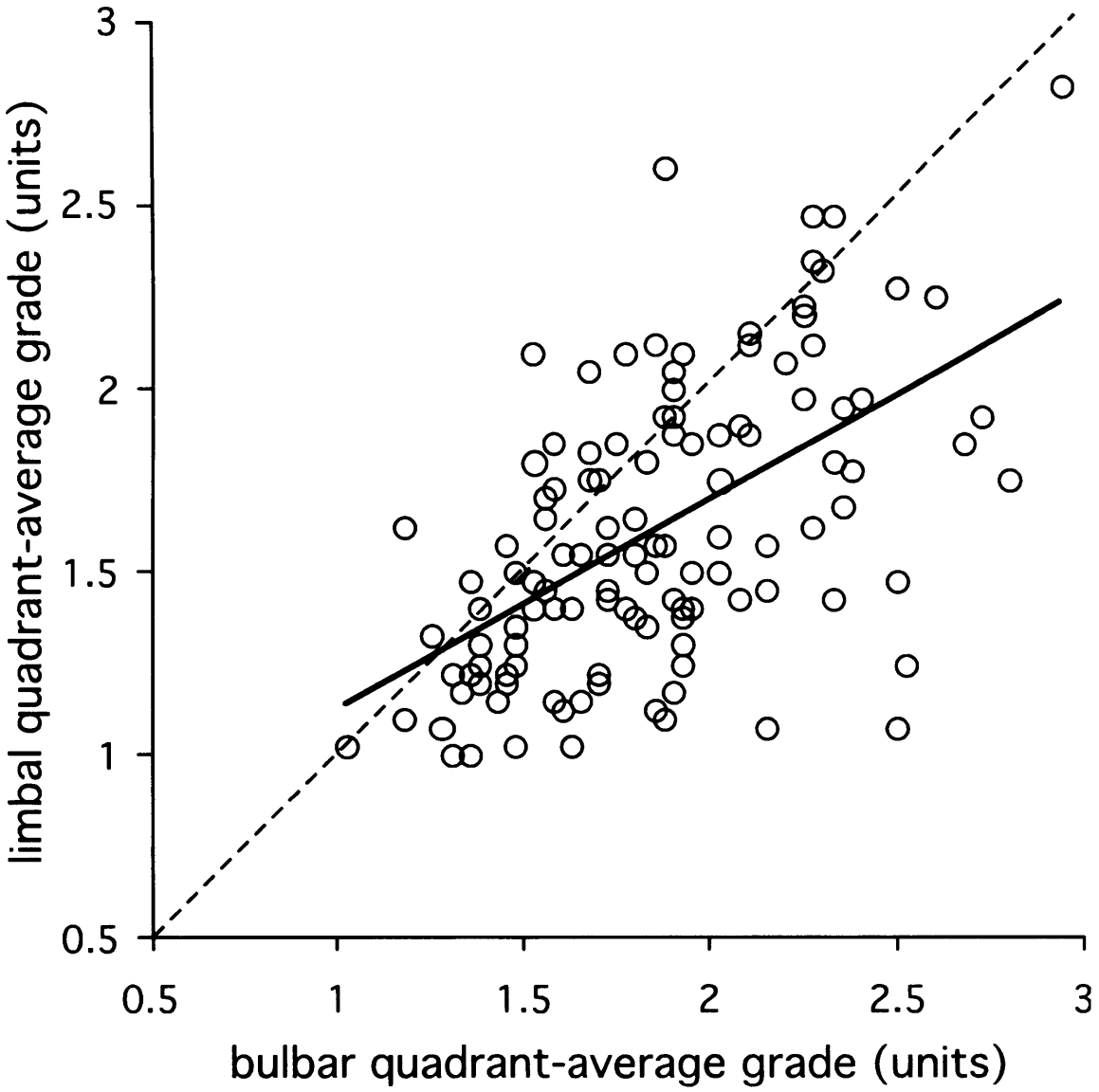
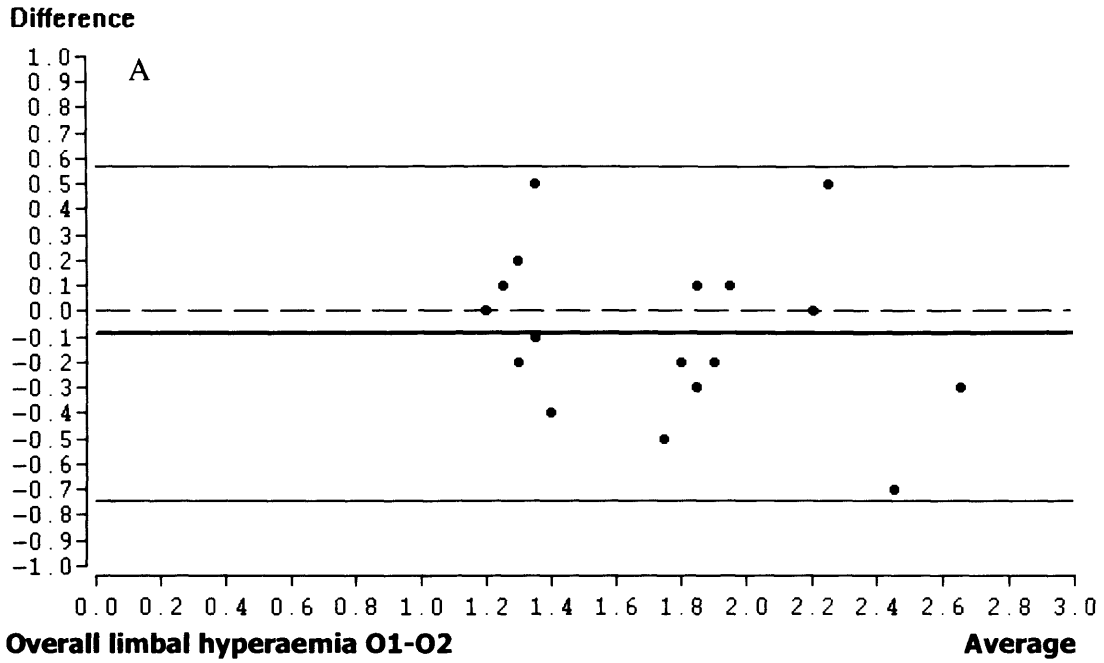


Figure 4.6: The relationship between limbal and bulbar quadrant-average redness. The dashed line shows a slope of 1 (i.e. limbal redness equals bulbar redness), while the solid line illustrates the line of best fit (slope = 0.57, Pearson r = 0.56, $p < 0.001$).

4.4.2 Inter-observer agreement study

In general, the 95% agreement coefficients were larger and more variable for individual quadrants than the quadrant-average, were larger for overall than quadrant-average, and did not vary between the start and end of the prevalence study (Figure 4.7 and 4.8). The agreement coefficients were similar between the two groups (first and last 20 subjects) (O'Brien's test for homogeneity of variance, $F<1.44$, $p>0.23$), except for limbal nasal quadrant (0.82 versus 0.55; $F=3.7$, $p=0.06$) and limbal quadrant-average (0.35 versus 0.22; $F=4.8$, $p=0.03$), when the agreement was better for the last 20 group. When both groups were combined, agreement coefficients for limbal and bulbar redness were not significantly different ($F<2.74$, $p>0.10$), except for the nasal quadrant (0.70 versus 0.53; $F=4.0$, $p=0.05$), when agreement was better for bulbar than limbal redness. When limbal and bulbar scores were combined, the agreement coefficient was better for quadrant-average (0.28) than overall (0.57) redness ($F=36$, $p<0.0001$). The power calculation of the completed inter-observer study resulted in a power of 1.00.



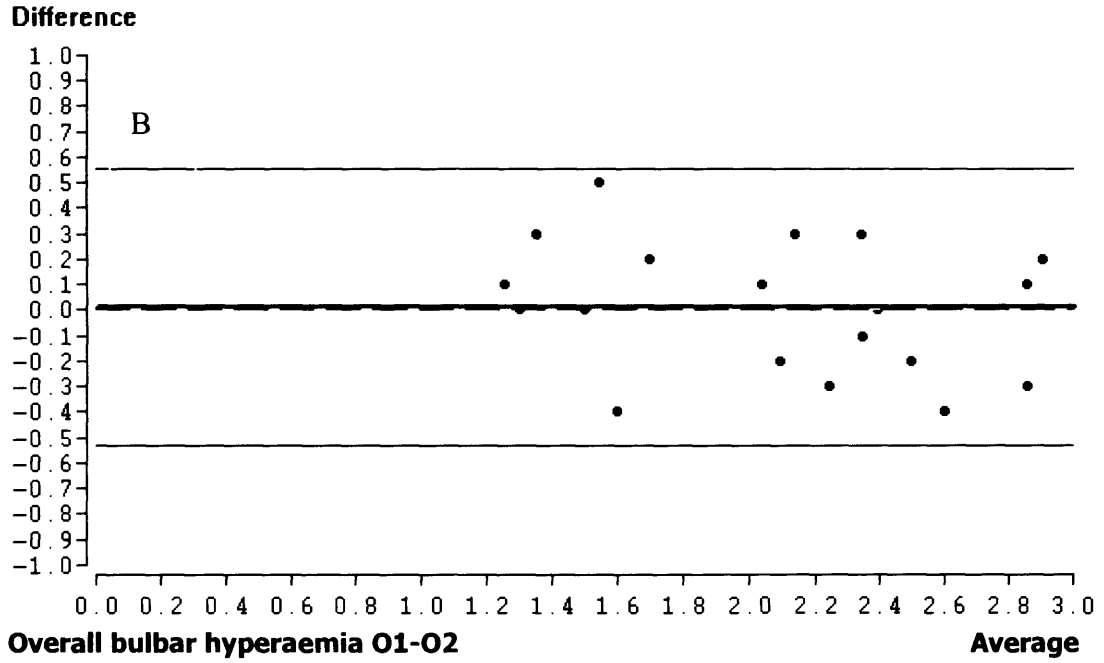
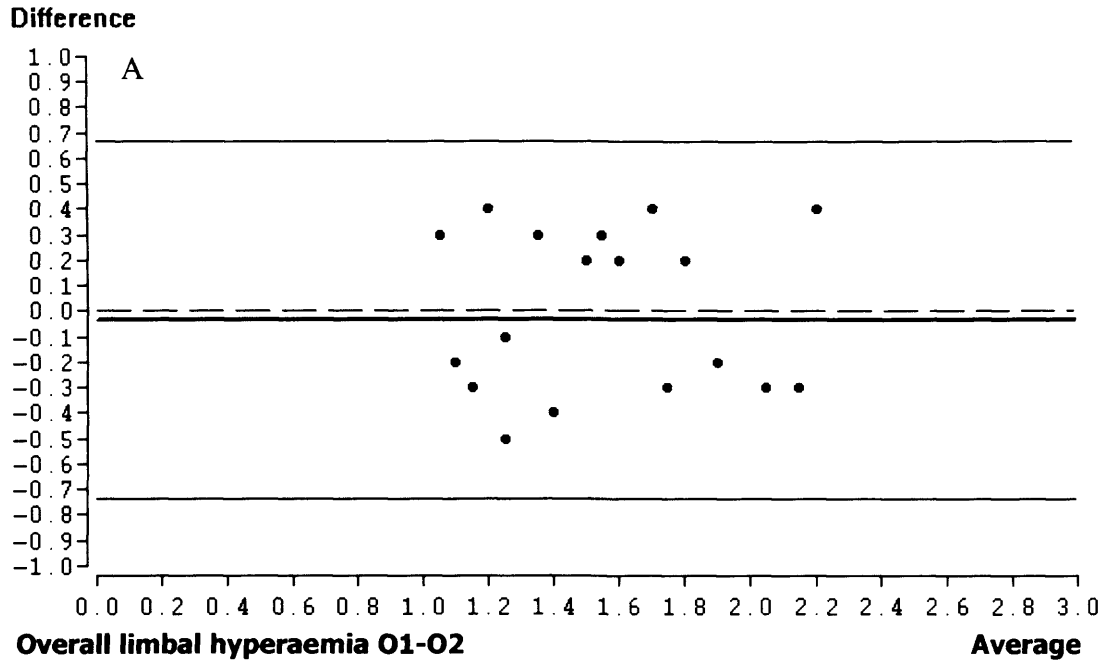


Figure 4.7: Inter-observer agreement (O1 - O2) of the first 20 patients. 95% limit of agreement in overall limbal hyperaemia (A) was 0.60 and overall bulbar hyperaemia (B) 0.50.



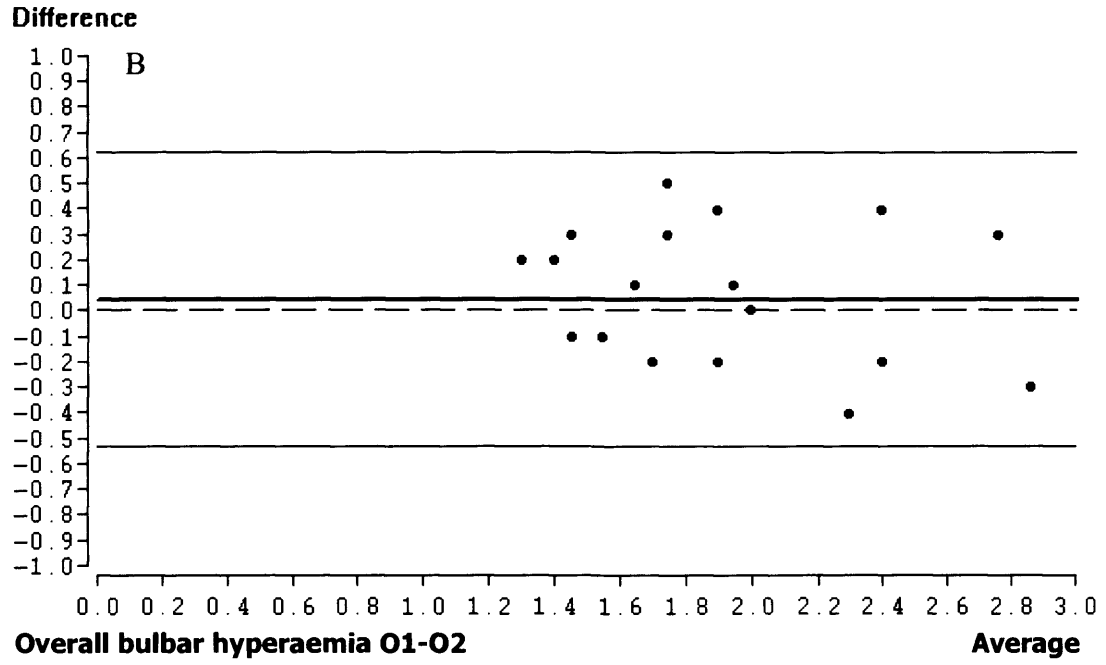
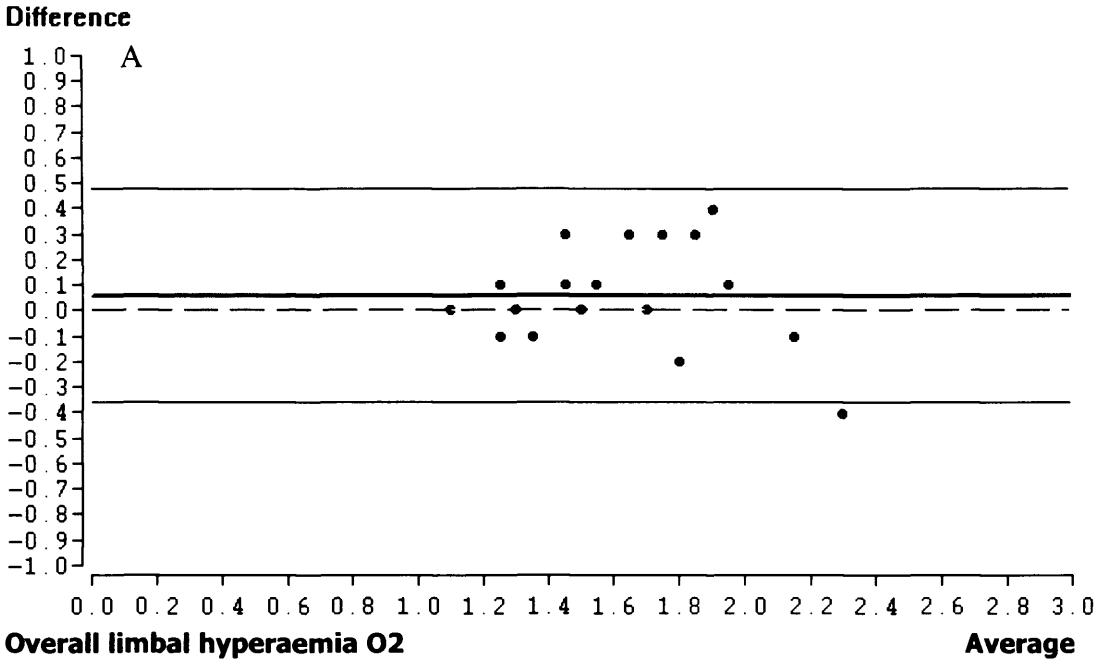


Figure 4.8: Inter-observer agreement (O1 - O2) of the last 20 patients. 95% limit of agreement in overall limbal hyperaemia (A) was 0.64 and overall bulbar hyperaemia (B) 0.53.

4.4.3 Intra-observer agreement study

The 95% agreement coefficient was 0.56/0.56 (O1/O2) units for overall bulbar redness, and 0.46/0.31 for quadrant-average bulbar redness, and 0.47/0.38 for overall limbal redness, and 0.30/0.31 quadrant-average limbal redness. The overall bulbar redness grade was 1.7/1.6 (± 0.34/0.36 sd) units, overall limbal redness grade was 1.7/1.6 (± 0.36/0.34 sd). No significant differences were found between the classification of bulbar as well as limbal redness in session one and two for overall grade zone average or for each quadrant (paired t-test $0.05>p>0.95$) (Figure 4.9 and 4.10). The power calculation of the completed intra-observer study resulted in a power of 1.00.



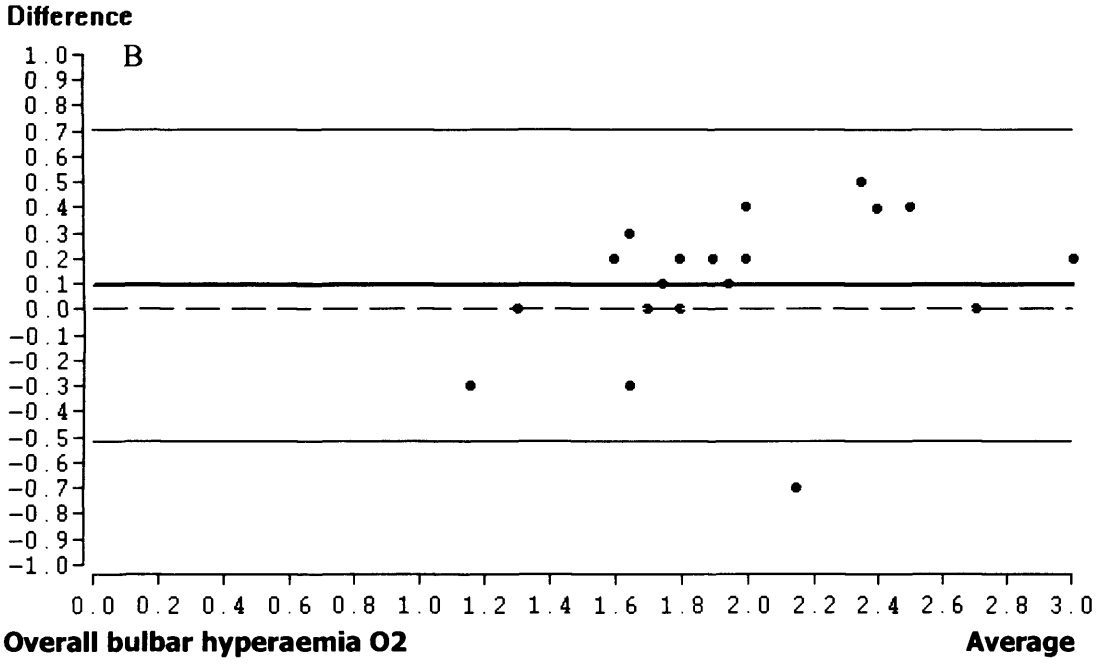


Figure 4.10: Intra-observer agreement of Observer 2 (Session 1 – 2). 95% limit of agreement in overall limbal hyperaemia (A) was 0.38 and overall bulbar hyperaemia (B) 0.56.

4.5 Discussion

This study has described the typical findings in normal subjects concerning limbal and bulbar hyperaemia, using the CCLRU grading scales. Firstly, the limbal redness grades were significantly lower than the bulbar redness grades, when assessed either by quadrant, quadrant-average or overall grade. Secondly, for bulbar hyperaemia, the overall redness grade was significantly higher than the quadrant average; while for limbal hyperaemia, there was no significant difference. Thirdly, the temporal and nasal quadrants were significantly redder than the superior and inferior quadrants for both limbal and bulbar hyperaemia. However, there were significant correlations between limbal and bulbar redness grades across all measured parameters.

The significant differences between limbal and bulbar redness may reveal either a consistent difference in the redness of these two ocular areas or it may be a feature of the grading scales used[221]. The review of current literature suggests that increased limbal redness, while an associated feature of bulbar redness, can also be produced separately by conditions associated with corneal stress[226,228,233-238]. This suggests that the control of vasodilation may be different between these two ocular areas and that, under normal conditions in healthy subjects, the baseline redness of the two areas may not necessarily be similar, even when there is no physiological stress. This is supported by the finding of moderate (albeit significant) correlations between the two areas (Figure 4.6). Thus, the difference between redness grades could have an underlying physiological basis.

However, it is more likely that the differences in redness grades are a feature of the CCLRU grading scales, and the correlation might also be weakened by subjects' grading variability, although this is likely to be a small amount. Each grading scale is represented by a series of four labelled, sample images, of progressing severity of condition. Grading scales are typically divided into four or five grades. Nevertheless, interpolating the scales into decimal intervals increases their sensitivity[268,269]. The selection of these images was by expert opinion, but the intervals between successive images may[311] or may not[314] be equal and the grading scales may not be aligned (i.e. same score for same level of severity as proposed by Woods[266]). Papas[311] reported, that by decimalising the CCLRU grading scale for bulbar redness, the grading approximated an interval scale. If scales are aligned, a single grading result can be more easily interpreted with respect to normal limits, as the user need not remember different confidence limits for different clinical appearances[221]. Differences between the appearances of normal healthy eyes for corneal fluorescein staining[220], palpebral conjunctival roughness[303], and bulbar conjunctival redness [232] shown in Figure 4.2, suggests that the CCLRU scales are not aligned. In other words, each clinical appearance of the CCLRU grading scale has an individual grade level for 'normal' that may not correspond with the 'normal' grade for other clinical appearances (Figure 4.2). A similar situation found in this study (Figure 4.5) suggests that the limbal and bulbar redness scales may not be inter-related (aligned). Also, the slope of the correlations between limbal and bulbar redness (Figure 4.6) may indicate that the two redness scales do not change at the same rate. Overall, this suggests that the authors of the CCLRU grading scale did not create grading scales with a universal scaling[266], despite the use of universal language for naming of the sample images. This study, and previous similar studies[220,232,303], show that each CCLRU grading scale has the potential to detect

changes in clinical appearance, but comparisons between the scales requires some form of calibration.

When considering the redness scores themselves, the quadrant-average bulbar redness scores compared well with a previous study of normal bulbar hyperaemia which also used the CCLRU grading scale[232]. The mean quadrant-average bulbar redness was 1.8 units, and the upper 95% confidence limit for normality was 2.6 units, while Murphy et al [232] found mean quadrant-average score of 1.9 units, and an upper 95% confidence limit of 2.6 units (Figure 4.1). The two distributions are slightly different (Kolomorogov-Smirnov, z=1.39, p=0.042), with eyes in this study tending to be slightly less red (t-test, t=1.87, p=0.06). In a previous study of 40 subjects, an average overall bulbar redness of 0.78 units and an upper 95% confidence limit of 2.3 units, using a 6-level grading scale, were found[260]. In this study, the mean limbal redness quadrant-average score was 1.6 units and the upper 95% confidence limit was 2.4 units. Thus from this study a bulbar redness score of greater than 2.6 units or a limbal redness score of greater than 2.4 units may be considered unusual, when derived from the quadrant average, using the CCLRU scales. Although the time of the day may influence the grade of normal hyperaemia, the time of observation in this study was restricted to office-hours (10:00am to 6:00pm), and ocular redness is reported to be relatively constant in that period[309].

However, the quadrant-average score is not the typical method of achieving a score, more commonly the clinician makes a single overall judgment of the redness, even though the images used on the CCLRU scale are of the temporal quadrant. For bulbar hyperaemia, this produced a significantly higher average redness score of 2.0 units, with an upper 95% confidence limit of 3.0 units. For limbal hyperaemia, the average overall

redness score was again 1.6 units, with an upper 95% confidence limit of 2.5 units. Magnification increases visibility of the conjunctival vasculature, so a person who when observed without a slit-lamp from 1m appears to have a white eye, will have a higher redness grade when viewed with a slit-lamp[232]. The CCLRU grading scale is commonly used during a slit-lamp examination[232,311,315]. This difference between quadrant-average and overall redness scores suggests that there was a difference in the grading criteria adopted by the two observers. When judging overall bulbar redness the less red superior and inferior quadrants were not visible, and thus the overall bulbar redness score may have been based on the redness of the nasal and temporal quadrants only. This hypothesis is supported by the lack of a significantly difference between the overall bulbar redness score and the average of the nasal and temporal quadrants (2.04 ± 0.46 units; post-hoc t-test: $t=1.47$, $p<0.14$). This effect was not seen for the overall limbal redness scores, possibly because it was easier to see more of the superior and inferior limbal regions when judging the overall limbal redness.

The third observation was that the temporal and nasal quadrants were redder than the superior and inferior quadrants. This is consistent with the findings of previous studies[232,233,259], possibly reflecting the greater exposure of these quadrants to environmental conditions.

The agreement between the quadrant-average redness grades found by the two observers (0.4 to 0.8 units) was comparable to similar studies that interpolated decimal (0.1 unit) increments of CCLRU grading scales[220,232,303], and was better than the inter-observer agreement of 1.0 units found when using a similar photographic grading scale that was not interpolated[260]. Bailey et al [268] described the benefits of using increments

that are related to the agreement between observations. As noted previously[220,303], the decimal interpolation of such grading scales can be learnt and applied effectively with only modest training by inexperienced observers[316,317]. The improvement in agreement for limbal nasal quadrant and quadrant-average at the end of the study may reflect just such a training effect for the observers in our study.

The intra-observer agreement demonstrates that the results of this study are reliable and the appearance of bulbar and limbal hyperaemia can be, and was, repeatably classified by trained observers using the CCLRU Grading Scale interpolated in 0.1 increments.

To our knowledge, this study was the first direct comparison of overall redness grading using the quadrant-average. As the agreement coefficients for overall redness were about twice as large as for quadrant-average redness, the additional effort required to grade each quadrant, then taking the average, may be worthwhile in the clinic and in research studies. This difference may explain some apparent differences in reported inter-observer agreement between studies of real eyes (that have used quadrant-average scores)[220,232,303] and studies of photographs of eyes (that have used overall scores)[264,311,318,319]. A difference of 0.3 units or more for quadrant-average redness, more than 0.6 units for limbal overall redness, and more than 0.5 units for bulbar overall redness, between two observations by two trained observers is likely to represent a real difference in the hyperaemia of that eye.

4.6 Conclusions

In conclusion, normal limbal redness appearance has been described for the first time. Although higher than expected, it has a lower grade than that for normal bulbar redness, which was observed to be similar in appearance to previous studies using the CCLRU grading scales. However, these higher redness grades observed for ocular hyperaemia are not necessarily due to a greater physical redness, but may be due to features of the grading scale used. Bulbar redness and limbal redness were inter-related, although the strength of this relationship is weakened by the poor alignment of the CCLRU grading scales.

For similar populations, a limbal redness above 2.5 may be considered abnormal. A bulbar redness above 2.6 (quadrant-average) or 3.0 (overall) may be considered abnormal. The good inter- and intra-observer agreement in this study demonstrates that the CCLRU allows reliable and repeatable grading by a group of trained individuals.

(The published form of this chapter can be found in the appendices)

5. Clinical Tests for Successful Contact Lens Wear: Relationship and Predictive Potential in Experienced Soft Contact Lens Wearers

5.1 Introduction

Contact lens wear comfort depends on a number of factors, including the interaction between the tear film and the ocular surface. Nearly half of contact lens wearers claim symptoms of dryness in lens wear[12]. Unfortunately, even though the primary reasons for discontinuing contact lens wear are dryness and discomfort[5], current clinical tests are barely able to predict these symptoms[13,14]. The literature review showed that tear film and ocular signs like hyperaemia, lid wiper epitheliopathy (LWE) and lid parallel conjunctival folds (LIPCOF) are important indicators of dry eyes in contact lens wearers. Relations between tear meniscus height and tear film stability (non-invasive break-up time (NIBUT)) and LWE and LIPCOF were investigated by prior studies[77,78,80,250,284]. Although LWE and LIPCOF are related clinical signs in contact lens-induced dry eye[77,80], relations between LWE and LIPCOF and pre-lens break-up time and the ocular surface are unknown.

LWE is a clinically observable alteration in the epithelium of the advancing lid margin, the lid wiper. In patients with dry eye, the thickness of the tear film is insufficient to separate the ocular surface and lid wiper[78]. Due to this deficiency, the lid wiper is subjected to trauma during the entire lid movement, as a result of the continual rubbing of the narrow surface area of lid wiper tissue against the corneal surface, including any contact lens[77,78].

LIPCOF are sub-clinical folds in the lateral, lower quadrant of the bulbar conjunctiva, parallel to the lower lid margin[79,80,239] (Figure 5.1), easily observable by slit-lamp.

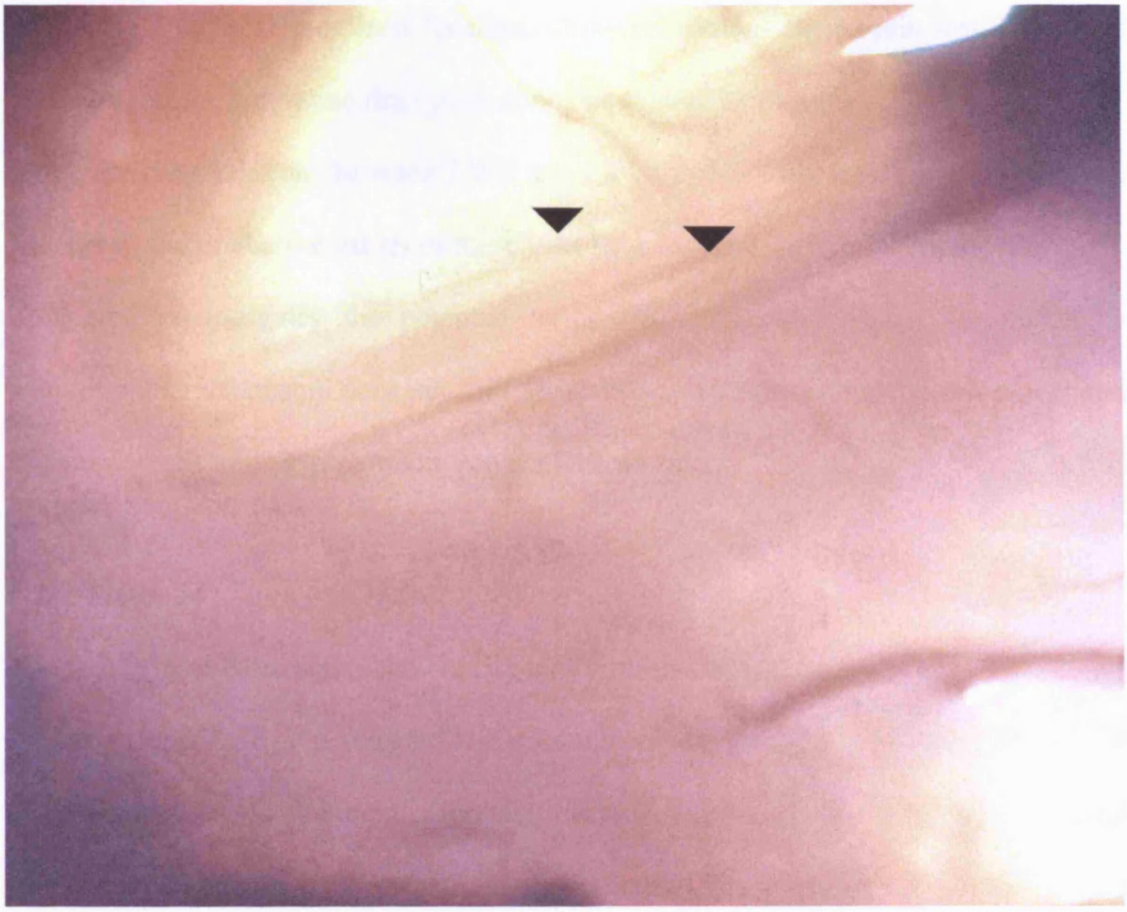


Figure 5.1: LIPCOF degree 2, two parallel conjunctival folds at the temporal quadrant of the eye.

Several causes of bulbar conjunctival folds are hypothesised: conjunctival 'looseness' as a result of inflammatory processes, a decrease of elastic fibres, aging, and lymphatic dilation by mechanical forces between the lower lid and conjunctiva that gradually interfere with lymphatic flow[207,240-244]. Bulbar conjunctival folds were first described by Hughes[245] and named conjunctivochalasis. Age does not appeared to be correlated with sub-clinical conjunctival folds[79]. Höh et al[79] described these as LIPCOF, as distinct from conjunctivochalasis, where an age-association was suspected by the authors. To avoid confusion, in this study LIPCOF refers only to sub-clinical

conjunctival folds at a defined location, observed without fluorescein instillation and used as a test for predicting dry eye in non- and contact lens wearers[79,80,243].

There are clear relations between LWE and LIPCOF, but their nature is still unknown. Moreover, the predictive values of these tests have not been reported in literature.

This study investigates the potential to predict contact lens wear discomfort by assessing the relationship between LIPCOF, LWE and standard clinical tests in a cohort of symptomatic and asymptomatic contact lens wearers.

5.2 Methods

The right eye of 61 experienced contact lens wearers (male=23, female=38; median age 29 years, range= 18-55), selected from volunteers attending the optometry practice of Horst Riede GmbH, Weinheim, Germany, were examined. The subjects were grouped into symptomatic and asymptomatic patients according to their response to the Contact Lens Dry Eye Questionnaire (CLDEQ)[119].

5.2.1 Inclusion and exclusion criteria

Subjects were excluded if they had any ocular/systemic pathology or allergy known to affect the conjunctiva, e.g. Sjögren's Syndrome, rheumatoid arthritis, diabetes, infections, hay fever, or if they were taking any medication known to affect the ocular surface or tear film. Subjects were also excluded if they had undergone ocular surgery or were pregnant. All subjects had worn soft monthly disposable lenses (24%-62% water content) for at least 6 months; high water content lenses were excluded. The lenses must have been worn for three weeks prior to the evaluation visit and used at least four times a week in normal wearing modality. Time of examination was between 3:00pm and 6:00pm. All procedures were conducted in accordance with the

Declaration of Helsinki (1983), and approval for the study was given by the Cardiff School of Optometry and Vision Sciences Ethics Committee. All subjects signed an informed consent form before participating in the study.

5.2.2 Techniques

Ocular hyperaemia

Limbal and bulbar hyperaemia of the ocular surface (summed quadrants) were evaluated by slit-lamp microscope (x12 mag) and classified using the four grades, interpolated in 0.1 increments of the CCLRU grading scale (Cornea and Contact Lens Research Unit, University of New South Wales, Sydney, Australia)[220,232,268].

Pre-lens tear break-up time (PLBUT)

PLBUT was determined non-invasively using a Tearscope (Keeler, UK Ltd) with a fine grid insert[202]. PLBUT was the time measured, in seconds, between the full opening of the eyelids after a complete blink and the first observed break in the tear film. Three consecutive readings eye were taken and the median noted.

Lid parallel conjunctival folds (LIPCOF)

LIPCOF was evaluated in the area perpendicular to the temporal and nasal limbus on the bulbar conjunctiva above the lower lid (temporal and nasal LIPCOF, respectively, Figure 5.2) with a slit-lamp microscope using 18 to 24 x magnification, as necessary. The grading score of Höh et al[79], adapted by Pult and Sickenberger[80] (Table 5.1) was employed. A further combined LIPCOF score (LIPCOF Sum) was calculated by adding together the nasal LIPCOF grade and temporal LIPCOF grade. Care was taken

to differentiate between parallel, permanent conjunctival folds (LIPCOF) and disrupted micro-folds or conjunctival flaps[159,248,249].

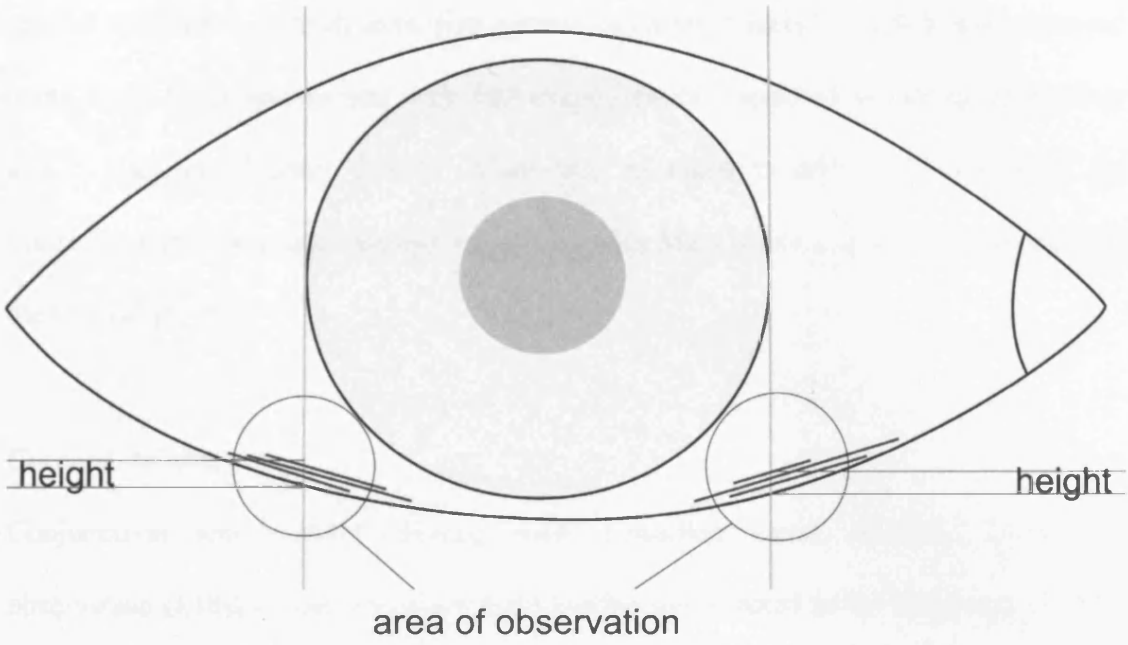


Figure 5.2: Areas of observation of temporal and nasal LIPCOF.

	LIPCOF Grade
No conjunctival folds **or** disrupted micro-folds in one line	0
One permanent and clear parallel fold **or** one permanent and clear parallel fold plus disrupted micro-folds above	1
Two permanent and clear parallel folds up to a height of 0.2mm **or** two permanent and clear parallel folds plus disrupted micro-folds above up to a height of 0.2mm	2
More than two permanent and clear parallel folds higher than 0.2mm **or** more than two permanent and clear parallel folds plus disrupted micro-folds above higher than 0.2mm	3

Table 5.1: Grading Scale of LIPCOF[80].

Lid wiper epitheliopathy (LWE)

LWE was made visible using a combination of instilled fluorescein and lissamine green (instillation separated by 1-2min), and evaluated for both upper and lower lids. A second instillation of both dyes was carried out after 5 mins[255]. LWE was observed using a slit-lamp microscope with 18x magnification classified according to Korb et al[77,78], also see Chapter 2.5.11. Care was to taken to differentiate between the fluorescein and lissamine staining associated with Marx's line and that from staining of the true lid wiper[77].

Corneal staining

Conjunctival and corneal staining were classified under slit-lamp microscope observation (x18-24 mag) using the four grades, interpolated in 0.1 increments, of the CCLRU grading scale[220,232,268]. Corneal and conjunctival staining were visualised using sodium fluorescein and lissamine green strips, respectively.

5.3 Statistical analyses

Since the data was ordinal and not normally distributed, non-parametric analyses were used, as appropriate, on WinSTAT 2005.1-Software (R Fitch Software, Bad Krozingen, Germany) and SPSS 14.0 (SPSS Inc. Chicago, USA). Correlations were calculated using Spearman Rank and differences were analysed by U-Test (Mann-Whitney). The validity of the Bonferroni correction for data analysed here is debated in the statistical literature[320-323]. Where significance at 5% is lost after applying the Bonferroni correction, this has been indicated. Predictive values were calculated for all significant clinical tests. The discrimination of the tests was evaluated by calculating the area under receiver operating characteristic curve (AUC).

5.4 Results

Thirty-eight subjects were classified as asymptomatic, and 23 as symptomatic.

Upper-lid LWE, temporal and nasal LIPCOF, and LIPCOF Sum severity scores were significantly increased in symptomatic patients ($0.01<p<0.03$) (Figure 5.3), whilst no significant differences were found between groups for lower-lid LWE, PLBUT, corneal staining or hyperaemia ($0.29<p<0.93$).

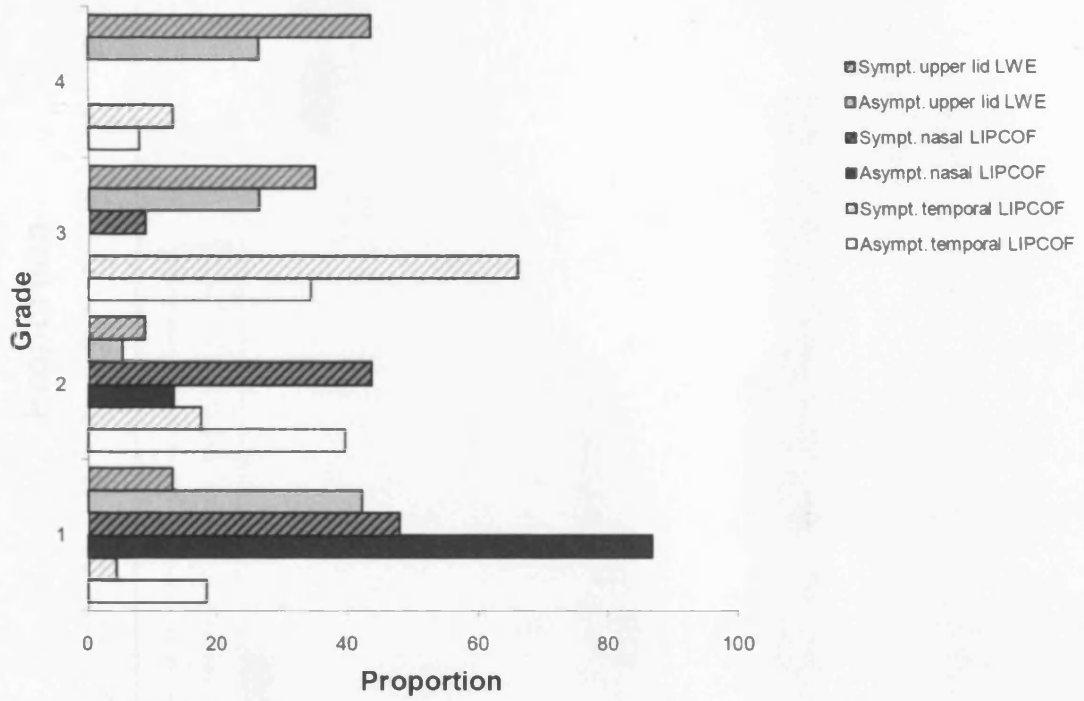


Figure 5.3: Distribution of LIPCOF and LWE grades in contact lens wearers.

Significant positive correlations were found between upper-lid LWE and LIPCOF scores (Figure 5.4 and Table 5.2). Lower-lid LWE was correlated to temporal, but not nasal LIPCOF. Upper-lid LWE, but not lower-lid LWE or temporal LIPCOF, was correlated to bulbar and limbal hyperaemia. Nasal LIPCOF was correlated to limbal hyperaemia, but not to bulbar hyperaemia. LWE scores and LIPCOF scores were not correlated to PLBUT or staining. LIPCOF was related to age (temporal $r=0.36$, $p<0.002$; nasal $r=0.45$, $p<0.001$). The power calculation of the completed study resulted in a power of >0.83.

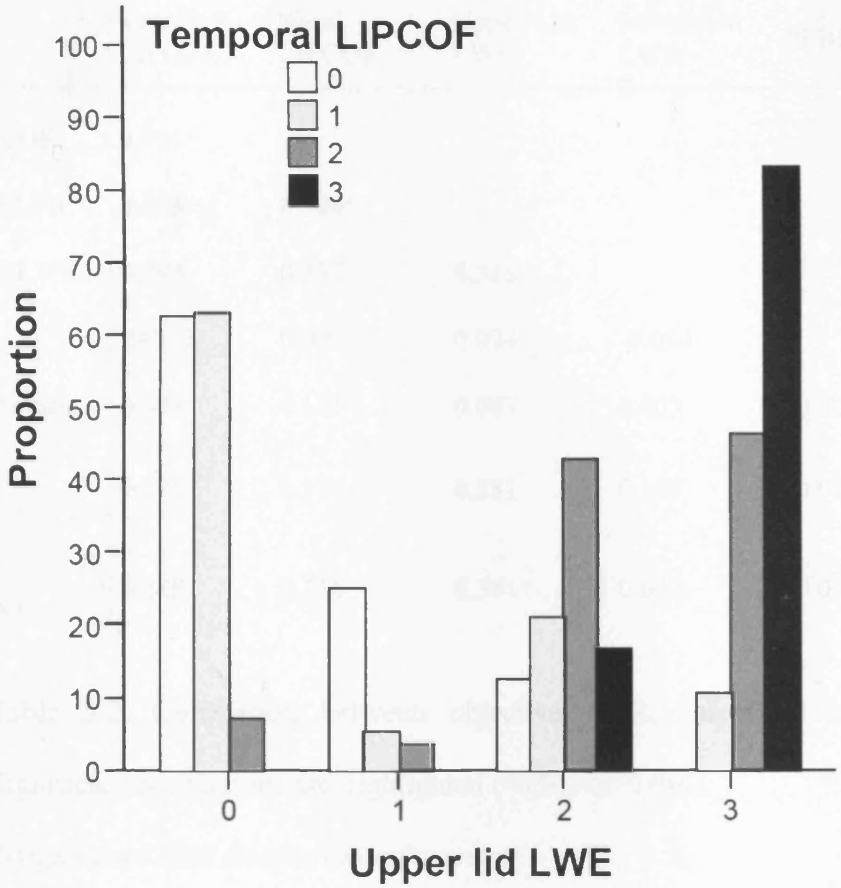


Figure 5.4: Relation between temporal LIPCOF grade and upper lid LWE.

	Temporal LIPCOF	Nasal LIPCOF	Upper-Lid LWE	Lower-Lid LWE	PLBUT	Corneal Staining	Bulbar Hyperaemia
Nasal LIPCOF	**0.511***						
Upper-Lid LWE	**0.675***	**0.390***					
Lower-Lid LWE	**0.296**	0.173	**0.315**				
PLBUT	-0.042	0.095	0.074	-0.050			
Corneal Staining	0.048	0.171	0.087	0.003	-0.022		
Bulbar Hyperaemia	0.175	0.174	**0.281**	0.107	-0.030	0.197	
Limbal Hyperaemia	**0.218**	0.116	**0.361***	0.007	-0.070	0.068	**0.739***

Table 5.2: Correlation between objective signs, calculated by Spearman Rank. Significant correlations are highlighted (<0.001<p>0.046).

**(significant after Bonferroni adjustment).*

The predictive values for symptoms of temporal LIPCOF were positive=56.9% (PPV; for a prevalence of 43% dry eye symptoms, Guillon at al[12]); nasal LIPCOF 74.9%, LIPCOF Sum 79.7% and LWE (upper lid) 53.1%. The AUC of temporal LIPCOF was 0.685, nasal LIPCOF 0.701, LIPCOF Sum 0.746, and LWE (upper-lid) 0.654 (Figure 5.5 and Table 5.3). Symptomatic patients were significantly older (p=0.049; 35.9 ±11.8 SD years) than asymptomatics (29.8 ±10.6 SD years), and there were more females in the symptomatic group (p=0.047).

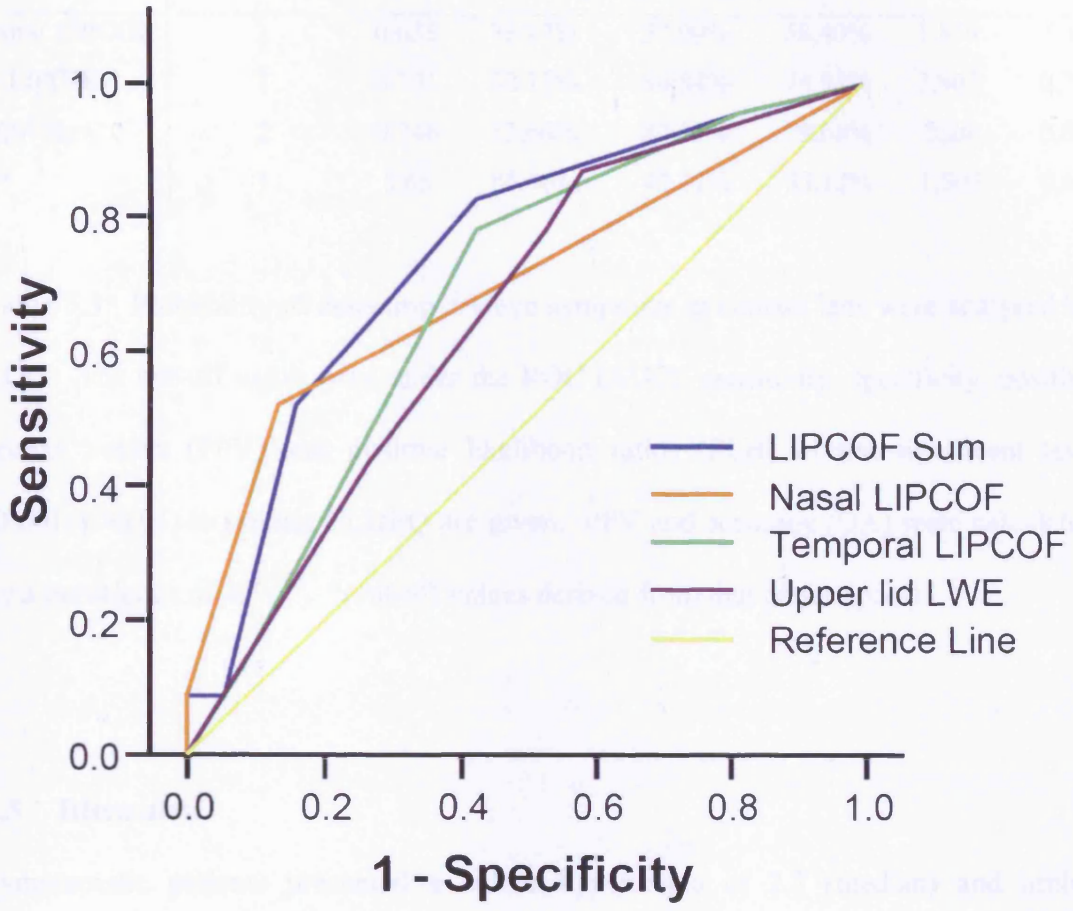


Figure 5.5: Probability of detecting dry eye symptoms in contact lens wear. The reference line is the line of non-discrimination and represents an AUC of 0.50.

	Cut-off value	AUC	Sensitivity	Specificity	PPV	PLH	Accuracy
Temporal LIPCOF	2	0.685	78.27%	57,90%	58,40%	1,859	0,667
Nasal LIPCOF*	1	0.701	52,17%	86,84%	74,94%	3,965	0,7193
LIPCOF Sum*	2	0.746	82,60%	84,11%	79,68%	5,20	0,8346
LWE*	1	0.65	86,96%	42,11%	53,12%	1,502	0,6140

Table 5.3: Probability of detecting dry eye symptoms in contact lens were analysed by ROC. The cut-off value, area under the ROC (AUC), sensitivity, specificity, positive predict values (PPV) and positive likelihood ratios (PLH) of the significant tests ($0.001<p>0.03$) to predict CLDEQ are given. PPV and accuracy (OA) were calculated by a prevalence of 43%[12]. *(cut-off values derived from that patient pool).

5.5 Discussion

Symptomatic patients presented a bulbar hyperaemia of 2.7 (median) and limbal hyperaemia of 2.2 (median) and asymptomatic patients presented grades of 2.6 and 2.2 for bulbar and limbal hyperaemia (median), respectively. This difference between groups is not statistical significant. According to prior investigation (Chapter 4), the bulbar hyperaemia grades in this study are borderline abnormal (greater than 2.6), but the limbal hyperaemia grades are not (greater than 2.5)[306]. This confirms previous studies which found that although pre-lens tear break-up time, corneal staining and hyperaemia are frequently reported signs of dry eye, their usefulness as predictors of the development of contact lens-induced dry eye is unclear[7,287,324].

In this study, no significant differences were found between symptomatic and asymptomatic lens wearers for these clinical signs. The pre-lens break-up time probably relates more to the surface properties of the lens than to individual lens

wearers[120,155,324,325]. As all subjects were experienced, successful, contact lens wearers, it may be assumed that extreme values for redness, staining, etc. would not be seen amongst such a population and, as such, significant differences and correlations may be less apparent.

In contrast, LIPCOF and LWE were significantly increased in symptomatic contact lens wearers. LWE of the upper-lid appears to correlate well with LIPCOF and hyperaemia, but not to corneal staining or pre-lens tear break-up time. No significant correlations were found between LIPCOF and bulbar hyperaemia, or staining and pre-lens tear break-up time.

The significant correlation between LWE and LIPCOF supports the suggestion by Watanabe et al[244] that both signs have a similar aetiology: induced by friction during blinking. There is evidence for direct contact of the marginal conjunctiva with the surfaces of the oculus bulbi[251,326,327]. Stratified squamous epithelium, which is seen in LWE, is a characteristic feature of other body tissues that experience frequent rubbing (e.g., cornea, skin, and oral mucosa)[78] and its presence in the particular region of the lid wiper[327] infers that the marginal conjunctiva is intimately and mechanically associated with the surfaces of the oculus bulbi.

As negative correlations between LIPCOF and non-invasive break-up time or tear meniscus height are reported[80], this friction may result from deficient tear film stability or volume. Tear volume may genuinely be reduced in cases with LIPCOF, or the tear film may be partly bound in the folds. However, improvements in tear film stability have been accompanied by a reduction in LIPCOF when phospholipid liposome eye sprays have been used in dry eye patients[328,329], suggesting that tear stability is a factor, and certainly inserting a contact lens reduces tear film stability[330]. However, pre-lens

tear film stability and LIPCOF were not significantly related in this study. It may also be reasonable to suggest that it is the mechanical influence of the lens edge that produces conjunctival folds in these cases, but no relationships between fitting criteria and different corneo-scleral profiles have previously been found[250].

Nevertheless, the relationships between LIPCOF and tear film stability, volume and LWE in contact lens wearers strongly points to LIPCOF being a result of mechanical forces during blinking: the bulbar conjunctiva will be stretched, rubbed and massaged during the blink, which, in turn, may result in an over-expansion or/and lymphatic dilation[244] that is visible as bulbar conjunctival folds. The resulting friction may also present as staining of the lid wiper[77,78], but further investigation is needed to determine which arises first, LWE or LIPCOF. The stronger relationship observed between temporal LIPCOF and LWE, than that with nasal LIPCOF, may result from the temporal bulbar surface presenting a larger surface area of exposed epithelium in most subjects, and thus may be more susceptible to drying that will further increase friction.

The results indicate a positive correlation between LWE and hyperaemia. Hyperaemia is the result of an increase in the volume of blood in the anterior scleral, bulbar conjunctival and limbal vessels, and occurs in response to inflammation, irritation and systemic disease[84,151,226-232]. It seems likely that irritation can be a factor in soft contact lens wear that may progress to inflammation. The lack of correlation between bulbar hyperaemia and LIPCOF suggests a progressive pathogenesis where LWE and redness increase, but LIPCOF is not seen until later in the inflammatory spectrum/processes.

Even though LWE and LIPCOF are significantly increased in symptomatic patients, for the researchers as well as for the clinician, it is important to know how predictable these tests are for contact lens-induced dry eye [331]. This can be analysed by the predictive values, which are produced from the sensitivity and specificity of the test; with these

depending on the cut-off value of the test and the prevalence of the syndrome itself. For this study, the cut-off values used to discriminate asymptomatics from symptomatics were determined from the data pool, except for temporal LIPCOF which was taken from previous research[80]. As the fine calculation of cut-off values is an iterative process, the predictability of these tests was additionally clarified by the receiver operating characteristic curve (ROC).

While Schirra et al reported the importance of evaluating LIPCOF at the lateral quadrant of the eye close to the lower lid[80,239], in this study the nasal LIPCOF was also a good predictor for contact lens-induced dry eye. Indeed, the sum of temporal and nasal LIPCOF has a higher predictive value than regional LIPCOF scores, or the other objective signs. Thus it is proposed that LIPCOF Sum can be included as an improved test to predict contact lens-induced dry eye. In contrast, despite LWE being significantly increased in symptomatic lens wearers, the predictive values indicate that LWE, with lissamine green as a second dye serves better to exclude dry eye symptoms. These outcomes are confirmed by the ROC analyses, which indicate that while LWE and LIPCOF are significant predictors of contact lens-induced dry eye, LIPCOF Sum is the best, as reflected in the greatest AUC (0.746). Since there are differences of opinion on whether thin high-water content lenses are associated with patient comfort and ocular signs ([7,133,332] vs[9]), further study is required to extend the investigation of these relationships in high-water content contact lens wearers. Nevertheless, using a set of tests to diagnose dry eye symptoms in contact lens wearers might increase the predictability and should be considered in further studies.

5.6 Conclusions

In conclusion, this study has shown that symptomatic, experienced, soft contact lens wearers exhibit significantly more LWE and LIPCOF, but not corneal staining, bulbar hyperaemia or decreased PLBUT. LWE and LIPCOF are significantly correlated, suggesting that LIPCOF results from friction during blinking. Among contact lens wearers, older women are more likely to present symptoms. LIPCOF Sum appears to be more predictive of symptoms than other clinical tests.

These results give rise to several further questions:

1. Can the model of "friction in blink" be confirmed by laboratory tests, e.g. analyses of the mucin layer of the tear film, since mucin are pivotal in lubrication of the ocular surface[38,122]?
2. Can similar relations (objective and subjective) been seen in non-contact lens wearers?
3. How do these tests perform in prediction of symptoms of dryness in naïve soft contact lens wearers?

(The published form of this chapter can be found in the appendices)

6. Mucin and Ocular Signs in Symptomatic and Asymptomatic Contact Lens Wearers

6.1 Introduction

The Chapter 5 study confirmed that lid wiper epitheliopathy (LWE) and lid parallel conjunctival folds (LIPCOF) are predictive clinical signs in contact lens-induced dry eye[77,80,333]. LWE is an alteration in the epithelium of the advancing lid margin, the lid wiper, caused by friction during lid movement[77,78]. LIPCOF are sub-clinical folds in the lateral, lower quadrant of the bulbar conjunctiva, parallel to the lower lid margin[79,80,239], easily observable with a slit-lamp.

Since the previous study (Chapter 5) found significant correlations between LWE and LIPCOF, it may be logical to assume these two clinical signs share the same aetiology - frictional mechanical forces in blink[333]. Therefore this study seeks to investigate whether the "friction in blink" model can be confirmed by analyses of the mucin layer of the tear film. Surface mucins lubricate and anchor the tear film to surface epithelia. Further protection from friction is provided by shear thinning[31]. Lubrication of the ocular surface is one of the functions ascribed to mucins[38,122] and relations between mucins and LWE and LIPCOF might confirm they share the same aetiology, friction in blink.

Changes in the quantity or quality of mucins are also among the likely causes of ocular discomfort, because of the biophysical characteristics of fluids are affected by these components. It is not clear whether mucin (MUC) species composition, or their glycosylation or quantity affect comfort. Changes in the composition or quantity of the pre-ocular fluid, as a result of excessive evaporation, hyperosmolarity[104,121], decreased tear clearance, or changes in the morphology of the ocular surface epithelia, might all influence the comfort of wearing contact lenses.

In this chapter the relationship between anatomical changes, mucins and ocular discomfort in contact lens wear is considered. To determine any relationship between the composition of surface and lens-adherent mucins and dry eye symptoms in contact lens wearers, mucins on worn soft contact lenses, and from the ocular surface of the same individual, were analysed in respect to the gene products present and mobility on electrophoresis. The latter is determined by the distribution of size and or size-charge ratios for each MUC species. It has been shown that mucins adherent to contact lenses can be analysed at the level of gene product and molecular characteristics[334]. In LIPCOF or LWE there is an assumed failure of tear film protection. In this study we are evaluating whether this failure is reflected in mucins from the individual ocular surface.

6.2 Methods

Fifty experienced contact lens wearers (male=19, female=31, median age = 30 years, range = 18-55), selected from volunteers attending the optometry practice of Horst Riede GmbH, Weinheim, Germany, were examined. The subjects were grouped into symptomatic (n=19) and asymptomatic (n=31) patients according to their response to the Contact Lens Dry Eye Questionnaire (CLDEQ)[119].

6.2.1 Inclusion and exclusion criteria

All subjects included in the study had worn hydrogel monthly disposable lenses (24%-62% water content) for at least 6 months, and for three weeks prior to the evaluation visit used at least four times a week in normal wearing modality. Time of examination was between 3:00pm and 6:00pm.

Subjects were excluded if they had any ocular/systemic pathology or allergy known to affect the conjunctiva, e.g. Sjögren's Syndrome, rheumatoid arthritis, diabetes,

infections, hay fever, or if they were taking any medication known to affect the ocular surface or tear film. Ocular surgery and pregnancy were also exclusion criteria.

All procedures were conducted in accordance with the Declaration of Helsinki (1983), and approval for the study was given by the Cardiff School of Optometry and Vision Sciences Ethics Committee. All subjects signed an informed consent form before participating in this study.

6.2.2 Technique

Symptoms

Comfort was evaluated using the Contact Lens Dry Eye Questionnaire (CLDEQ).

Lid parallel conjunctival folds (LIPCOF)

LIPCOF was measured in the right eye, in an area perpendicular to the temporal and nasal limbus on the bulbar conjunctival above the lower lid[333], using the grading score of Höh et al[79], adapted by Pult and Sickenberger[80]. A further combined LIPCOF score (LIPCOF Sum) was calculated by adding the nasal and temporal LIPCOF grades.

Lid wiper epitheliopathy

LWE was visualised with fluorescein and lissamine green (instillation separated by 1-2min), with a second application five minutes after the first[255]. This method was chosen to reflect common clinical practice, and because in evaluating LWE the frequently of instillation is more important than the volume of dye[255]. LWE was evaluated for upper and lower lids, using a slit-lamp microscope and classified according to Korb[77,78], also see Chapter 2.5.11. Care was taken to differentiate between the fluorescein and lissamine staining associated with Marx's line and that from staining of the lid wiper[77].

Corneal staining

Conjunctival and corneal staining were classified under slit-lamp microscope observation (x18-24 mag) using the four grades, interpolated in 0.1 increments, of the CCLRU grading scale[220,232,268]. Corneal and conjunctival staining were visualised using sodium fluorescein and lissamine green strips, respectively.

Sample collection and extraction of adherent material

Mucus collected from the normal ocular surface, as well as other mucosal surfaces, contains some mucins that require pre-treatment to elute in aqueous buffers[16, 21, 22]: the latter are necessary for mucin analysis. Earlier study indicated that mucins adhering to contact lenses require a similar treatment[334]. Although the significance of the "insoluble" fractions is not clear, they are part of the physiological mucin complement. For these reasons two extractions were performed, as described below, to ensure that the entire mucin complement of the ocular surface was analysed.

Ocular surface fluid samples were obtained by gently pressing Schirmer strips onto the temporal bulbar conjunctiva. Contact lenses were collected from each subject after 4 weeks of daily wear. Strips and contact lenses were individually stored at -20°C until analysed.

Each lens was extracted with a 3:1 mixture of 4M Guanidinium Chloride (Sigma, Poole, UK) with protease inhibitors and RIPA buffer (Sigma). RIPA buffer was used to extract adherent material from Schirmer strips. A second extraction, with the addition of dithiotreitol (DTT), was used to solubilise mucins from any remaining macromolecular assemblies. Reactivity with antibodies against mucin peptide core epitopes (Table 6.1) was probed in dot-blots on PVDF membranes (Immobilon-P, Millipore, Watford, UK), and in Western blots after electrophoresis. After incubation

with appropriate secondary antibodies, reactivity was visualised with either a colour substrate (DAB or BCIP/NBT as required, Sigma. Poole, UK) or with a fluorescent substrate (Duo-Lux, Vector Laboratories, Peterborough, UK). Images were acquired on a UVP High Performance Transilluminator (Ultra-Violet Products Ltd., Cambridge, UK) and quantified with LabWorks4 (UVP). For large mucins, electrophoresis was performed on 1% agarose, for 4h at 60V, followed by vacuum blotting for 1.5h on Immobilon. The smaller mucins were evaluated on 4-12% NuPAGE® Novex Bis-Tris gels (Invitrogen, Paisley, UK), after electrophoresis for 35 min at 200V[23] and semi-dry blotting (Trans-Blot SD, BioRad Laboratories, Hercules, Ca., USA) on Immobilon.

Antibody	Mucin	Epitope	Source	Control
BC2	MUC1	VNTR	Santa Cruz	conjunctiva
P-18	MUC2	near C terminus	Santa Cruz	blocking peptide
P-20	MUC4	N terminus	Santa Cruz	blocking peptide
45M1	MUC5AC	near C terminus	Sigma	gastric mucin
CLH2	MUC5AC	VNTR	Santa Cruz	
Man5BIII	MUC5B	nonVNTR	D Thornton, Manchester	saliva
G-16	MUC5B	N terminus	Santa Cruz	blocking peptide
EurMUC7a	MUC7	histatin-like domain	D Swallow, London	saliva
V-20	MUC7	internal	Santa Cruz	blocking peptide
CA125	MUC16	glyco-epitope	Dako	saliva
N-20	MUC16	N terminal	Santa Cruz	blocking peptide

Table 6.1: Antibodies to mucins used to assess the reactivity of mucins in extractions from strips and contact lenses. Blocking peptides (all from Santa Cruz) inhibited the reactivity of each antibody at the concentration used, but did not abolish it (except for MUC7, not shown). Control saliva was collected from 4 healthy individuals, centrifuged and separated from the microbial pellet.

6.3 Statistical analyses

Where data was ordinal or not normally distributed, non-parametric analyses were performed, using WinSTAT 2005.1 (R Fitch Software, Bad Krozingen, Germany) and SPSS 14.0 (SPSS Inc., Chicago, USA). Correlations were calculated using Spearman rank analysis, and differences by Mann-Whitney U-Tests. When data has been normalised (as proportions or percentages) ANOVA and post-hoc tests were performed on Prism4 for Macintosh, (Graph Pad, San Diego, California, USA).

6.4 Results

All mucin species described at the ocular surface could be detected adhering to contact lenses and Schirmer strips. These are: MUC1, MUC4 and MUC16 associated with the cell surface, and MUC2, MUC5AC, MUC5B and MUC7 that are secreted mucins, with origins in either conjunctiva, cornea or lacrimal glands (for recent reviews see[24, 25]). Not all species were detected in every extraction. For example, in the first extraction, MUC7 could not be detected on 23% of impressed strips, and 27% of contact lenses, though not both from the same individual; MUC5AC was below detection in 28.1% of contact lenses from asymptomatics, and in 36.8% of lenses from wearers with dry eye symptoms. The proportions of lenses that were positive for mucins were different for asymptomatics and wearers with dry eye symptoms ($p=0.047$, Friedman analysis of variance), and there was also a significant difference when analysed by mucin species ($p=0.0016$, two-way ANOVA on percentage positive contact lenses), as shown in Table 6.2. Dithiotreitol has been used to free mucins from macromolecular aggregates[335]. As in previous studies[16, 18], extraction with DTT yielded more mucins. For asymptomatics, 17.4% of lenses that were positive for MUC5AC in the first extraction were negative in

the second, as opposed to only 7.7% lenses from contact lens wearers with dry eye symptoms. In contrast, 34.9% and 38.5% of lenses (asymptomatics and dry, respectively) were positive for MUC5AC after the DTT extraction, though negative in the first, suggesting that this mucin adhered to lenses in a manner that prevented it from eluting native in aqueous solutions. The power calculation of the completed study resulted in a power of >0.75.

	MUC16	MUC1	MUC2	MUC4	MUC5AC	MUC5B	MUC7
1st extraction							
CLDEQ-dry	92.31	30.77	38.46	23.08	46.15	84.62	46.15
asymptomatics	95.65	56.52	56.52	21.74	65.22	91.30	78.26
DTT extraction							
CLDEQ-dry	100	100	92.31	92.31	61.54	100	100
asymptomatics	100	100	95.83	87.5	79.17	100	100

Table 6.2: Percentage of positive contact lenses for each mucin species in extractions from the same lenses. The second extraction, in the presence of dithiothreitol, dissolved material in macromolecular aggregates.

6.4.1 Distribution of reactivity with mucin antibodies

To compare the proportions of different mucin species in individuals, reactivity with each mucin antibody was expressed as a percentage of the summed reactivities in that extraction. Schirmer strips impressed on the ocular surface yielded first-extraction mucins in similar proportions, except for MUC7, which represented a much smaller proportion of the total reactivity (Figure 6.1A). Mucin proportions were similar in

asymptomatic and patients with dry eye symptoms (two-way ANOVA with repeated measures, p= 0.97).

The second extraction (DTT) from Schirmer strips was comprised largely of MUC1, MUC5AC and MUC4, while MUC2, MUC5B, and MUC16 represented less than 0.5% each of the total mucin population (Figure 6.1B, C). The proportions of mucin species solubilised by DTT were not different in the two groups (two-way ANOVA with repeated measures, p=0.61), nor could any interaction be detected between the proportion of a given mucin and patient classification by the dry eye questionnaire CLDEQ. Thus, at the ocular surface, some of the mucin species are easily soluble in aqueous buffers, while MUC1, MUC5AC and MUC4 are also found in "insoluble" complexes.

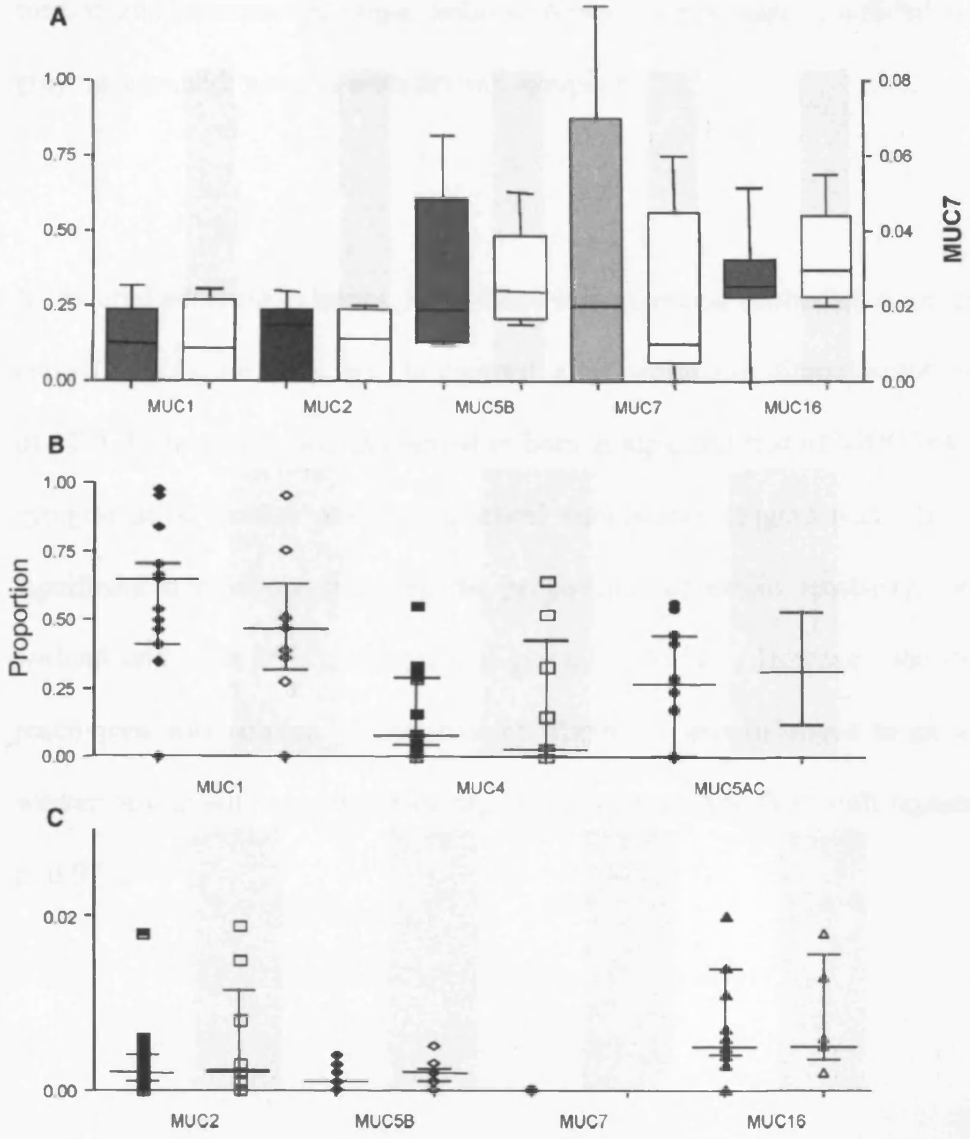


Figure 6.1: Proportions of mucin species in extractions from impressed strips. Each strip was extracted and analysed individually: conditions and reagents were constant in all analyses. A. First extraction: all mucins except MUC7 are expressed in relatively equal proportions (median and interquartile range). MUC7 proportions are shown on the right Y scale. B. and C. Extraction with dithiothreitol: MUC1, MUC4 and MUC5AC encompass most of the reactivity (B); while MUC2, MUC5B, MUC7 and MUC16 represent a small fraction only (C). Data presented as aligned scatter graphs, lines

median and interquartile range. Solid symbols: asymptomatics; unfilled symbols, light grey background: patients with dry eye symptoms.

In material adherent to lenses, reactivities with all mucin antibodies were similar, except antiMUC7 (Figure 6.2A, B). In contrast, after addition of dithiothreitol, the proportion of MUC16 reactivity was decreased in both groups, and that of MUC5AC increased in symptomatics: neither reached statistical significance (Figure 6.2C, D). There were significant interactions between the proportions of mucin reactivity, extraction (i.e. without and with DTT), and patient group ($p<0.001$). However, the distribution of reactivities was similar, irrespective of whether a lens belonged to an asymptomatic wearer or one with symptoms of dry eye, (two-way ANOVA with repeated measures, $p=0.077$).

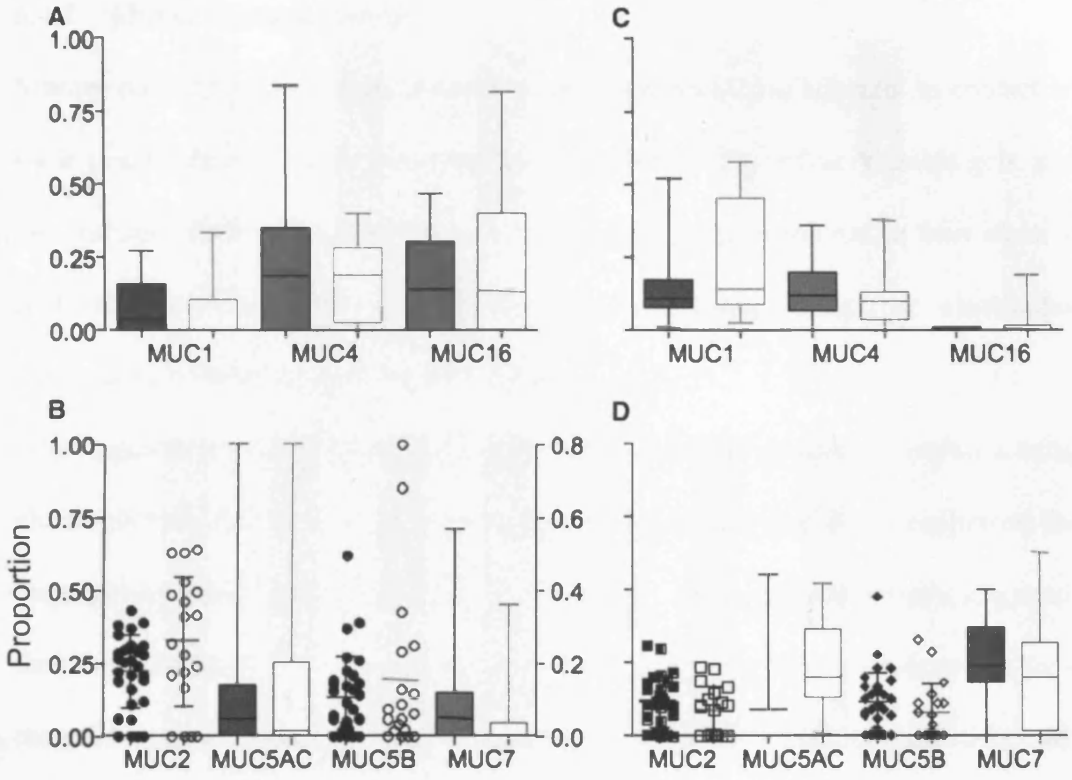


Figure 6.2: Distribution of mucin species in extractions from contact lenses. The same antibodies and conditions were used to probe reactivities after each extraction; each contact lens was extracted and tested individually. Mucin species distribution is similar in lenses from asymptomatic or symptomatic patients (A, B first extraction and C, D extraction with dithiothreitol). Note the much smaller proportion of MUC16, and increased MUC7 in the DTT extraction. MUC7 reactivity corresponds to the right Y scale; solid symbols: asymptomatic patients; unfilled symbols: patients with dry eye symptoms. Bars on scatter distributions represent means ± standard deviations. Box and whiskers plots show median and inter-quartile ranges.

6.4.2 Mucin characteristics

Mucins on the ocular surface, sampled with strips, and those adherent to contact lenses were characterised by their electrophoretic mobilities. On polyacrylamide gels, such as the NuPage gels used in this study, molecules migrate proportional to their sizes: small molecules migrate further than larger ones. Mobility on agarose electrophoresis depends on both molecular size and charge.

After agarose electrophoresis, MUC5AC from impression strips revealed a range of electrophoretic mobilities, indicative of different size-charge ratios, as expected for this large gel-forming mucin (not shown). MUC5AC from lens extractions migrated less than the 250KDa molecular weight marker in agarose gels, with glycoforms in the same range in asymptomatics as in patients with dry eye symptoms (Figure 6.3). MUC4 mobility on NuPage gels was surprisingly high (Figure 6.4A), with either a single band or doublet around 40KDa, indicating proteolytic cleavage of the molecule. MUC4 in saliva, run on the same gel, shows a single or a doublet of bands of much lower mobility. MUC7 resolved in a doublet of bands of low mobility (around 150KDa), similar to saliva controls (Figure 6.4B).

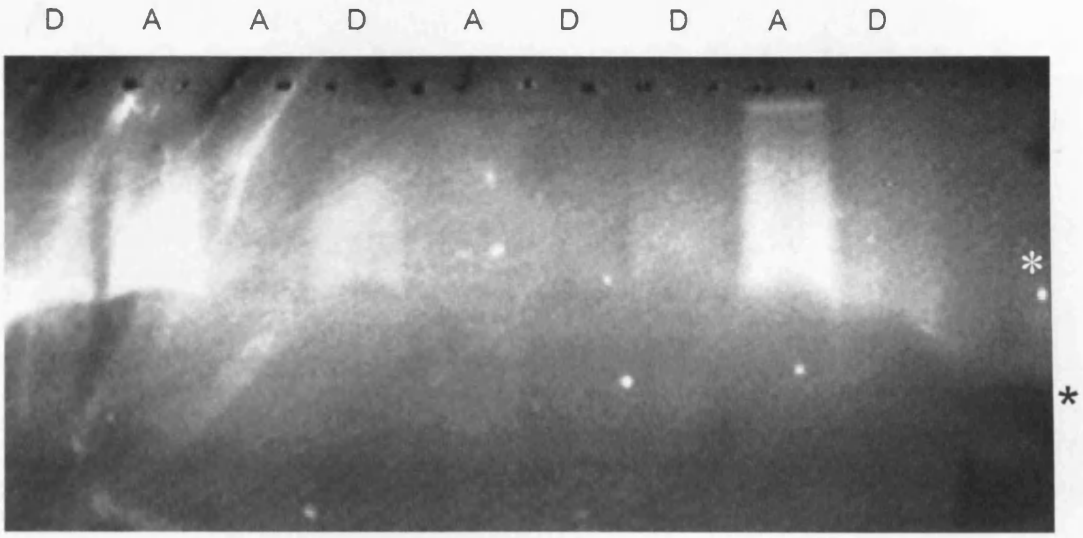


Figure 6.3: Electrophoretic mobility of MUC5AC; vacuum bolts after electrophoresis on 1% agarose. (D) indicates samples from patients with dry eye symptoms, (A) indicates samples from asymptomatic patients. All MUC5AC on lenses migrated less than the 250KDa molecular weight marker (*). The smear, here visualised with Duo-Lux fluorescent substrate, denotes the presence of multiple glycoforms. Agarose gels separate molecules according to their size/charge ratios, most mobile are the most charged mucins relative to their size. Overall mobility ranges were similar in asymptomatics and dry eye patients. The frown across the gel indicates a high protein content of the extraction. Separating mucins from other proteins will have left insufficient material for analysis.

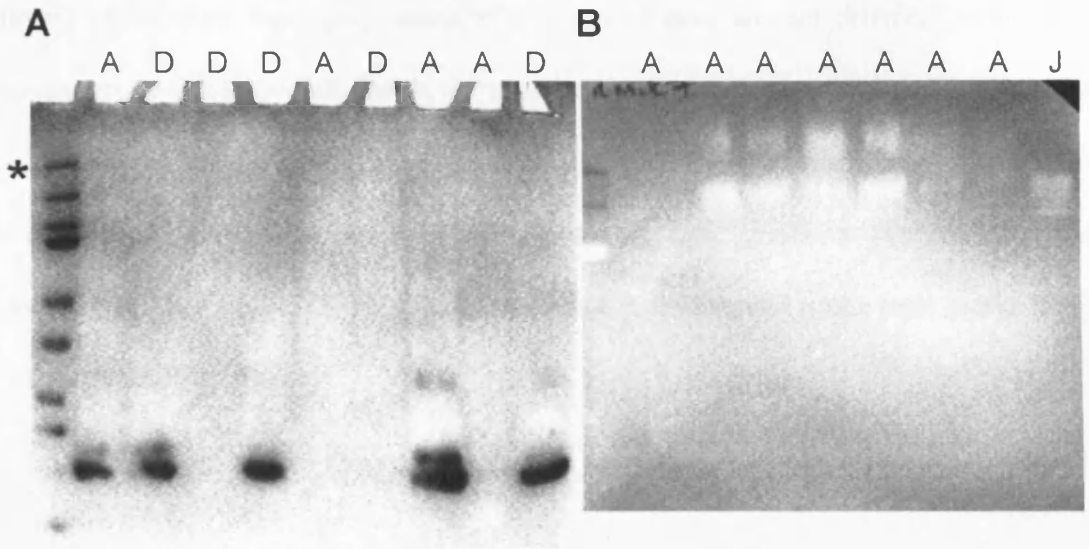


Figure 6.4: Electrophoretic mobility of smaller mucins was not affected by dry eye symptoms. Semi-dry blots of MUC4 and MUC7 from impressed strips after electrophoresis on NuPage Bis-Tris gels. (D) indicates samples from patients with dry eye symptoms, (A) indicates samples from asymptomatic patients.

A. MUC4 migrated far into the gel, sometimes as two close bands equivalent to less than 20KD molecular weight marker. This is an unexpectedly high mobility for a mucin. A less mobile band was observed only occasionally. Reactivity was visualised with BCIP/NBT.

B. MUC7 consistently migrated as two distinct bands, one above and one below 250KD in impressed strips. Conjunctival extractions (J) show a single band of similar mobility with the more mobile MUC7 glycoform extracted from strips. Reactivity visualised with Duo-Lux fluorescent substrate.

Molecular weight markers in the first lane of both blots are: 250(*); 150; 100; 75; 50; 37; and 25KD.

Having established that gross characteristics of mucins are not different in the two patient groups, the overall levels of mucins in the two groups of patients can be addressed. More mucin adhered to contact lenses in asymptomatics: however, mucins in aggregates were increased in wearers with dry eye symptoms (Figure 6.5), and especially MUC4 and MUC16. This pattern was not observed in the total mucin lifted by the impressed strip.

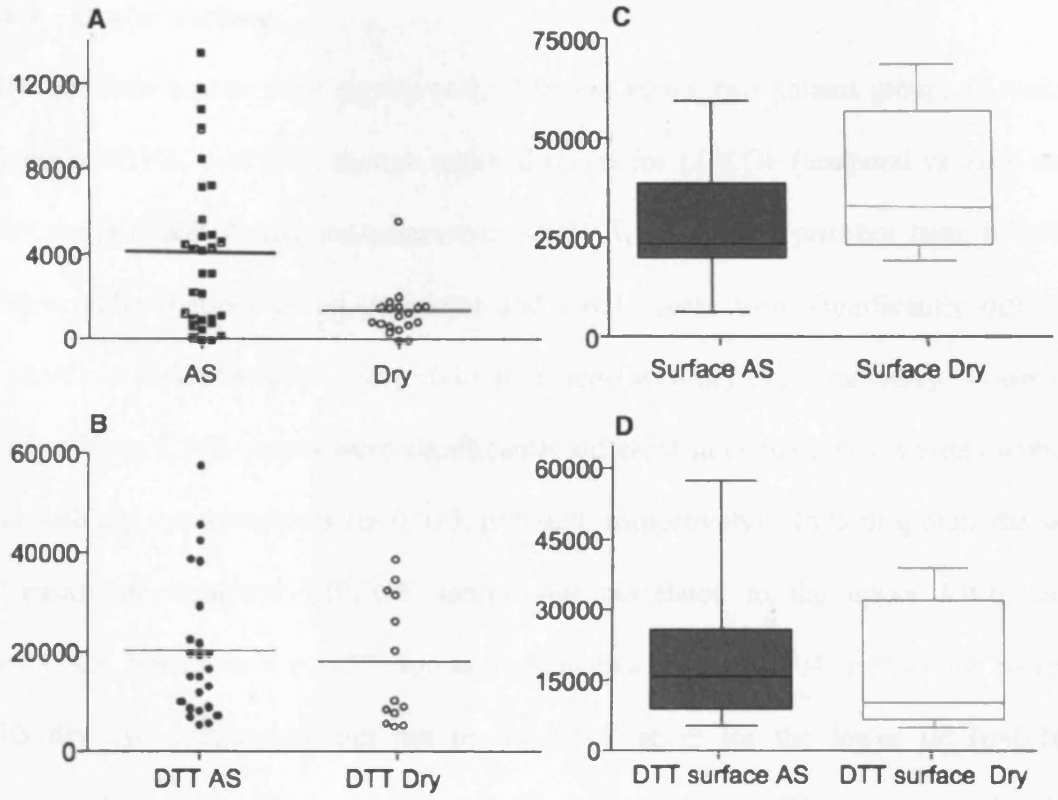


Figure 6.5: Total mucin adhering to contact lenses. Mucin concentration expressed as intensity of reactivity with anti-mucin peptide-core antibodies (integrated grey pixels).

A and B. Extractions from the same contact lenses without (A) and with DTT solubilisation of macromolecular aggregates.

C and D. Extraction from the same strips impressed on the conjunctiva, without (C) and with DTT solubilisation of macromolecular aggregates.

Filled symbols: asymptomatics; open symbols: patients with dry eye symptoms.

6.4.3 Ocular surface

LIPCOF Sum scores were significantly different in the two patient groups (Kruskal-Wallis ANOVA, $p<0.001$), though regional scores for LIPCOF (temporal vs nasal etc.) were not (Kruskal-Wallis non-parametric ANOVA, and Dunn's post-hoc tests, $p>0.05$). Within either patient group, temporal and nasal scores were significantly different ($p<0.001$ in asymptomatics, and $p<0.01$ in patients with dry eye symptoms). Upper lid, but not lower LWE scores were significantly different in contact lens wearers without and with dry eye symptoms ($p<0.035$, $p<0.929$, respectively). In both groups the sum of nasal and temporal LIPCOF scores was correlated to the upper LWE score ($p=0.0005$, Spearman's $r=0.57$, for asymptomatics, and $p=0.004$, $r=0.73$ for patients with dry eye symptoms), but not to the LWE score for the lower lid ($p=0.165$, Spearman's r=0,18, and, $p=0.091$, $r=0.39$ respectively). MUC4 was correlated to temporal LIPCOF and LWE, ($p<0.01$, Spearman's $r=-0.47$ and -0.46), while MUC16 and MUC5AC correlated with corneal staining (Spearman's $r=-0.36$ and -0.53; $p<0.04$). These correlations are, however, not significant after Bonferroni correction.

When the patients were ordered first by LIPCOF scores, and then by LWE scores, a clear relationship emerged between mucin levels and severity of scores. This relationship was also seen in the asymptomatic group, but not in the group of contact-lens wearers with dry eye symptoms (Figure 6.6).

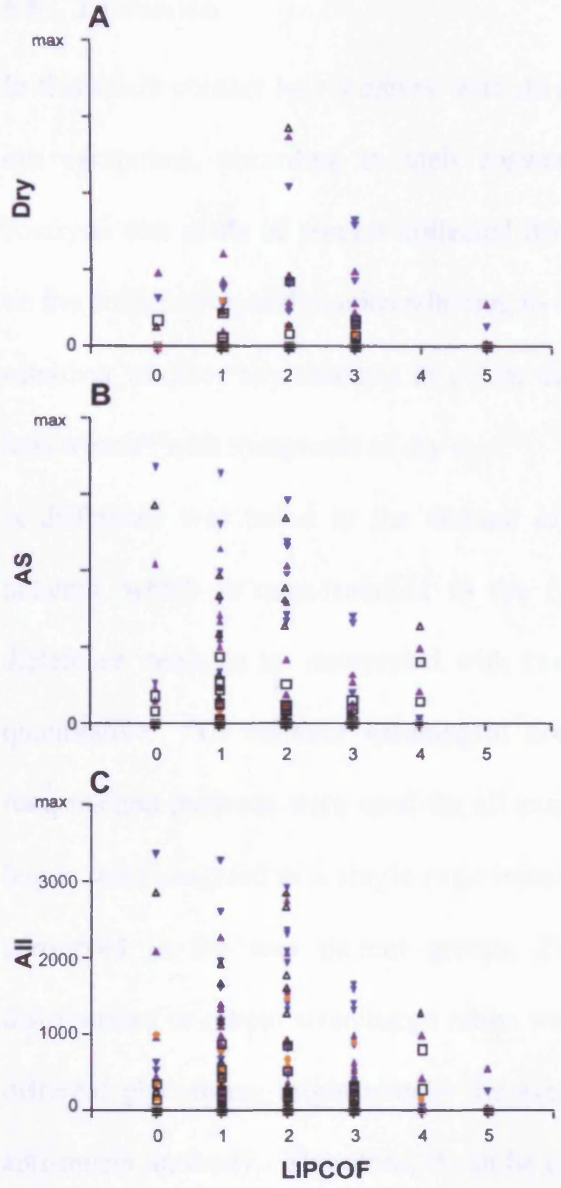


Figure 6.6: Relation between mucins level and LIPCOF Sum and LWE scores.

Patients were ordered first by increasing LIPCOF scores and then by increasing scores of lower lid LWE. (A. Patients with dry eye symptoms, B. Asymptomatic patients, C. Distribution irrespective of symptoms). In the entire population, as in asymptomatics, there is a clear decrease in mucin levels with the increase in pathology scores. This is less clear in the group of patients with dry eye symptoms.

6.5 Discussion

In this study contact lens wearers were divided into two groups: with and without dry eye symptoms, according to their answers to the CLDEQ dry eye questionnaire. Analysis was made of mucins collected from the ocular surface with a strip impressed on the conjunctiva, and mucins adhering to the contact lenses of the same individuals, to establish whether any changes in ocular surface mucins could be detected in contact lens wearers with symptoms of dry eye.

A difference was noted in the amount of mucin collected from the two groups of patients, which is most marked in the first extraction from contact lenses. This difference needs to be interpreted with caution: mucin quantification is at best semi-quantitative. To conduct meaningful comparisons between individuals, the same reagents and methods were used for all extractions, and the vast majority of strips and lenses were analysed in a single experiment. Mucin species and their proportions were conserved in the two patient groups. Furthermore, and importantly, mucin size distributions or mucin size/charge ratios were also similar in the two groups, although different glycoforms might restrict the availability of the peptide core epitope to the anti-mucin antibody. Therefore, it can be concluded that contact lens wearers with dry eye symptoms had decreased mucin concentrations at the ocular surface, and that more of their mucins were contained in macromolecular aggregates (solubilised with dithiotreitol).

Upper lid LWE and LIPCOF scores were significantly higher in contact lens wearers with dry eye symptoms, as reported in previous studies[77,80], and confirmed in Chapter 5. Overall, a pattern of decreasing mucin levels in relation to increasing scores of LIPCOF and LWE was noted. Some subjects have low mucin levels with grade 0 for LIPCOF

(Figure 6.6). If those with high mucin levels but low LIPCOF are assumed to be outliers, the relations are more quadratic than linear with a gradual decrease in mucins with increasing LIPCOF. A possible explanation might be that, in the early stages (1-2), mucin production is up-regulated by mechanical forces in blink[39], but that this cannot be sustained in the higher grades since chronic over-production of mucins is too expensive. This was lost in patients with dry eye symptoms, suggesting more subtle mucin changes in this group, probably related to specific alterations in their oligosaccharides. The results show changes in mucin production irrespective of symptoms, and suggest that increased stimulation, as in increased friction, might trigger a change in mucin production.

It would have been surprising to detect differences in overall mucin characteristics, for two reasons. The first is the mildness of dry eye in these contact lens wearers: presence of symptoms and reversible signs of dry eye are classified as mild[27, 28]. The second is technical: the small quantity of material precluded unveiling differences in a potentially small mucin fraction.

Well-balanced, i.e. containing the normal spectrum of mucins, and sufficient mucus is considered crucial for lubrication of the conjunctiva and cornea, and for contact lens comfort[15, 16, 29]. In this study, despite all mucins being present, it has been shown that decreased mucin quantity is associated with LWE and LIPCOF severity, rather than dry eye symptoms. These results support the concept that LWE and LIPCOF follow from a failure of the tear film[5, 6, 14], and indicate the need for the presence of a sufficient quantity of mucins for the maintenance of a healthy ocular surface.

6.6 Conclusion

Symptomatic soft contact lens wearers exhibit significantly more severe LWE and LIPCOF, while ocular surface mucin composition is conserved. These increased scores of ocular pathology are accompanied by decreased mucins, which might explain the increased friction manifesting in changes to the ocular surface morphology. The contact lens clinician should therefore consider including LWE and LIPCOF as part of their standard clinical examination routine for contact lens wearers, noting the clinical grade of these indicators of mucin insufficiency. Any progressive change in grade can then prompt intervention by the clinician to promote wearing comfort by altering lens type, wearing schedule or providing supplementary tear film lubrication.

(The published form of this chapter can be found in the appendices)

7. Ocular Signs and Tear Film in Non-Contact Lens Wearers: Inter-relations and Relations to Symptoms

7.1 Introduction

Dry eye patients suffer from many symptoms such as stinging, burning, itching, light sensitivity and blurry vision[94-98], limiting quality of life as well as occupation; for example computer work, dominant in many professions, is known to increase dry eye symptoms in those patients[59]. Dry eye prevalence is suggested to be about 10-15%[93], and these patients often show more severity in symptoms than visible in ocular signs in clinical practice[13].

Lid wiper epitheliopathy (LWE) and lid parallel conjunctival folds (LIPCOF) are related to dry eye symptoms in contact lens wear[77,80,333,336]. In Chapter 5, an improved method was found to predict dry eye symptoms of contact lens wearers, by observing both temporal LIPCOF and nasal conjunctival folds, and combining them to produce a new score named LIPCOF Sum[333]. Whilst LWE has been reported to be predictive of dry eye symptoms in non-lens wearers too[78,333], LIPCOF has only been related to dry eye patients using an objective SICCA score[79] (which has a 12 point scale combining the results from Schirmer I, tear-film break-up time, rose Bengal and fluorescein staining, lysozyme test and impression cytology).

Thus the relationship between temporal LIPCOF, nasal LIPCOF and LIPCOF Sum to symptoms in marginal dry eye of non-contact lens wearers is unknown, but appraisal of these signs forms an important part of any assessment amongst the general population. This pilot study evaluates the relationships between clinical tear film tests, ocular signs and dry eye symptoms in a cohort of non-contact lens wearers.

7.2 Methods

The right eyes of 38 healthy, non-lens wearers (male = 15, female = 23, median age = 32 years, range = 19-44) who had never worn contact lenses previously were selected from volunteers attending the optometry practice of Horst Riede GmbH, Weinheim, Germany. The tear film and ocular surface of the eyes was evaluated during a single session.

7.2.1 Inclusion and exclusion criteria

Subjects were excluded if they have had Sjögren's Syndrome, rheumatoid arthritis, diabetes, recent ocular infections, hay fever, and history of ocular surgery, use of any medication or eye drops known to affect the ocular surface, or were pregnant. All procedures were conducted in accordance with the Declaration of Helsinki (1983), and approval for the study was given by the Cardiff School of Optometry and Vision Sciences Ethics Committee. All subjects signed an informed consent form before participating in the study.

7.2.2 Techniques

Tear meniscus height (TMH)

TMH was measured by a slit-lamp microscope (with a graticule in 0.05mm units) at the centre of the lower lid margin. The slit has to be positioned horizontal to the lower lid with indirect illumination, to exclude invasive triggers like glaring or heating. Three consecutive readings were evaluated and the median noted.

Non-invasive break up time (NIBUT)

NIBUT was determined non-invasively using a TearScope Plus™ (Keeler Ltd, Windsor, UK) with a fine grid insert[202]. NIBUT was the time measured, in seconds, between the full opening of the eyelids after a complete blink and the first break in the tear film. Three consecutive readings were evaluated and the median noted.

Ocular hyperaemia

Limbal and bulbar hyperaemia of the horizontal segment of the ocular surface was evaluated by slit-lamp microscope using 12x magnification and classified using the CCLRU grading scale (University of New South Wales, Sydney, Australia)[220,232,268], interpolated in 0.1 increments. The assessment of the horizontal segment reflects common clinical practice. Since grading by quadrant averages increases reliability[306] (Chapter 4), temporal and nasal grades were averaged to give a horizontal grade of hyperaemia.

Lid parallel conjunctival folds (LIPCOF)

LIPCOF was evaluated in the area perpendicular to the temporal and nasal limbus on the bulbar conjunctiva above the lower lid (temporal and nasal LIPCOF, respectively)

with a slit-lamp microscope using 18 to 24 x magnification, as necessary[333,336]. The grading score of Höh et al[79], adapted by Pult and Sickenberger[80] was employed. A further combined LIPCOF score (LIPCOF Sum; temporal and nasal LIPCOF summarised) was calculated by adding together the nasal LIPCOF grade and temporal LIPCOF grade. Care was taken to differentiate between parallel, permanent conjunctival folds (LIPCOF) and disrupted micro-folds or conjunctival flaps[159,248,249].

Phenol red thread test (PRTT)

Patients were asked to keep their eyes open (blinking gently, if necessary) for 15 seconds while a phenol-red–impregnated cotton thread (Zone-Quick; Menicon Co. Ltd., Nagoya, Japan) was placed in their lower conjunctival sac. This test is based on the Hamano cotton thread test measuring tear volume in the lower meniscus sac[212].

Corneal and conjunctival staining

Conjunctival and corneal staining were assessed by applying 1% lissamine green and 2% fluorescein, and classified into four grades, interpolated in 0.1 increments (CCLRU grading scale, University of New South Wales, Sydney, Australia)[220,232,268].

Lid wiper epitheliopathy (LWE)

LWE was made visible using a combination of instilled 1% lissamine green and 2% fluorescein, and evaluated for the upper lid. A second instillation of both dyes was carried out after 5 mins[255]. LWE was observed using a slit-lamp microscope with 18x magnification classified according to Korb et al[77,78]. Care was to taken to differentiate between the fluorescein and lissamine staining associated with Marx's line and that from staining of the lid wiper[77].

7.3 Statistical analyses

Data was examined for normality and tests used as appropriate. Correlations between tear film tests were evaluated by Pearson correlation or Spearman rank, if variables were parametric or non-parametric, respectively, differences were evaluated by Mann-Whitney U-Test.

The data were analysed using SPSS 16.0 (SPSS Inc., Chicago, USA) and BiAS 8.4.2 (Epsilon Verlag, Frankfurt, Germany).

7.4 Results:

NIBUT was significantly correlated to TMH (Spearmans Rank; $r=0.388$, $p=0.009$) and PRTT (0.526, $p<0.001$). Temporal LIPCOF was significantly correlated to nasal LIPCOF (0.487, $p=0.001$) and bulbar hyperaemia (0.342, $p=0.0191$). LWE was significantly correlated to LIPCOF (0.482 and 0.477, $p<0.001$, temporal and nasal, respectively) (Table 7.1). No significant effects were found between any tear film test or ocular sign and age (Spearmans Rank; $0.106<p>0.458$) and gender (U-Test, $p>0.05$). Subjects presented with a median OSDI score of 6.25 (mean=8.3 ±8.6 sd). Table 7.2 shows the correlations between OSDI total score and its subsets with the measured tear film and ocular surface parameters.

NIBUT (Figure 7.1) and TMH were significantly negatively correlated with OSDI scores. The positive correlations between OSDI scores (environmental section) and LIPCOF were significant, but only NIBUT and nasal LIPCOF were significantly correlated with total OSDI score (Figure 7.2 and 7.3). The power calculation of the completed study resulted in a power of >0.89.

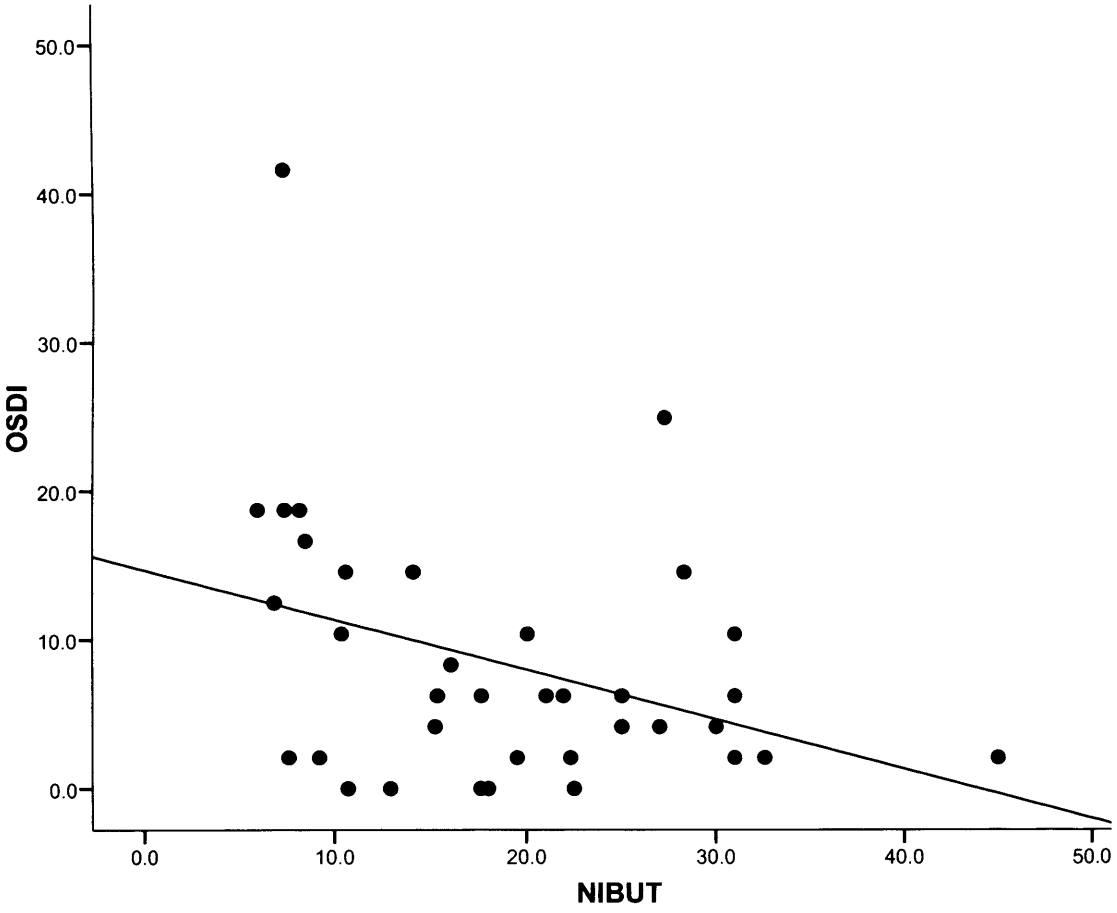


Figure 7.1: NIBUT was significantly correlated to OSDI scores.

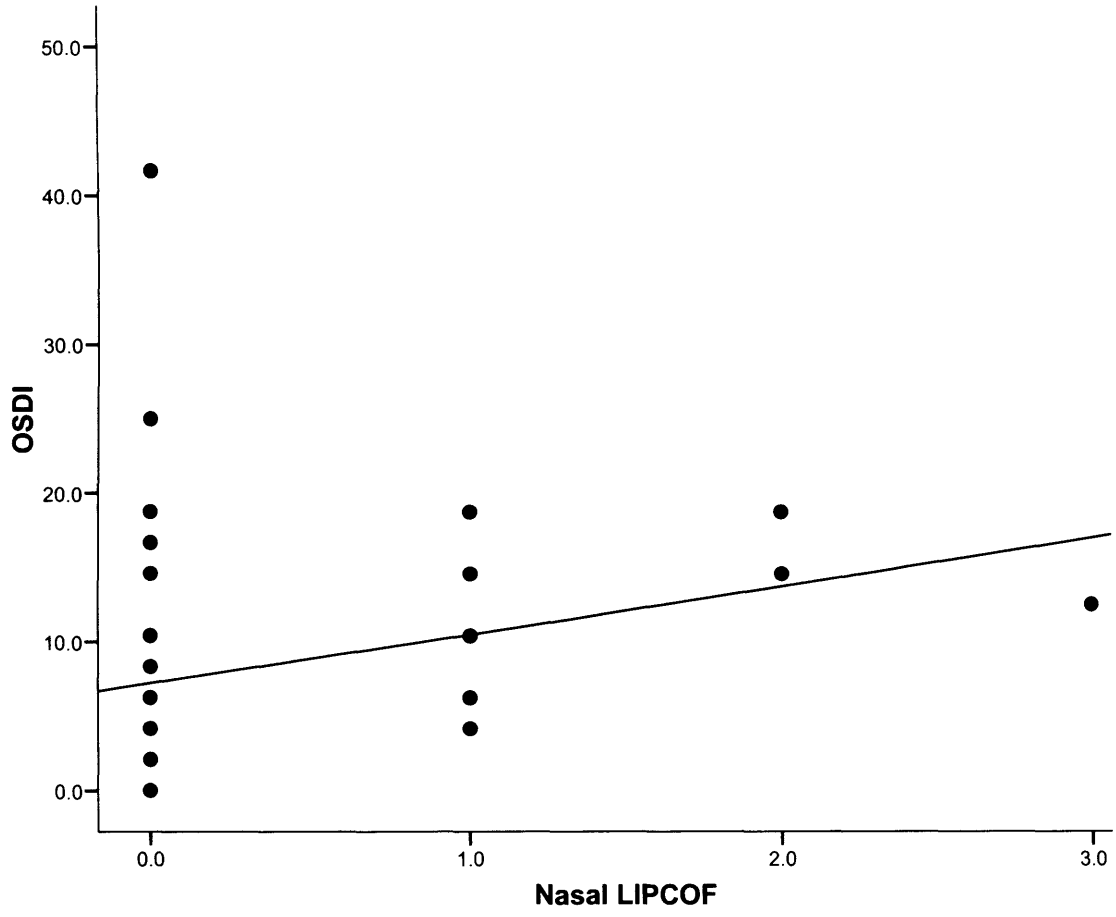


Figure 7.2: Nasal LIPCOF was significantly correlated to OSDI scores.

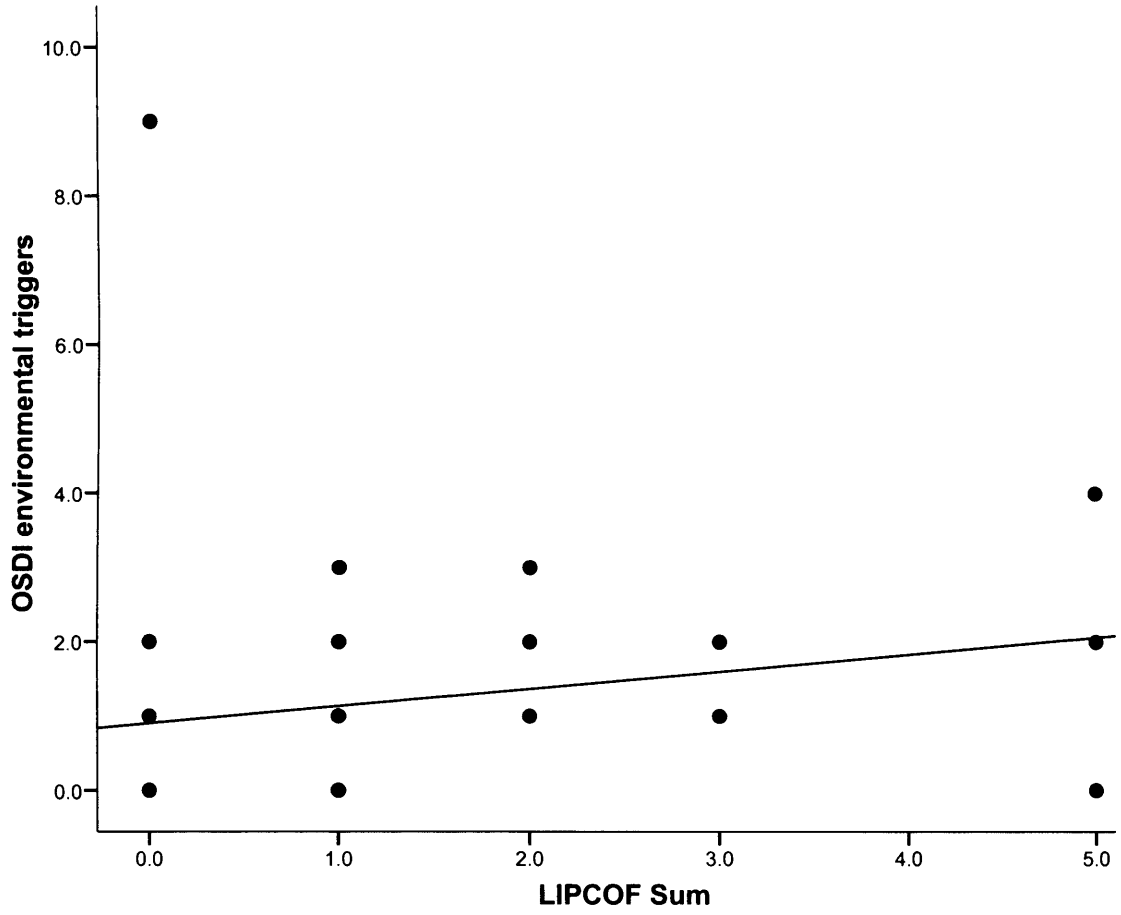


Figure 7.3: LIPCOF Sum was significantly correlated to environmental triggers of the OSDI (sum of questions 10-12).

	Age	TMH	NIBUT	Temp. LIPCOF	Nasal LIPCOF	Bulbar Hyp.	Limbal Hyp.	PRTT	Corneal Staining	Conj. Staining
TMH	0.176									
NIBUT	-0.045	**0.388**								
Temporal LIPCOF	0.116	0.176	-0.176							
Nasal LIPCOF	0.205	0.051	-0.193	**0.487***						
Bulbar Hyp.	0.078	0.214	-0.011	**0.342**	0.016					
Limbal Hyp.	-0.045	0.055	-0.055	0.266	-0.008	**0.792***				
PRTT	-0.048	0.221	**0.526***	-0.099	-0.111	0.096	0.050			
Corneal Staining	-0.210	0.006	-0.135	0.136	0.153	0.090	0.250	-0.173		
Conj. Staining	0.018	0.046	-0.008	0.062	-0.125	0.012	-0.083	0.065	-0.094	
LWE	0.044	0.124	0.007	**0.482***	**0.477***	-0.006	-0.150	-0.048	0.130	**0.333**

Table 7.1: Correlations between tests of only the right eye, (r-value). Correlation coefficients in bold indicate significance levels were 0.001<p>0.019.

**(Significant after Bonferroni adjustment)*

	TMH	NIBUT	Temp LIPCOF	Nasal LIPCOF	LIPCOF Sum	Bulbar Hyp.	Limbal Hyp.	PRTT	Corneal Staining	Conj. Staining	LWE
OSDI: Ocular sympt.	**-0.342**	**-0.308**	-0.150	0.126	-0.081	-0.075	0.128	-0.082	-0.052	0.086	-0.202
OSDI: Vision related	-0.215	**-0.350**	0.126	0.399	0.211	-0.103	-0.075	-0.221	0.030	-0.006	**0.286**
OSDI: Environmental triggers	-0.087	-0.135	**0.439**	**0.343**	**0.453***	0.186	0.149	0.094	0.050	0.231	0.168
OSDI: Score	**-0.311**	**-0.344**	0.166	**0.419***	0.244	-0.047	0.053	-0.113	-0.009	0.088	0.169

Table 7.2: Correlations between tests and symptoms.

Correlation coefficients in bold indicate significance levels were $0.001<p>0.04$.

** remain significant after appropriate Bonferroni adjustment)*

7.5 Discussion

This sample of a normal non-lens wearing population presented mostly with no LWE, less than Grade 1 of temporal LIPCOF, and no nasal LIPCOF. Observed bulbar hyperaemia of Grade 2.3 (median) was relatively similar to reported horizontal scores, but limbal hyperaemia (median grade, 2.0) was slightly increased compared to published norms (median horizontal bulbar hyperaemia and limbal hyperaemia, CCLRU Grade 2.1 and 1.7 respectively[232,306] (Chapter 4)). Corneal staining and conjunctival staining in this population were uncommon and less reported than in other studies[220]. However, the smaller sample size of this study may have had some influence on these results.

Tear film volume, which was assessed by TMH and PRTT, is an important contributor to a stable tear film[83,337], and in this study the positive correlations observed between

NIBUT and both TMH and PRTT (supported by other studies[83,204,205,213]) support this theory.

LIPCOF was significantly positively correlated to LWE and bulbar hyperaemia. This reflects the hypothesised mechanical origin of LIPCOF and LWE[78,336]. In LWE squamous cells are visible at the lid wiper, which is in contact with the bulbar conjunctiva where the tear film is insufficient[78] and/or reduced mucin quantity exists[336] (Chapter 6). Squamous epithelial cells feature in tissues that experience frequent rubbing[78], and their presence in the particular region of the lid wiper[327] infers that the marginal conjunctiva is intimately and mechanically associated with the surfaces of the oculus bulbi.

Age and gender did not appear to have significant effects on any of the clinical signs or tear film tests in this population, but the limited age group (18-44yrs) may have had some bearing here. Older people and females over 45 years of age are more likely to present with dry eye symptoms and signs, than younger people and men[93].

OSDI scores correlated with tear film stability and tear volume tests, but only with nasal LIPCOF. This is somewhat surprising given that previous work demonstrates an association between temporal LIPCOF and an objective SICCA score in dry eye patients[79], and LIPCOF Sum has been found to be more predictive for dry eye symptoms in contact lens wear than temporal LIPCOF or nasal LIPCOF[333] (Chapter 5).

It is interesting to note that certain aspects of the OSDI correlate better with LIPCOF measures than others. This may suggest that different tests reflect specific symptoms in non-lens wearers, although the small study numbers limits the strength of these conclusions.

7.6 Conclusions

LIPCOF and LWE are correlated, as found in contact lens wearers in prior investigation in this PhD. The tear film tests TMH and NIBUT, as well as the ocular signs LIPCOF and LWE, each indicate different factors of symptomatology (ocular symptoms, vision, environment) in marginal dry eye. Increased OSDI scores are related to decreased TMH and NIBUT, and to severe nasal LIPCOF.

8. A Novel Method to Predict the Development of Contact Lens Induced Dry Eye (CLIDE) in Naïve Contact Lens Wearers

8.1 Introduction

This study directly addresses the main aim of this PhD: how the tests, evaluated in this thesis, perform in predicting dryness symptoms in new soft contact lens wearers.

Through the initial investigation of bulbar and limbal hyperaemia, the expected grades for normality and abnormality were found (Chapter 4), but further investigation showed that hyperaemia of the ocular surface does not appear to be related to symptoms, nor is it an indicator for CLIDE in experienced lens wearers (Chapter 5). However, LWE and LIPCOF showed acceptable levels for prediction of dryness symptoms in experienced contact lens wearers, particularly that of LIPCOF Sum.

In non-lens wearers, the symptoms of dryness were additionally related to tear meniscus height (TMH), non-invasive break-up time (NIBUT) and nasal LIPCOF, but not LWE (Chapter 7). Therefore, this study investigates whether a combination of these tests, plus subjective evaluation prior to contact lens fitting, is able to predict CLIDE, and what combination will achieve the best predictive values. Furthermore, the response of naïve lens wearers to lens wear through a period of adaptation and intervention, using objective signs as well as symptoms, will be investigated.

To summarise, this chapter describes an investigation of potential test combinations for predicting dry eye symptoms in new patients embarking on hydrogel contact lens wear for the first time, as well as examining the changes in wearing comfort and clinical signs when such patients are subsequently re-fitted with silicone hydrogel contact lenses.

8.2 Methods

Thirty-three subjects (male = 12, female = 21, median age = 30.5 years; range = 19-44), who had never worn contact lenses previously were recruited from the patient pool of Horst Riede GmbH, Weinheim, Germany for a prospective longitudinal study. Subjects in the study were required to wear their lenses at least 4 times per week for a minimum wearing period of 6 hours each time throughout the study, and to use a hydrogen peroxide system as directed by the manufacturer (AO-Sept, Ciba Vision Vertriebs GmbH, Grossostheim, Germany), with saline solution (SoftWear, Ciba Vision Vertriebs GmbH, Grossostheim, Germany) for rinsing and rubbing the lenses. Subjects were excluded from the study if lens fit was unacceptable, or visual acuity was reduced by more than one line with contact lenses.

All subjects were supplied with lenses for a total wearing period of 8 weeks, split between the wear of 2 different lens designs and materials (Figure 8.1).

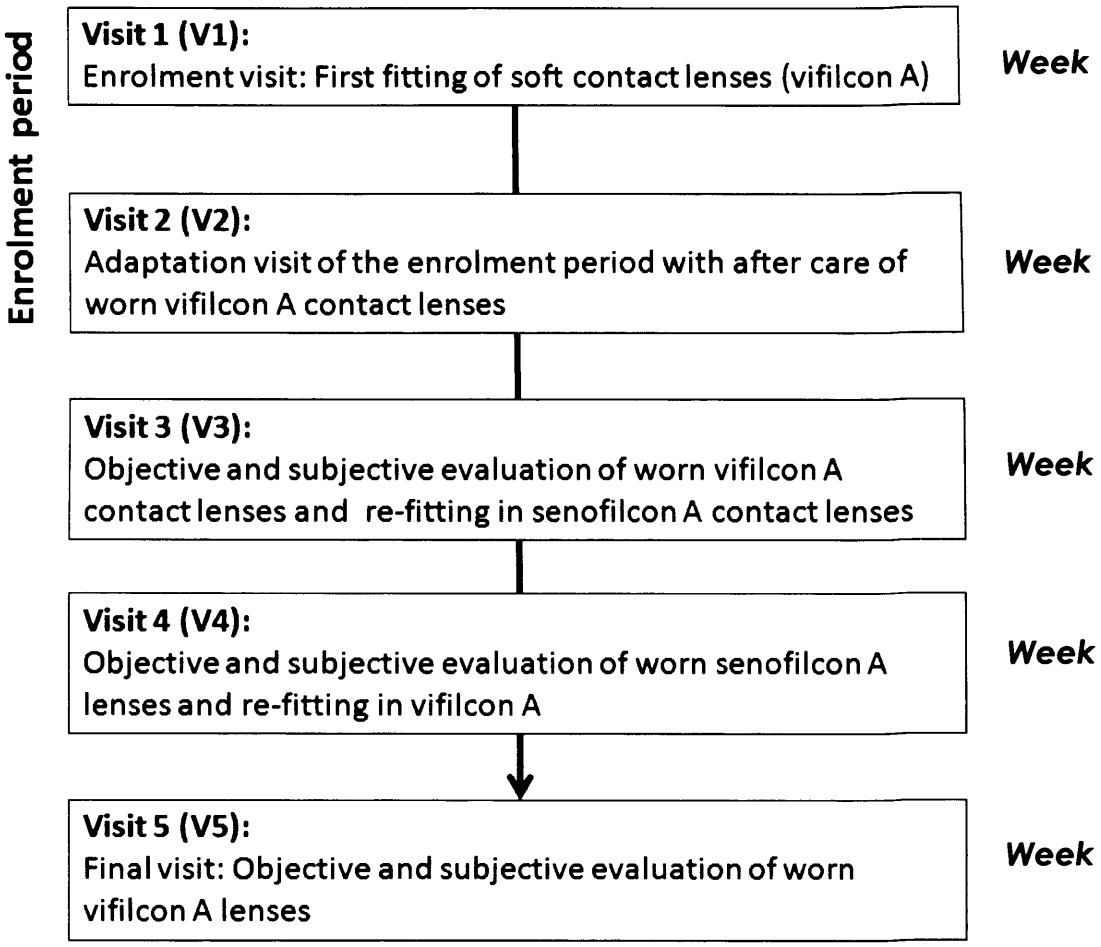


Figure 8.1: The principle schedule of the study.

To follow common clinical practice, subjects were first fitted with Vifilcon A hydrogel contact lenses (Ciba Vision Vertriebs GmbH, Grossostheim, Germany) for an initial 2 week adaptation period to allow subjects to become accustomed to hydrogel contact lens wear. These lenses were then replaced and a new pair inserted for an additional 2 week period. Then subjects were refitted with Senofilcon A silicone hydrogel (Si-Hy) contact lenses (Johnson & Johnson Medical GmbH, Norderstedt, Germany) and asked to wear the lenses for a 2 week period. Finally, the subjects were fitted again with Vifilcon A lenses for a 2 week period.

Property Name	Focus Visitint	Oasys
USAN	Vifilcon A	Senofilcon A
Manufacturer	Ciba Vision	Johnson & Johnson
Water Content (%)	55	38
Base Curve / Diameter	8.9-8.6 / 14.0	8.8 – 8.4 / 14.0
Oxygen Transmissibility (Fatt Units)	20	103
Centre Thickness (mm) - 3.00 DS	0.10	0.07
FDA Group	IV	I
Surface Treatment	None	None. Internal wetting agent (PVP) that also coats the surface
Principal Monomers	HEMA + PVP + MA	mPDMS + DMA + HEMA + siloxane macromer + PVP + TEGDMA

Table 8.1: Properties of contact lens materials evaluated in the study *(DMA, N,N-dimethylacrylamide; HEMA, poly(2-hydroxyethyl methacrylate); MA, methacrylic acid; mPDMS, monofunctional polydimethylsiloxane; PVP, polyvinyl pyrrolidone; TEGDMA, tetraethyleneglycol dimethacrylate).*

8.2.1 Inclusion and exclusion criteria

Subjects were excluded if they had Sjögren's Syndrome, rheumatoid arthritis, diabetes, recent ocular infections, hay-fever, history of ocular surgery, use of any medication or eye drops known to affect the ocular surface, or were pregnant. All procedures were conducted in accordance with the Declaration of Helsinki (1983), and approval for the study was given by the Cardiff School of Optometry and Vision Sciences Ethics Committee. All subjects signed an informed consent form before participating in the study.

8.2.2 Technique (Table 8.2)

Symptoms

The OSDI questionnaire was used to measure patients' symptoms at the start and during the study, on a 0-100 scale[96,119]. The Contact Lens Dry Eye Questionnaire (CLDEQ) was used to group patients into asymptomatic and symptomatic contact lens wearers by its dichotomous outcome[10] during the contact lens wear period only. Both questionnaires were completed independently by the subjects and the author was blind to the results.

Tear meniscus height (TMH)

TMH (centre of lower lid) was measured by slit-lamp microscope (x18 magnification) with a objective lens graticule in 0.05mm units. Three consecutive readings were taken and the median noted.

Non-invasive break-up time (NIBUT)

NIBUT was determined non-invasively using a Tearscope (Keeler Ltd, Windsor, UK) with a fine grid insert[202]. NIBUT was the time measured, in seconds, between the full opening of the eyelids after a complete blink and the first observed break in the tear film. Three consecutive readings eye were taken and the median noted.

Ocular hyperaemia

Limbal and bulbar hyperaemia of the horizontal segment of the ocular surface was evaluated by slit-lamp microscope using 12x magnification and classified using the CCLRU grading scale (University of New South Wales, Sydney, Australia)[220,232,268], interpolated in 0.1 increments. The assessment of the horizontal segment reflects common clinical practice. Since grading by quadrant averages increases reliability[306] (Chapter 4), temporal and nasal grades were averaged to give a horizontal grade of hyperaemia.

Lid parallel conjunctival folds (LIPCOF)

LIPCOF was evaluated in the area perpendicular to the temporal and nasal limbus on the bulbar conjunctiva above the lower lid (temporal and nasal LIPCOF, respectively) with a slit-lamp microscope (x18-24 magnification, as necessary). The grading score of Höh et al[79], adapted by Pult and Sickenberger[80] was employed. A further combined LIPCOF score (LIPCOF Sum) was calculated by adding together the nasal LIPCOF grade and temporal LIPCOF grade (see Chapter 5). Care was taken to differentiate between parallel, permanent conjunctival folds (LIPCOF) and disrupted micro-folds or conjunctival flaps[159,248,249].

Phenol red thread test (PRTT)

Patients were asked to keep their eyes open (blinking gently, if necessary) for 15 seconds while a phenol-red–impregnated cotton thread (Zone-Quick; Menicon Co Ltd, Nagoya, Japan) was placed in their lower conjunctival sac, with the thread looped over the lower lid margin. This test is based on the Hamano cotton thread test for assessing tear volume in the lower meniscus sac[212].

Corneal and conjunctival staining

Conjunctival and corneal staining were classified under slit-lamp microscope observation (x18-24 magnification) using the four grades of the CCLRU grading scale[220,232,268], interpolated to 0.1 intervals. Corneal and conjunctival staining were visualised using sodium fluorescein and lissamine green strips, respectively.

Lid wiper epitheliopathy (LWE)

LWE was observed using the recommended technique[255] and evaluated for the upper lid. LWE was observed using the slit-lamp microscope (x18 magnification), and classified according to Korb et al[77,78]. Care was to taken to differentiate between the fluorescein and lissamine staining associated with Marx's line and that from staining of the true lid wiper[77].

Corneal topography

Corneal topography was performed using the Oculus Keratograph (OCULUS Optikgeräte GmbH, Wetzlar, Germany), to evaluate horizontal and vertical radii, as well as eccentricity.

First Examination (V1):	After Care (V3-5):
• Tear meniscus height (TMH) • Non-invasive break up time (NIBUT) • Temporal and nasal lid parallel conjunctival folds (LIPCOF) • Horizontal bulbar and limbal hyperaemia • Phenol red thread test (PRTT) • Conjunctival and corneal staining • Lid wiper epitheliopathy (LWE) • Eye lids • Refraction • Keratometry • Subjective evaluation by the OSDI • Inserting of lenses • Evaluation of the fitting performance and refraction • Visual acuity with lenses • Training of handling an care of the lenses	*With worn Lenses:* • Fitting criteria • Contact lens surface and deposits • Visual acuity • Horizontal bulbar and limbal hyperaemia • Refraction *Without Lenses:* • LIPCOF • Corneal staining • LWE • Eye lids • Evaluation of symptoms by the CLDEQ and OSDI

Table 8.2: Order of tests.

8.3 Statistical analyses

Differences between visits were analysed by repeated measures ANOVA (with post-hoc analysis) or Friedman test and Wilcoxon tests; differences between groups by unpaired t-test or Mann Whitney U-test, depending on whether variables were parametric or non-parametric respectively. Post-hoc pair-wise comparisons between visits were made, where appropriate.

Different combinations of the objective tests were analysed for their predictive power by logistical regression, and the predictive value assessed by the receiver operating characteristic (ROC) curve, and the area under the ROC curve (AUC).

The data was analysed using WinSTAT 2007.1-Software (R Fitch Software, Bad Krozingen, Germany) and SPSS 16.0 (SPSS Inc. Chicago, USA).

8.4 Results

8.4.1 Significant differences between visits

There were significant changes in limbal hyperaemia and LWE during the study (Repeated measures ANOVA; $p<0.001$; Friedman test; $p=0.004$, respectively)

There was no significant variation in LIPCOF (Friedman; $p\geq0.318$; temporal, nasal and Sum), bulbar hyperaemia (repeated measures ANOVA; $p=0.432$), staining ($p=0.060$) (Figure 8.2A-G), OSDI (repeated measures ANOVA; $p=0.126$) or CLDEQ (McNemar; $p=0.317$, $\kappa=0.279$) during the study.

Even though when subjects were grouped according to symptomatic status (CLDEQ), there was no alteration in the parameters demonstrating significant change. The power calculation of the completed study ('differences between visits' – section) resulted in a power of >0.74.

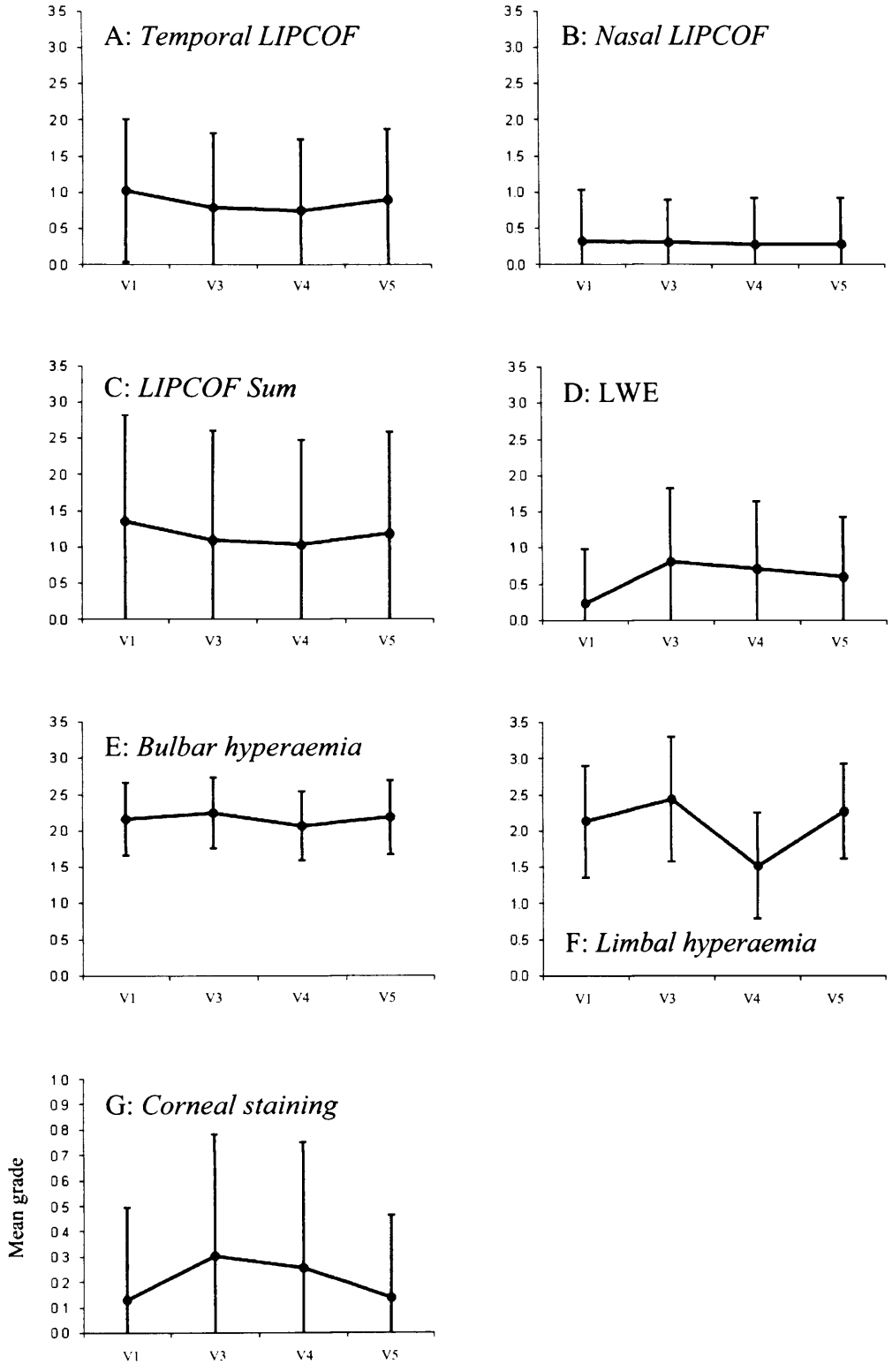


Figure 8.2: Variations of objective signs between visits 1-3-4-5 (Mean, SD).

Here follows the post-hoc pair wise comparisons from repeated measures analysis (where appropriate):

8.4.2 Differences between hydrogel lens wear (Visit 3) and silicone hydrogel lens wear (Visit 4)

Limbal hyperaemia was significantly decreased after wearing Senofilcon A contact lenses compared to Vifilcon A lenses (repeated measures ANOVA Bonferroni post-hoc; $p<0.001$) (Figure 8.2F).

8.4.3 Differences between silicone hydrogels (Visit 4) and hydrogel lens wear (Visit 5)

Limbal hyperaemia increased significantly when subjects were re-fitted with Vifilcon A contact lenses (repeated measures ANOVA Bonferroni post hoc; $p<0.001$; Figure 8.2F).

8.4.4 Contact lens comfort

When analysing the overall subject symptom score using the CLDEQ, there was no significant difference between Vifilcon A and Senofilcon A (McNemar; $p=0.317$, $\kappa=0.279$). However, when only symptomatic subjects were analysed, a significant improvement in the quantitative score CLDEQ question 1 (wearing comfort) was observed when changing from Vifilcon A to Senofilcon A, but not in the quantitative score from CLDEQ question 2 (dryness) (repeated measures ANOVA Bonferroni post-hoc; $p=0.005$ and $p=0.511$, respectively).

8.4.5 Analyses of best clinical test combination

Regression analyses

Results from the enrolment visit (Visit 1) were analysed for the test combination that best predicted future symptomatic status during contact lens wear. Since there were no statistical differences in symptoms (OSDI or CLDEQ) between Visits 3-4-5 of the ungrouped patient pool, the subjects were considered to be symptomatic if the CLDEQ was positive in any one of the contact lens visits. Twenty subjects were classified into symptomatic and thirteen as asymptomatic. Subjects who became symptomatic in contact lens wear were also found to have presented at the enrolment visit, with significantly decreased NIBUT and increased scores in LIPCOF and OSDI compared to subjects free of symptoms later on (Table 8.3). The power calculation of the completed study ('discrimination of later contact lens symptoms' – section) resulted in a power of >0.95.

	All Subjects		Asymptomatics		Symptomatics		
	Mean/Median	S.D.	Mean/Median	S.D.	Mean/Median	S.D.	P
TMH	0.21/0.20	±0.831	0.243/0.20	± 0.075	0.188/0.20	± 0.082	0.113
NIBUT	19.189/18.0	±9.516	22.735/22.40	± 9.141	14.930/14.0	± 8.103	**0.016**
PRTT	19.324/20.0	±5.094	19.85/22.0	± 6.192	19.077/20.0	± 3.328	0.387
Temporal LIPCOF	1.027/1.0	±0.986	0.60/0.0	± 0.821	1.538/1.0	± 1.050	**0.030**
Nasal LIPCOF	0.324/0	±0.709	0.0/0.0	±0.0	0.615/0.0	± 0.768	**0.027**
Sum LIPCOF	1.351/1.0	±1.476	0.60/0.0	± 0.821	2.154/2.0	± 1.57	**0.003**
Bulbar Hyperaemia	2.160/2.30	±0.505	2.14/2.15	± 0.464	2.223/2.30	± 0.487	0.619
Limbal Hyperaemia	2.132/2.0	±0.773	2.055/2.0	± 0.811	2.315/2.0	± 0.607	0.311
Corneal Staining	0.130/0.0	±0.364	0.159/0.0	± 0.433	0.125/0.0	± 0.306	0.645
Conj. Staining	0.135/0.0	±0.365	0.150/0.0	± 0.671	0.154/0.0	± 0.555	0.912
LWE	0.230/0.0	±0.751	0.150/0.0	± 0.671	0.384/0.0	± 0.961	0.632
OSDI	8.359/6.250	±8.669	3.968/2.0833	± 5.648	14.481/14.583	± 9.672	**<0.001**

Table 8.3: This table displays the median and average values (standard deviation (SD)) and p-values for subjects at enrolment visit (Visit 1), and when grouped by symptomatic status in contact lenses.

Considering the sample size, it was appropriate to include only those significant clinical measures in the combinations for logistic regression analyses (all other parameters were found to be insignificant to regression analyses). The different combinations are shown with their resultant formulae related to CLDEQ are shown below. The final combination was analysed by the manual 'Enter' method, whilst the others were calculated by using the 'Forwards Likelihood Ratio (LR)' method.

Formula I (logistical regression, Forwards LR):

Variables: NIBUT, LIPCOF (temporal, nasal, Sum).

CLDEQ = 1.314 x **LIPCOF Sum** -0.146 x **NIBUT** + 0.571

Formula II (logistical regression, Forwards LR):

Variables: NIBUT, LIPCOF (temporal, nasal, Sum) and OSDI.

CLDEQ = 1.559 x **LIPCOF Sum** + 0.228 x **OSDI** - 4.118

Formula III (logistical regression, Enter):

Variables: NIBUT, Sum LIPCOF and OSDI.

CLDEQ = 2.025 x **LIPCOF Sum** - 0.175 x **NIBUT** + 0.276 x **OSDI** - 1.582

Receiver Operating Characteristic (ROC) Curves:

The tests NIBUT, temporal LIPCOF, nasal LIPCOF, LIPCOF Sum and OSDI show poor to excellent discrimination in isolation for symptomatic status (AUC: 0.248, 0.760, 0.731, 0.813 and 0.910, respectively; $<0.001<p>0.027$). The combination of these tests shows an improved AUC of 0.877 in Formula I, 0.948 in Formula II and 0.950 in Formula III ($<0.001<p>0.003$) (Table 8.4, Figure 8.3 and 8.4).

Combining LIPCOF Sum with NIBUT (Formula I) improved the excellent discrimination of the single use of LIPCOF Sum by 6.4%. Adding the subjective evaluation (OSDI) to Formula I results in an improvement of 7.3% (Formula III).

Even though these improvements in AUC seem to be small, they can be further improved by using the 'Enter' method. Formula III shows an additional improvement in the balance of sensitivity, specificity, predictive values and accuracy. Formula III performs best as analysed by positive predictive value and accuracy and has been named the Pult-Predictive-Test (P-Test).

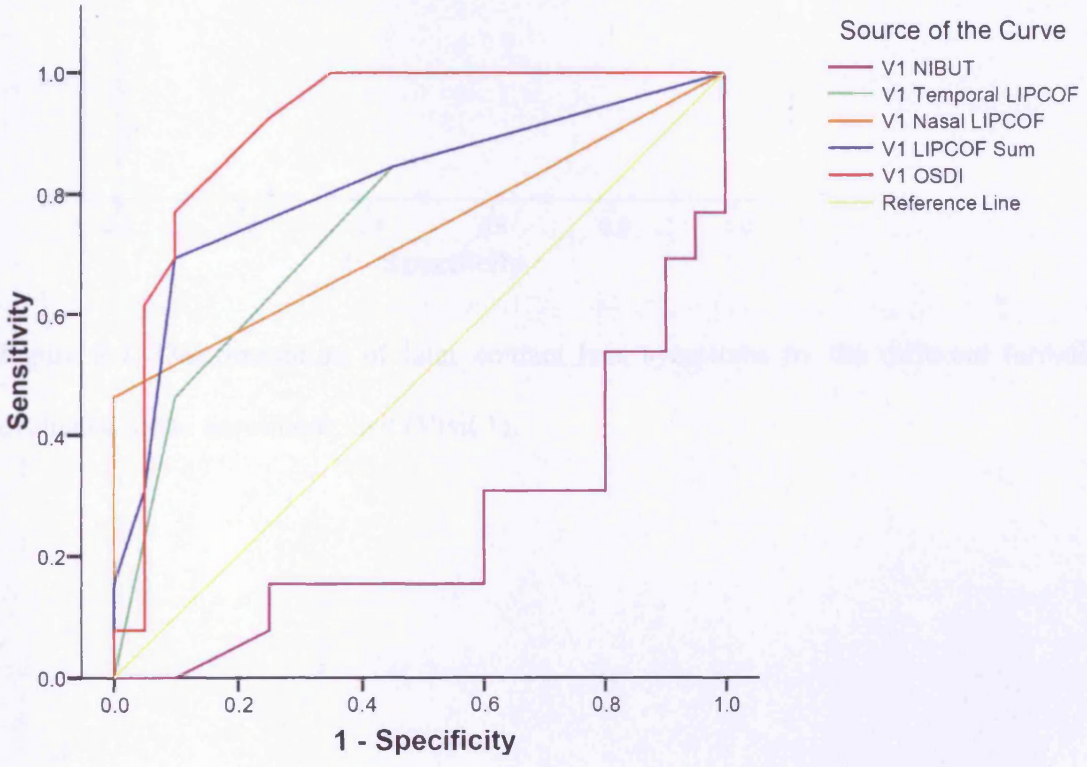


Figure 8.3: Discrimination of later contact lens symptoms by clinical tests, evaluated at the enrolment visit (Visit 1).

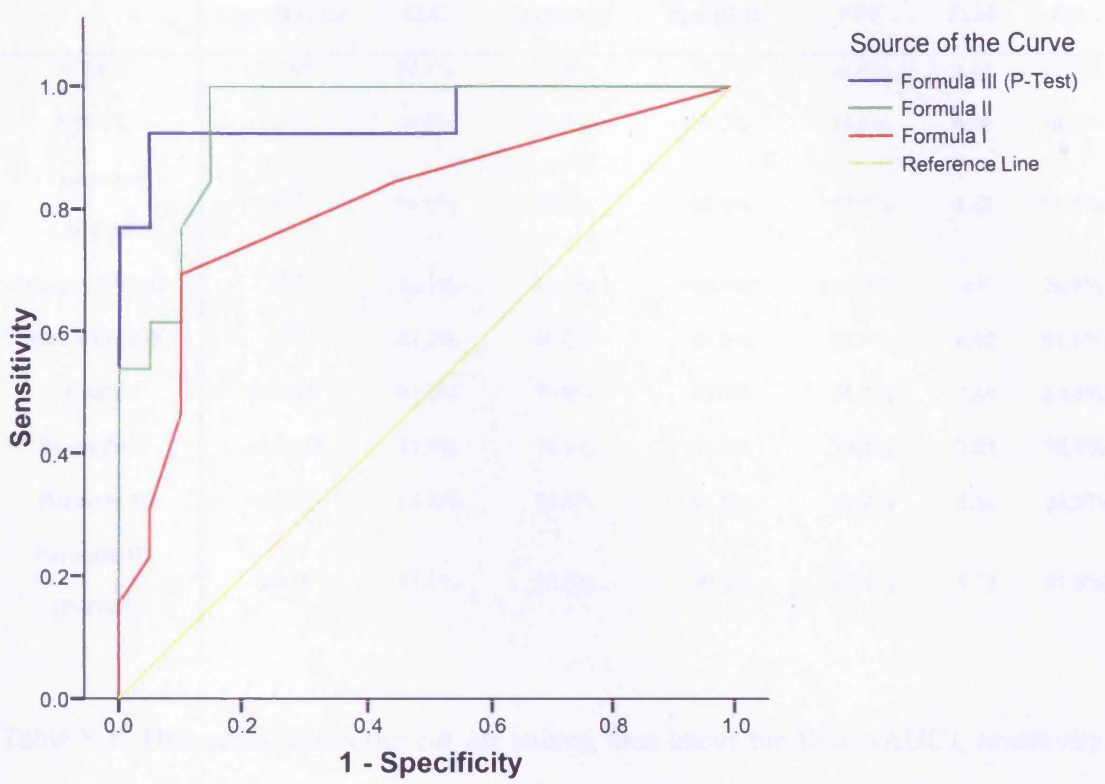


Figure 8.4: Discrimination of later contact lens symptoms by the different formula, evaluated at the enrolment visit (Visit 1).

	Cut-off value	AUC	Sensitivity	Specificity	PPV	PLH	OA
NIBUT	17.8*	24.8%	30.8%	30.0%	**24.9%**	**0.44**	**32.2%**
NIBUT	10.0^{2}	24.8%	61.5%	10.0%	**34.0%**	**0.68**	**30.3%**
Temporal LIPCOF	2^{333}	76.0%	46.2%	90.0%	**77.7%**	**4.62**	**71.2%**
Nasal LIPCOF	1^{333}	73.1%	46.2%	100.0%°	**100.0%°**	∞°	**76.9%**
Sum LIPCOF	2^{333}	81.3%	69.2%	90.0%	**83.9%**	**6.92**	**81.1%**
OSDI	6.534*	91.0%	76.9%	90.0%	**85.3%**	**7.69**	**84.4%**
Formula I	-0.305*	87.7%	76.9%	80.0%	**74.4%**	**3.85**	**78.7%**
Formula II	-0.527*	94.8%	84.6%	85.0%	**81.0%**	**5.64**	**84.8%**
Formula III (P-Test)	-0.475*	95.0%	92.3%	90.0%	**87.4%**	**9.23**	**91.0%**

Table 8.4: This table shows the cut-off values, area under the ROC (AUC), sensitivity, specificity, positive predict values (PPV), positive likelihood ratios (PLH) and accuracy (OA) of the significant tests and for the calculated formula, to predict CLDEQ status in subjects who go on to wear contact lenses. PPV and accuracy (OA) were calculated using a prevalence of 43%[12].

**(cut-off values derived from that patient pool)*

°*(These values might be artificially inflated due to a lack of "misdiagnosed asymptomatics").*

8.5 Discussion

A combination of the tests NIBUT, LIPCOF Sum plus OSDI was the most effective way to predict dry eye symptoms in these new lens wearers. Secondly, LWE appeared to increase when wearing contact lenses, and limbal hyperaemia increased in Vifilcon A lens wear compared to Senofilcon A lens wear. Senofilcon A lenses appear to offer better comfort in symptomatic patients compared to vifilcon A lenses.

NIBUT, LIPCOF and OSDI are known predictive tests for dry eye symptoms[79,80,94,96,200,292,298,333], but this is the first time that these tests have been used to predict later contact lens symptoms in a cohort of naïve lens wearers. Contact lenses can induce dry eye symptoms in otherwise asymptomatic patients[88,338], and it is vital to know prior to lens fitting, how comfortable patients will be after they have adapted to lens wear. Previous work has been limited to more conventional clinical tests and this is the first study that evaluates this question in naïve lens wearers[89]. Individual measures of NIBUT, LIPCOF and OSDI were able to discriminate between asymptomatic and symptomatic subjects, whilst measures of TMH, PRTT, LWE, ocular hyperaemia and staining were not. However, the significant tests differ in their predictive values. Hosmer and Lemeshow[3] suggest the following classification of ROC: 0.5 indicates no discrimination, between 0.7 and 0.8 indicates acceptable discrimination, greater than 0.8 indicates excellent discrimination and >0.9 outstanding discrimination. Whilst NIBUT was significantly different between groups, its AUC of 0.248 demonstrates poor predictive ability. In contrast, LIPCOF Sum and OSDI showed excellent to outstanding predictive ability, since their areas under the curve were the greatest (0.813 and 0.910, respectively). A further improved ability to discriminate and predict symptomatic status was obtained using Formula III, which contained objective as well as subjective tests (NIBUT, Sum LIPCOF and OSDI). This is supported by previous work that suggests a

combination of tests can lead to increased prediction[89,109,339,340]. It also confirms that theoretical models analysed in logistical regression by the manual 'Enter' method are preferable, as they reduce noise in the analyses[341].

Although the single tests were excellent in their discrimination, the results indicate the use of the Formula III (NIBUT plus Sum LIPCOF plus OSDI), named "P-Test", can potentially improve clinical management by better prediction of symptoms in new contact lens wearers.

The "P-Test" showed an excellent ability to discriminate between those with dry eye, and those without, and has great potential as a predictive test. However, a larger, long term study is required to actually determine the value of the P-Test at predicting the onset of symptoms.

As well as dryness symptoms, the results of this study also suggest that hydrogel lenses induce changes in the ocular surface. This is the first time that LWE and LIPCOF have been monitored in naïve lens wearers. LWE, but not LIPCOF, significantly increased in lens wear during the initial adaptation period, but within clinically acceptable levels[77,306,333]. The increase in LWE alongside relative stability in LIPCOF during contact lens wear in this study is supported by previous work that suggests that LWE is caused by mechanical forces in blink when the tear film is insufficient[77]: contact lenses decrease tear film stability in lens wear[330,342-344]. Conjunctival folds, however, are not thought principally to be induced by mechanical forces of the lens edge[80,333], but negative correlations to tear film stability and membrane associated mucins[333,336] (also see Chapter 6) perhaps suggest that LIPCOF may be occurring later than LWE in the development of CLIDE [333], a progression that this study duration did not permit.

Limbal hyperaemia significantly increased in mid-water hydrogels (Mean grade 2.6), but decreased in the silicone hydrogel lenses (SiHy) (Mean grade 1.3), even below baseline levels. It must be acknowledged that mean baseline limbal hyperaemia grade in this study was higher than that found in Chapter 4 and published[306]. It is generally accepted that eyes wearing silicone hydrogel lenses demonstrate decreased limbal redness[233]. Limbal hyperaemia is an important signal of corneal stress, e.g. hypoxia[226,227,233,258,306].

When refitting subjects with Senofilcon A lenses, symptomatic patients experienced better wearing comfort which is supported by recent literature[155,163,345,346], but not less dryness. Even though SiHy are known to be, in many cases, more comfortable than soft lenses because of their increased oxygen permeability and lower surface dehydration[125,157,158,345-348], Senofilcon A lenses did not appeared to "cure" contact lens related dry eye specifically.

8.6 Conclusion

The best method to forecast later wearing comfort in new lens wearers is a combination of NIBUT, LIPCOF Sum and OSDI, which has been named the P-Test. Bulbar hyperaemia, TMH, PRTT, staining and LWE are not significant discriminators for dry eye symptoms in naïve lens wearers, but temporal, nasal, LIPCOF Sum, NIBUT and OSDI are sufficient measures in isolation. Silicone hydrogel lenses can, in some cases, improve comfort in contact lens wear, but are not generally able to cure dry eye symptomatology in contact lens wear.

9. Overall Summary

This PhD had four principal aims (as stated in Section 3.4), which were addressed in the series of experimental chapters. From the results of these studies the following conclusions can be made:

1. Normal limbal redness appearance has been described for the first time. Although higher than expected, it has a lower grade than that for normal bulbar redness, which was observed to be similar in appearance to previous studies using the CCLRU grading scales. The higher redness grades observed for ocular hyperaemia are not necessarily due to a greater physical redness, but may be due to features of the grading scale used. Bulbar redness and limbal redness were inter-related, although the strength of this relationship is weakened by the poor alignment of the CCLRU grading scales. A limbal redness grade above 2.5 may be considered abnormal. A bulbar redness above 2.6 grade (quadrant-average) or 3.0 (overall) may be considered abnormal.

2. Symptomatic, experienced, soft contact lens wearers exhibit significantly more LWE and LIPCOF, but not corneal staining, bulbar hyperaemia or decreased PLBUT. LWE and LIPCOF are significantly correlated, suggesting that LIPCOF also results from friction during blinking. The sum of temporal and nasal LIPCOF scores appears to be more predictive of symptoms than other clinical tests. These increased scores of ocular pathology are accompanied by decreased mucin concentration, which might explain the increased friction manifesting in changes to the ocular surface morphology.

3. LIPCOF and LWE are also positively correlated with symptoms amongst non-contact lens wearers. The tear film tests TMH and NIBUT, as well as the ocular surface signs LIPCOF and LWE, relate to different kinds of symptomatology (ocular symptoms, vision, environment) in marginal dry eye. Increased OSDI scores are related to decreased TMH and NIBUT, and to increased nasal LIPCOF.
4. The best method of detecting dry eye symptoms of naïve lens wearers is a combination of NIBUT, LIPCOF Sum and OSDI, named the "P-Test". Bulbar hyperaemia, TMH, PRTT, staining and LWE do not appeared to be predictive tests in naïve lens wearers.

But how do these results fit within the overall aim of this PhD, which was "Is it possible to predict symptoms of dryness in soft contact lens wearers?". To answer this question, it is necessary to return to the DEWS proposed mechanism for the development of dry eye[2].

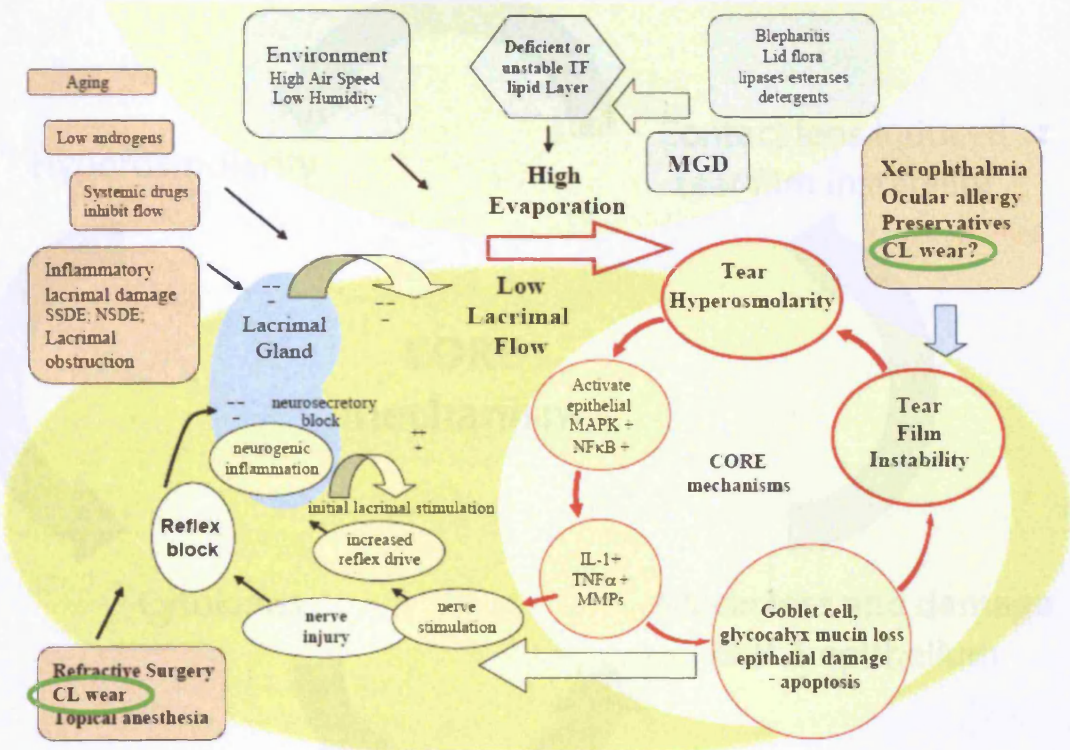


Figure 9.1: Mechanisms for the development of dry eye[2].

The diagram reveals a complicated network of interacting systems, which emphasises the underlying difficulty of diagnosing dry eye. Contact lens wear can interact with this mechanism in two principal ways: reducing tear film stability by interfering with the pre-ocular tear film layer formation, and lowering tear production by affecting corneal sensory nerve action. Simplifying the diagram allows the core mechanism to be clarified.

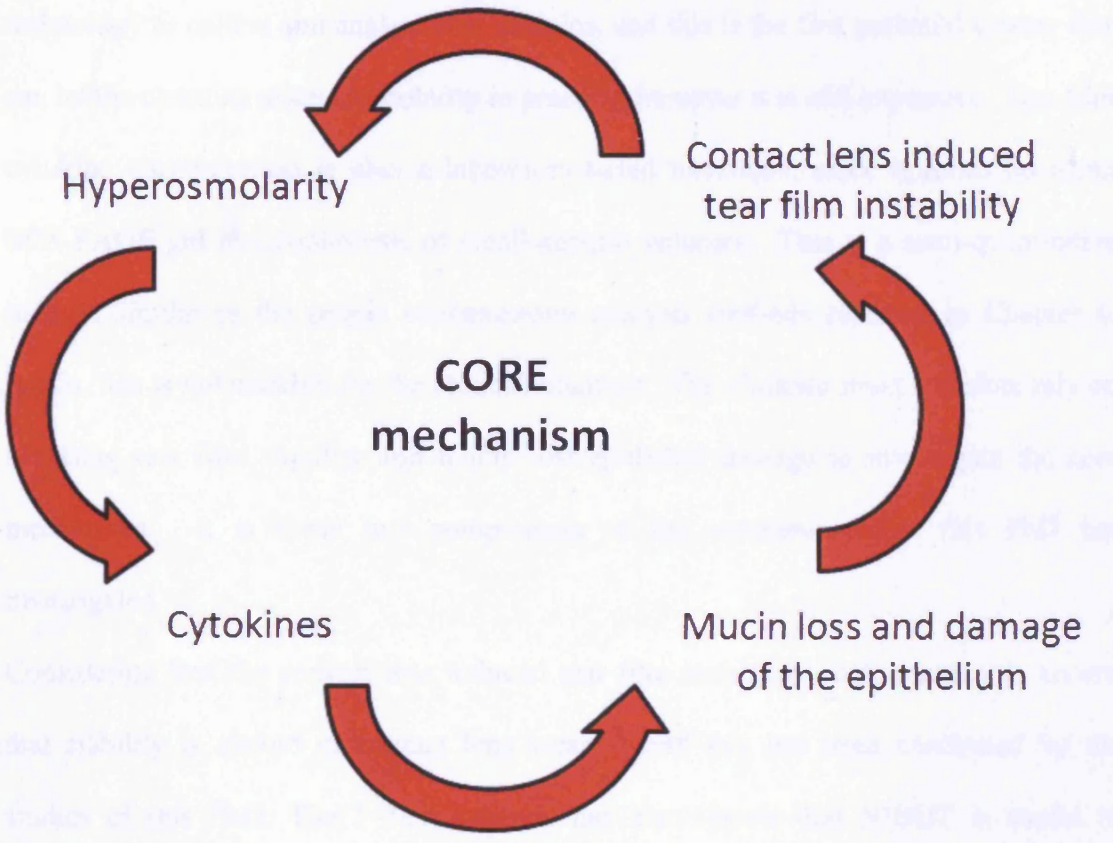


Figure 9.2: The core mechanism for development of dry eye.

Explaining the core mechanism in simple terms, contact lens wear induces tear film instability leading to increased tear film evaporation and its associated hyperosmolarity. This in turn causes ocular surface inflammation resulting in the release of cytokines. The cytokines impact on tear mucin production, which further destabilises the tear film, closing the loop and perpetuating the destructive cycle.

For the clinician, investigating this mechanism in a clinical setting poses several difficulties. Tear film osmolarity of small volume tear film samples is a significant technical challenge, and traditional methods have relied upon freezing point osmometry. This is an intensive laboratory-based approach, unsuitable for clinical practice. The new TearLab™ system from OcuSense Inc (San Diego, USA) uses "lab-on-a-chip"

technology to collect and analyse tear samples, and this is the first potential system that can let the clinician assess osmolarity in practice, however it is still expensive. Tear film cytokine concentration is also a laboratory-based technique, since it relies on using SDS-PAGE gel electrophoresis of small sample volumes. This is a semi-quantitative method similar to the mucin concentration analysis methods reported in Chapter 6. Again, this is not suitable for the clinical situation. The clinician must therefore rely on assessing tear film stability and mucin loss/epithelial damage to investigate the core mechanism. It is these two components of the mechanism that this PhD has investigated.

Considering first the contact lens induced tear film instability component, it is known that stability is altered in contact lens wear[330], and this has been confirmed by the studies of this PhD. The P-Test analysis has also shown that NIBUT is useful in predicting the development of dryness symptoms in contact lens wearers. For the clinician then, assessing NIBUT must be included in the pre-lens fit assessment and in subsequent routine aftercare visits.

The mechanism also proposes that tear film stability is dependent on the quality and quantity of the tear mucins. It is proposed that the aetiologies of LWE and LIPCOF are related to mechanical forces during blinking caused by a deficiency of the mucin layer. So using these two tests as a surrogate, the clinician can, for the first time, have a useful indication of the mucin layer in contact lens patients. This is the crucial additional piece of information that is the missing link in the available range of clinical dry eye tests. Furthermore, by evaluating LWE and LIPCOF, the extent of influence of the core mechanism on each patient's contact lens induced dry eye (CLIDE) can be measured. For the clinician, including LWE and LIPCOF in their routine will give additional guidance on the appropriate lens choice and management of symptoms.

As a final possibility, it could be proposed that the cytokine component of the core mechanism could be assessed using ocular hyperaemia as an indirect measure of inflammation[234,349]. However, the inherent transient variability of this measurement makes this unreliable. Similarly, the variability of ocular surface staining means that it cannot be used as a surrogate indicator for mucin deficiency[17,122,225], by revealing epithelium damage.

Returning again to the fundamental question – "Can the development of dryness symptoms in soft contact lens wearers be predicted?" – this PhD has found that, to meet this question, the optimum combination of tests is LIPCOF Sum plus NIBUT plus OSDI, termed the P-Test. Corneal staining, ocular hyperaemia and decreased pre-lens tear film stability do not provide any individual or additional discrimination for dry eye symptoms even though these tests are recommended for dry eye diagnoses. This was felt to be due to the many other potential influences on these signs hindering their predictive power. The single test LIPCOF Sum or dry eye questionnaire OSDI proved to be excellent tests for predicting and evaluating later dry eye symptoms in contact lens wearers, but the P-Test shows outstanding[3] discrimination and prediction of contact lens induced dry eye.

The value of the P-Test now needs to be investigated in longitudinal studies, where symptomatic and asymptomatic soft contact lens wearers are monitored over several years. In this way the predictive power of the P-Test can be assessed and optimised, and the aetiology of LWE and LIPCOF more clearly determined. The P-Test should also be assessed against common types of contact lens materials with the aim of

analysing if the P-Test can provide a clearer recommendation of the optimum contact lens for any individual patient. This should include an assessment of the latest third-generation silicone hydrogels (SiHy), since the results of this PhD have indicated that the current second-generation materials can improve contact lens wearing comfort.
It would also be useful to investigate the benefit of non-lens treatment options, such as liposomal eye sprays and oil-in-water emulsions. These treatments have already been shown to benefit LIPCOF[329] and LWE[283], respectively.

In conclusion, this PhD provides a new view on the prediction of contact lens induced dry eye (CLIDE). Using the P-Test, clinicians can now gain some indication of the potential success that a new patient may have in future contact lens wear. It is recommended that the clinician includes the use of LWE and LIPCOF in their routine, and that they ask their patients to complete the OSDI questionnaire. It is also recommended that, when using the P-Test, since it is important to avoid influencing the tear film, the following order of examination be followed:

1. Tear film stability using the TearScope Plus™ (3 measurements, averaged)
2. Evaluation of the LIPCOF Sum (temporal plus nasal LIPCOF)
3. OSDI last, to exclude bias.

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Appendices:

Appendix 1: Research Presentations

1.1 Lid Wiper Epitheliopathy, Ocular Surface and Tear Film in Symptomatic Contact Lens Wearers

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Conference talk at: European Association for Vision and Eye Research (EVER) Annual Congress 2008, Special Interests Symposium, Portoroz, Slovenia (2008)

Purpose: Lid wiper epitheliopathy (LWE) as well as lid parallel conjunctival folds (LIPCOF) are related to dry eye symptoms in contact lens wearers and are thought to be caused by mechanical forces during blinking. This study investigates whether any correlations are detectable between LWE and LIPCOF and the ocular surface and tear film in soft contact lens wearers. **Methods:** 38 subjects were classified asymptomatic and 23 symptomatic by the Contact Lens Dry Eye Questionnaire. Pre-lens break-up time, ocular hyperaemia, corneal staining, LWE and LIPCOF were assessed in the right eyes of 61 (23 males, 38 females; mean age = 32.1 ±11.4yrs) experienced lens wearers. Pre-ocular fluid was sampled using Schirmer strips pressed onto the temporal conjunctiva, and from harvested contact lenses. Mucins were assessed in dot-blots and in Western blots after electrophoresis on 1% agarose or 4-12% NuPAGE Gels. **Results:** LWE and LIPCOF were significantly increased in the symptomatic group ($p<0.03$). Significant correlations were found between LWE and both temporal LIPCOF ($r=0.67$, $p<0.001$), and nasal LIPCOF ($r=0.39$, $p<0.001$), and between LWE and bulbar

hyperaemia ($r=0.28$, $p<0.001$). MUC5AC reactivity was significantly decreased in symptomatics ($p=0.050$). MUC4 was negatively correlated to temporal LIPCOF and LWE ($r=-0.47$ and -0.46; $p<0.01$), MUC16 and MUC5AC correlated with corneal staining ($0.36<r<0.53$;$p<0.04$). **Conclusions:** Symptomatic contact lens wearers exhibit significantly more LWE and LIPCOF, and decreased MUC5AC reactivity. Decreased mucins are associated with LWE and LIPCOF severity. Correlations between LWE and LIPCOF may reflect their common frictional origin. Increased friction might follow from insufficient mucins at the ocular surface.

1.2 Lid Wiper Epithiolopathy, Lid Parallel Conjunctival Folds and Ocular Signs: Relationship, Predictive Potential and Clinical Impact in Contact Lens Discomfort

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Conference talk at: British Contact Lens Association (BCLA) Annual Clinical Conference, Birmingham, UK (2008)

Purpose: Although comfort is important for contact lens wearers, common clinical tests frequently fail to predict patients' symptoms. Lid wiper epitheliopathy (LWE) and lid parallel conjunctival folds (LIPCOF) appear to be related to dry eye symptoms in lens wearers. This study investigates the predictive value of LWE and LIPCOF as objective measures of discomfort, and their relation to the ocular surface in soft contact lens wearers. **Methods:** Subjects were classified as symptomatic or asymptomatic, using the Contact Lens Dry Eye Questionnaire (CLDEQ). Pre-lens break-up time (PLBUT), limbal and bulbar hyperaemia, corneal staining, LWE and LIPCOF were assessed in the right eyes of 61(23M, 38F; mean age 32.1yrs; range = 18-55) experienced contact lens wearers. Differences between groups, and relationships between LWE, LIPCOF (nasal, temporal and sum) and objective signs were examined using non-parametric analyses. The positive and negative predictive values (PPV and NPV) for symptoms of each objective measure were calculated. **Results:** 38 subjects were classified as asymptomatic, 23 symptomatic. LWE and LIPCOF severity scores were significantly

increased in symptomatic patients (U-test Mann-Whitney. $p<0.031$), whilst no significant differences were found between groups for PLBUT, corneal staining or hyperaemia ($0.29<p<0.88$). The predictive value of temporal LIPCOF was positive=56.9%, negative=77.1 % with a cut-off value of 2, of nasal LlPCOF 70.7%/75.0%/1 (PPV/NPV/cut-off value), of Sum LIPCOF 79.8%/86.5%/2, and of LWE 53.1%/81.1%/1. Significant positive correlations were found between LWE and LIPCOF scores (Spearman Rank, temporal $r=0.67$, $p<0.001$; nasal $r=0.39$, $p<0.0011$) and between LWE and hyperaemia (bulbar, $r=0.28$, $p<0.001$; limbal $r=0.36$, $p<0.001$).

Conclusions: Contact lens wearers with dryness symptoms exhibit significantly increased LWE and LIPCOF, but not increased corneal staining, bulbar hyperaemia or decreased PLBUT. LWE and LIPCOF are significantly correlated: this may reflect their common frictional origin. LlPCOF Sum appears to be most predictive for symptoms.

1.3 Clinical Signs of Discomfort in Contact Lens Wearers

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Poster presentation at: The Association for Research in Vision and Ophthalmology (ARVO) Annual Meeting, Fort Lauderdale, USA (2008)

Invest Ophthalmol Vis Sci 2008;49: E-Abstract 4842

Purpose: Lid wiper epithiolopathy (LWE) and lid parallel conjunctival folds (LIPCOF) are related to dry eye symptoms in contact lens wearers. This study investigates the relationship of LWE, LIPCOF and objective tests of the ocular surface and tear film to discomfort in soft contact lens wearers. **Methods:** Comfort was evaluated using the Contact Lens Dry Eye Questionnaire (CLDEQ). Pre-lens break-up time (PLBUT), ocular hyperaemia, corneal staining, LWE and LIPCOF were assessed in the right eyes of 61 (23M, 38F; mean age=32.1±11.4yrs) experienced lens wearers. The tear film was sampled using Schirmer strips pressed onto the temporal conjunctiva, and from harvested contact lenses. Mucins were assessed in dot-blots and Western blots after electrophoresis on 1% agarose or 4-12% NuPAGE Gels. Non-parametric analyses were used to study differences between groups, and correlations between objective tests, mucins and symptoms. **Results:** 38 subjects were classified asymptomatic and 23 symptomatic by the CLDEQ. LWE and LIPCOF were significantly increased in the

symptomatic group ($p<0.03$). No significant differences were found between groups for PLBUT, corneal staining or hyperaemia ($0.29<p<0.88$). Significant correlations were found between LWE and both temporal LIPCOF ($r=0.67$, $p<0.001$), and nasal LIPCOF ($r=0.39$, $p<0.001$), and between LWE and bulbar hyperaemia ($r=0.28$, $p<0.001$). The positive and negative predictive values (PPV/NPV/cut-off value) of temporal LIPCOF was 56.9%/77.1%/≥2; of nasal LIPCOF 70.7%/75.0%/≥1; of LIPCOF Sum 79.8%/86.5%/≥2; and of LWE 53.1%/81.1%/≥1.MUC5AC reactivity was significantly decreased in symptomatics ($p=0.050$). MUC4 was correlated to temporal LIPCOF and LWE, ($r=-0.47$ and -0.46; $p<0.01$). MUC16 and MUC5AC correlated with corneal staining ($0.36<r<0.53$; $p<0.04$). **Conclusions:** Symptomatic contact lens wearers exhibit significantly more LWE and LIPCOF, and decreased MUC5AC reactivity. LWE and LIPCOF are significantly correlated: this may reflect their common frictional origin. Increased friction might follow from insufficient, or an altered balance of, mucins at the ocular surface.

1.4 Mucins in Symptomatic and Asymptomatic Contact Lens Wearers

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Poster presentation at: Tear Film and Ocular Surface Society, 5th International Conference on the Tear Film and Ocular Surface: Basic Science and Clinical Relevance, Taormina, Sicily, Italy (2007)

Purpose: Lubrication of the ocular surface – one of the functions ascribed to mucins – is pivotal in contact lens comfort. We investigate the relationship between surface mucins, dry eye symptoms, lid wiper epitheliopathy (LWE) and lid parallel conjunctival folds (LIPCOF). **Methods:** Sixty-one experienced contact lens wearers (23M, 38F; age range 18-55 years) were recruited for the study. Ocular surface mucin samples were collected by gently pressing Schirmer strips onto the temporal bulbar conjunctiva. The worn contact lenses and strips were kept frozen until tested, when they were individually extracted in 4MGuHCl with protease inhibitors and RIPA buffer, respectively. Reactivity with antibodies against mucin peptide cores was probed in dot-blots and in western blots after electrophoresis on NuPage bis-tris gels, visualised with fluorescent substrates. **Results:** Subjects were divided into two groups, asymptomatic or symptomatic, according to their responses to the Contact Lens Dry Eye Questionnaire. Similar amounts of material, assessed by absorbances at 210 and 280nm, adhered to

contact lenses and impressions irrespective of dry eye symptoms (Kruskal-Wallis and Dunn post hoc tests, ns.). However, these absorbencies are significantly negatively correlated to PLBUT ($p<0.006$). All mucins described at the ocular surface could be detected, in different ratios in individual extractions. Dry eye symptoms could not be related to individual mucin species, i.e. MUC1, MUC2, MUC5AC, MUC5B or MUC7, presence or reaction intensity. MUC5B, a highly self-aggregating mucin, was positively correlated to LIPCOF ($p=0.006$) and LWE ($p=0.022$). MUC2 was more often undetectable in Schirmers of asymptomatics. **Conclusions:** In soft contact lens wearers, dry eye symptoms could not be simply related to mucin coverage of the ocular surface. The correlation of MUC5B to ocular surface pathology and tendency to higher MUC2 in symptomatics suggest that the mucin species composition of the pre-ocular fluid reflects, and may influence, specific ocular symptoms and signs.

1.5 An Investigation of Limbal and Bulbar Hyperaemia in Normal Eyes

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Poster presentation at: Tear Film and Ocular Surface Society, 5th International Conference on the Tear Film and Ocular Surface: Basic Science and Clinical Relevance, Taormina, Sicily, Italy (2007)

Purpose: To investigate the appearance of limbal and bulbar hyperaemia in normal eyes, their relationship, and the inter-observer agreement of clinical grading. **Methods:** Limbal and bulbar hyperaemia were assessed in four quadrants by two trained observers, using the CCLRU grading scale interpolated into 0.1 increments, on the right eyes of 120 healthy, non-contact lens-wearing subjects (m=57, f=63, median age=45 years, range 18-77). In addition, limbal and bulbar overall hyperaemia were assessed and quadrant-average hyperaemia calculated. Inter-observer agreement was assessed at the start and end of the study (20 subjects each). **Results:** For limbal hyperaemia, the overall grading (1.62 ± 0.46) (mean units ± sd) was not significantly different from the quadrant-average (1.61 ± 0.40). For bulbar hyperaemia, the overall grading (2.02 ± 0.49) was higher than the quadrant-average (1.82 ± 0.39; $p<0.0001$). Significant correlations were found between bulbar and limbal quadrants (Pearson: $r\geq0.43$ $p<0.0001$). Significant differences in hyperaemia were found between quadrants

(repeated measures, $p<0.0001$), with nasal and temporal redder than superior and inferior quadrants. Small effects of age and gender were found for limbal hyperaemia. The inter-observer 95% limits of agreement were similar at the start and end of the study, and were larger for overall (0.57) compared to quadrant-average (0.28) hyperaemia. **Conclusions:** 1) A limbal hyperaemia above 2.5 may be considered abnormal. 2) A bulbar hyperaemia above 2.6 units (quadrant-average) or 3.0 (overall) may be considered abnormal. 3) Limbal and bulbar hyperaemia were moderately correlated. 4) Grading 96 of overall hyperaemia was less repeatable than using a quadrant average.

1.6 The Relationship Between Lid Wiper Epitheliopathy, Lid Parallel Conjunctival Folds and Ocular Surface in Symptomatic and Asymptomatic Contact Lens Wearers

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Poster presentation at: Tear Film and Ocular Surface Society, 5th International Conference on the Tear Film and Ocular Surface: Basic Science and Clinical Relevance, Taormina, Sicily, Italy (2007)

Purpose: Lid wiper epithiolopathy (LWE) and lid parallel conjunctival folds (LIPCOF) are valuable tests in dry eye patients. This study investigates the relationship between lid wiper epithiolopathy (LWE) and lid parallel conjunctival folds (LIPCOF), and their relation to the ocular surface, in soft contact lens wearers. **Methods:** Subjects were divided into two groups (asymptomatic or symptomatic) according to their responses to the Contact Lens Dry Eye Questionnaire (CLDEQ). Pre-lens break-up time (PLBUT), limbal and bulbar hyperaemia, corneal staining, LWE, and temporal and nasal LIPCOF were assessed in the right eyes of 61 (23M, 38F; age 32,1 range= 18-55) experienced contact lens wearers. LWE and LIPCOF were classified using a four-grade scale, the further objective signs were classified into four grades, interpolated in 0.1 increments. Differences between groups and relationship between LWE, LIPCOF and objective signs were examined using non-parametric analysis. The predictive values (both

positive and negative predictive values; PPV and NPV) of each objective measure for symptoms were calculated. **Results:** 38 subjects were classified as asymptomatic, 23 symptomatic. LWE and LIPCOF severity scores were significantly increased in symptomatic patients (Utest, $p<0.03$), whilst no significant differences were found between groups for PLBUT, corneal staining or hyperaemia ($0.29<p<0.88$). The predictive value of LIPCOF (temporal) was 56.9%/77.1% (PPV/NPV), of LIPCOF (nasal) 70.7%/75.0%, and of LWE 53.1%/81.1%. Significant positive correlations were found between LWE and LIPCOF scores (temporal $r=0.67$, $p<0.001$; nasal $r=0.39$, $p<0.001$), and between LWE and hyperaemia (bulbar, $r=0.28$, $p<0.001$; nasal $r=0.36$, $p<0.001$). **Conclusions:** Contact lens wearers with dryness symptoms exhibit significantly more LWE and LIPCOF, but not corneal staining, bulbar and hyperaemia or decreased PLBUT. LWE and nasal LIPCOF appear to be valuable tests to predict dry eye in hydrogel contact lens wearers. LWE and LICPOF are significantly correlated.

Appendix 2: Papers

2.1 Limbal and Bulbar Hyperaemia in Normal Eyes

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Ophthal. Physiol. Opt. 2008 28: 13–20

Limbal and Bulbar Hyperaemia in Normal Eyes

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Abstract

Purpose: To investigate the appearance of limbal and bulbar hyperaemia in normal eyes, their relationship and the inter-observer agreement of clinical grading.

Methods: The right eyes of 120 healthy, non-contact lens-wearing subjects (m = 57, f = 63, median age = 45 years, range 18–77 years) were examined by two trained observers. Limbal and bulbar hyperaemia were scored using the Cornea and Contact Lens Research Unit (CCLRU) redness grading scales interpolated into 0.1 increments. Redness of four quadrants, and overall, were assessed, and quadrant-average redness was calculated. Inter-observer agreement was assessed at the start and end of the study (20 subjects each).

Results: For limbal redness, the overall (1.62 ± 0.46) (mean units ± S.D.) was not significantly different from the quadrant-average (1.61 ± 0.40) score. For bulbar redness, the overall (2.02 ± 0.49) was higher than the quadrant-average (1.82 ± 0.39) score ($p < 0.0001$). Significant correlations were found between bulbar and limbal quadrants (Pearson: $r \geq 0.43$, $p < 0.0001$). Significant differences in redness were found between quadrants ($p < 0.0001$), with nasal and temporal redder than superior and inferior quadrants. Small effects of age and gender were found for limbal redness. The inter-observer 95% limits of agreement were similar at the start and end of the study. They were larger for overall (0.57) compared with quadrant-average (0.28) redness.

Conclusions: For similar populations, a limbal redness above 2.5 or a bulbar redness above 2.6 (quadrant-average) or 3.0 (overall) may be considered abnormal. Limbal and bulbar redness were correlated. Quadrant-average scores are recommended instead of overall scores, as inter-observer agreement was better.

Keywords: bulbar hyperaemia, bulbar redness, clinical grading, limbal hyperaemia, limbal redness, normal

Introduction

Moderate to severe hyperaemia of the anterior eye is a common sign associated with an unhealthy eye. The redness observed is the result of an increase in the volume of blood in the anterior scleral, bulbar conjunctival and limbal vessels and occurs in response to inflammation, irritation and systemic disease (Papas, 1998; Albietz, 2001; Dumbleton *et al.*, 2001, 2006; Solomon *et al.*, 2001; Brennan *et al.*, 2002; Aasuri *et al.*, 2003; Coles *et al.*, 2004; Murphy *et al.*, 2007). A review of the literature suggests that bulbar hyperaemia is more typically caused by general ocular and systemic factors, while limbal hyperaemia is associated with corneal 'stress' (e.g. keratitis, infiltrates, staining, abrasion and hypoxia) (Kanski, 1994; Papas *et al.*, 1997; Papas, 1998; Wu *et al.*, 2000; Solomon *et al.*, 2001; Malet *et al.*, 2003; Stapleton *et al.*, 2003; Sweeney, 2003; Efron, 2004). Even though limbal hyperaemia is an important indicator of corneal stress, particularly in contact lens wear (Papas *et al.*, 1997; Papas, 1998; Dumbleton *et al.*, 2001, 2006; Brennan *et al.*, 2002; Malet *et al.*, 2003; Stapleton

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et al., 2003; Coles *et al.*, 2004) and normal levels of some appearances evaluated by the Cornea and Contact Lens Research Unit (CCLRU) grading scale are published (Dundas *et al.*, 2001; Mackinven *et al.*, 2001; Murphy *et al.*, 2007), the expected clinical appearance of the normal limbal vasculature is unknown. Furthermore, although there is an obvious anatomical link between limbal redness and bulbar redness, the literature review suggests that there may be some dissociation depending on the cause of the hyperaemia. Some studies have assessed both bulbar and limbal redness with contact lens wear (Papas *et al.*, 1997; Papas, 1998; Dumbleton *et al.*, 2001, 2006; Brennan *et al.*, 2002; Malet *et al.*, 2003; Stapleton *et al.*, 2003; Coles *et al.*, 2004) but not for healthy, non-contact-lens-wearing normal subjects. Also, to our knowledge, no previous studies have considered the relationship between limbal and bulbar hyperaemia.

As hyperaemia is an important clinical sign of ocular disease or inflammation, grading scales are frequently used to assess the severity or degree of change in bulbar and limbal redness (McMonnies and Chapman-Davies, 1987a,b; Papas *et al.*, 1997; Papas, 1998; Dumbleton *et al.*, 2001, 2006; Brennan *et al.*, 2002; Malet *et al.*, 2003; Stapleton *et al.*, 2003; Wolffsohn and Purslow, 2003; Coles *et al.*, 2004). These scales have utilised verbal description, photographs or paintings that illustrate an increasing level of hyperaemia, and they have been particularly used in clinical studies (McMonnies *et al.*, 1982; McMonnies and Chapman-Davies, 1987a,b; Begley *et al.*, 1996; Guillon and Shah, 1996; Papas *et al.*, 1997; Efron, 1998). With the introduction of digital imaging into clinical ophthalmic practice, it is possible to obtain a permanent record of the appearance of the eye. However, where this is not available to the clinician, verbal (Mandell, 1987; Woods, 1989) or pictorial (McMonnies and Chapman-Davies, 1987a; University of New South Wales School of Optometry, 1996; Efron, 1998) grading scales may be used to record ocular status and allow comparison across time. It is important that the clinician knows which grade or grades signify normality (in order to determine what is abnormal), and for the grading scale to be reliable and repeatable. Some of the commonly used grading scales (Terry *et al.*, 1993; University of New South Wales School of Optometry, 1996; Efron, 1998) imply that normality and abnormality are found at the same grading-scale level for each clinical appearance (i.e. the scales are aligned), as proposed by Woods (1989). For example, the CCLRU grading scale states that in general, a grade of slight (grade 2) or less is considered within normal limits. However, previous studies (McMonnies and Chapman-Davies, 1987a; Dundas *et al.*, 2001; Mackinven *et al.*, 2001; Murphy *et al.*, 2007) have shown that the normal ocular appearance is not necessarily the lowest level on a grading-scale, nor is the grading scale level the same for each clinical appearance. As shown in *Figure 1*, corneal fluorescein staining (Dundas *et al.*, 2001) was typically less than palpebral roughness (Mackinven *et al.*, 2001) (Kolomorogov–Smirnov, $z_{197} = 12.0$, $p < 0.001$) which was typically less than bulbar conjunctival hyperaemia (Murphy *et al.*, 2007) (Kolomorogov–Smirnov, $z_{216} = 5.0$, $p < 0.001$). Also, Efron *et al.* (2001) showed, in a comparison of four different grading scales, that cross-comparison between grading scales could not be made.

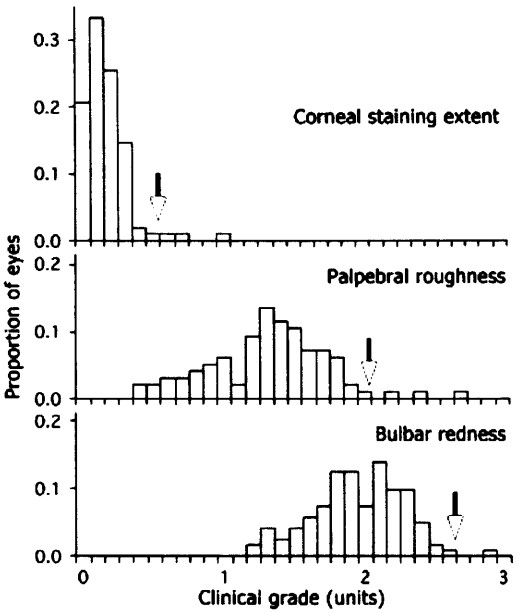

Figure 1. The typical grade of a normal eye of corneal staining (top panel, $n = 102$, Dundas *et al.*, 2001), palpebral roughness (middle panel, $n = 96$, MacKinven *et al.*, 2001) and bulbar redness (bottom panel, $n = 121$, Murphy *et al.*, 2007) is shown. For each of these studies the average of scored zones is shown. None of these studies of real eyes scored overall appearance. The arrows mark the upper 95% confidence limits, above which an eye may be considered to have an unusually high score.

Our study continues on from our previous reports (Dundas *et al.*, 2001; Mackinven *et al.*, 2001; Murphy *et al.*, 2007) on the normal clinical grading scores for limbal and bulbar hyperaemia, their relationship and the inter-observer agreement for such grading.

Methods

Subjects

One hundred and twenty subjects (male = 57, female = 63, median age = 45y, range = 18–78 years; *Figure 2*) were randomly selected from patients attending the optometry practice of Horst Riede GmbH,

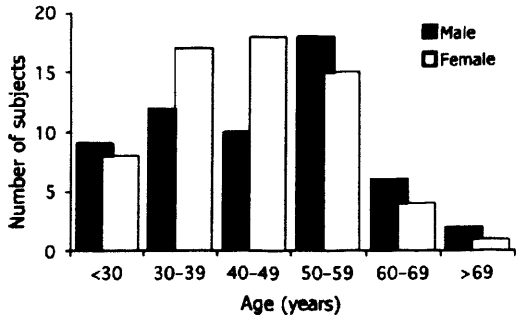

Figure 2. Frequency of age (median age = 45 years) and gender (male = 57, female = 63) among the 120 subjects.

Weinheim, Germany. All procedures obtained the approval of the Ethics Committee of the Cardiff University and were conducted in accordance with the requirements of the Declaration of Helsinki.

Since McMonnies and Ho (1991) described how conjunctival hyperaemia can vary with factors such as lack of sleep, eyestrain, wind, dust, smog, smoke and alcohol, we screened our subjects for these factors. All subjects had no current ocular disease, systemic disease, medication or allergy known to affect ocular hyperaemia. Contact lens wearers were included, if they had not worn contact lenses during the previous two weeks. Two weeks has been considered sufficient time for any contact lens related ocular hyperaemia to have resolved (Efron *et al.*, 2002).

To assess inter-observer agreement, the first twenty subjects (subject numbers 1–20) (male = 6, female = 14, median age = 41 years) and the last twenty subjects (subject numbers 101–120) (male = 7, female = 13, median age = 43 years) were assessed by both observers involved in the study.

Grading

Bulbar and limbal hyperaemia were assessed by two trained observers (optometrists) using the CCLRU grading scale (Johnson & Johnson Vision Products, Inc., Bracknell, Berkshire, UK) (Terry *et al.*, 1993; University of New South Wales School of Optometry, 1996), interpolated to 0.1 unit increments. The observers were instructed that, if they considered the ocular hyperaemia to be less than grade 1, they should attempt to grade between the pictured grade 1 and an imagined perfectly white eye, which would represent grade 0. The ability to extrapolate CCLRU grading scales has been demonstrated in previous studies (Dundas *et al.*, 2001; Mackinven *et al.*, 2001; see *Figure 1*). These photographic scales were developed by the CCLRU at the University of New South Wales (Sydney, Australia) and each of the ten anterior-eye-appearance scales comprises four photographs that increase in the appearance of severity and are labelled: 1. Very slight, 2. Slight, 3. Moderate and 4. Severe. Bulbar and limbal hyperaemia were graded using the bulbar and limbal redness scales, respectively. Since the CCLRU grading scale was designed for use with a slit-lamp bio-microscope (Andersen *et al.*, 1996), the right eye only of each subject was examined using a slit-lamp bio-microscope (×12 magnification). To provide consistent and even illumination over the eye, the slit-lamp diffuser was used, the beam-width was full and the brightness was set to maximum. Bulbar and limbal overall scores were evaluated by the observer making a judgement of the overall redness appearance. Then, the subject's position of gaze was directed to allow grading of four quadrants: superior, nasal, inferior and temporal. Bulbar and limbal quadrant-average scores were calculated as the average of the scores of the four quadrants.

Data analysis

Since the interpolated grading scales approximate an interval scale (Papas, 2000) and Barbeito and Simpson (1991) have argued that parametrical statistical tests can be applied to such data, we conducted both parametric and non-parametric tests (where there was an equivalent), but only report the parametric tests as the outcomes were similar. Differences between means were examined by *t*-test and ANOVA, relations were analysed by Pearson's correlation. Differences between distributions were evaluated with the two-sample Kolmogorov–Smirnov test. Inter-observer agreement was defined as the agreement coefficient of Bland and Altman (1986). The agreement coefficient is 1.96 times the standard deviation of the inter-observer difference scores (i.e. score from Observer 1 minus score from Observer 2). Differences of the agreement coefficients between first and last inter-observer group was evaluated by O'Brien's test for homogeneity of variance. The data were analysed by use of WINSTAT 2005.1-Software (R. Fitch Software, Bad Krozingen, Germany) and JMP IN 5.1.2 (SAS Institute, Belmont, Canada).

Results

Prevalence study

The distributions of overall and quadrant-average limbal and bulbar redness scores for the 120 subjects are shown in *Figure 3*. For bulbar redness, the quadrant-average grade (1.82 ± 0.39) (mean units ± S.D.) was significantly less than the overall grade (2.02 ± 0.49) (*post hoc t*-test: $t_{119} = 8.05$, $p < 0.001$), whereas, for limbal redness, the quadrant-average (1.61 ± 0.40) and overall (1.62 ± 0.46) grades were

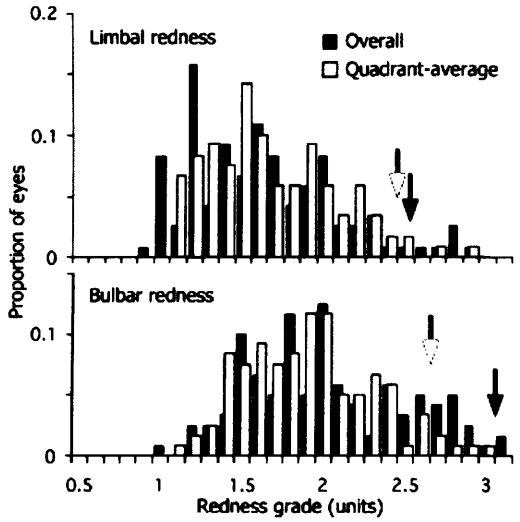

Figure 3. Distribution of overall and quadrant-average scores for limbal and bulbar redness (n = 120). The arrows show the 95% confidence limits, above which a redness score may be considered as unusual.

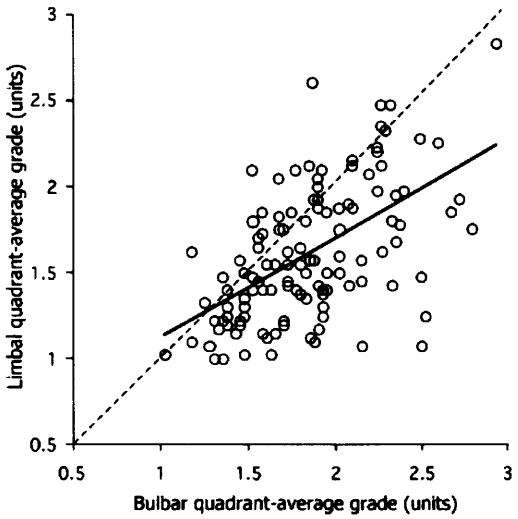

Figure 5. The relationship between limbal and bulbar quadrant-average redness. The dashed line shows a slope of 1 (i.e. limbal redness equals bulbar redness), while the solid line illustrates the line of best fit. (slope = 0.57, Pearson r (119) = 0.56, $p < 0.001$.

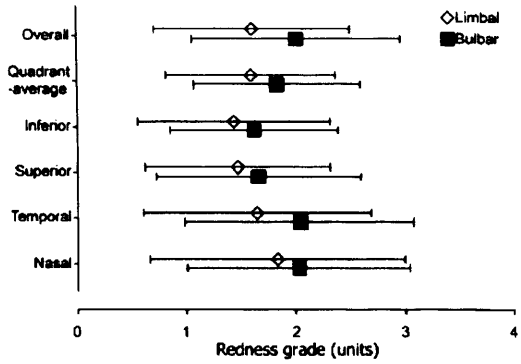

Figure 4. Mean and 95% confidence limit (error bars) of limbal and bulbar redness for overall, the quadrant-average and each quadrant.

similar (t_{119} = 0.88, p = 0.38) (*Figure 4*). There were significant differences between quadrants for both limbal (repeated measures ANOVA, $F_{3,116}$ = 19.7, $p < 0.0001$) and bulbar ($F_{3,166}$ = 49.0, $p < 0.0001$) redness. As shown in *Figure 4*, the nasal and temporal quadrants were redder than the superior and inferior quadrants for both limbal and bulbar redness ($t_{119} \geq 3.44$, $p < 0.001$). Significant correlations were found between bulbar and limbal redness scores in all quadrants (Pearson $r_{119} \geq 0.43$ $p < 0.0001$). For each of those correlations, as illustrated in *Figure 5*, on average, limbal and bulbar redness were similar for low grades, but as bulbar redness increased, limbal redness increased more slowly. Bulbar redness was significantly higher than limbal redness in all four quadrants and for the overall scores ($t_{119} \geq 4.21$, $p < 0.001$) (*Figure 3*). Females had slightly lower limbal redness quadrant scores than males (mixed-model ANOVA, $F_{1,116}$ = 3.78, p = 0.054) and there were small, but statistically significant, decreases in superior, inferior and overall limbal redness with age (multiple regression analysis, $F_1 \geq 3.96$, $p < 0.05$). There were no significant effects of age or gender on bulbar redness.

Inter-observer agreement study

In general, the 95% agreement coefficients were larger and more variable for individual quadrants than the quadrant-average, were larger for overall than quadrant-average and did not vary between the start and end of the prevalence study. The agreement coefficients were similar between the two groups (first and last 20 subjects) (O'Brien's test for homogeneity of variance, $F_{1,38} < 1.44$, $p > 0.23$), except for limbal nasal quadrant (0.82 vs 0.55; $F_{1,38}$ = 3.7, p = 0.06) and limbal quadrant-average (0.35 vs 0.22; $F_{1,38}$ = 4.8, p = 0.03), when the agreement was better for the last 20 group. When both groups were combined, agreement coefficients for limbal and bulbar redness were not significantly different ($F_{1,78} < 2.74$, $p > 0.10$), except for the nasal quadrant (0.70 vs 0.53; $F_{1,78}$ = 4.0, p = 0.05), when agreement was better for bulbar than limbal redness. When limbal and bulbar scores were combined, the agreement coefficient was better for quadrant-average (0.28) than overall (0.57) redness ($F_{1,158}$ = 36, $p < 0.0001$).

Discussion

This study has described the typical findings in normal subjects concerning limbal and bulbar hyperaemia, using the CCLRU grading scales. Firstly, the limbal redness grades were significantly lower than the bulbar redness grades, when assessed either by quadrant, quadrant-average or overall grade. Secondly, for bulbar hyperaemia, the overall redness grade was significantly higher than the quadrant-average; while for limbal hyperaemia, there was no significant difference. Thirdly, the temporal and nasal quadrants were significantly redder than the superior and inferior quadrants for both limbal and bulbar hyperaemia. However, there were significant correlations between limbal and bulbar redness grades across all measured parameters.

The significant differences between limbal and bulbar redness may reveal either a consistent difference in the redness of these two ocular areas or it may be a feature of the grading scales used (Efron *et al.*, 2001). Our review of current literature suggested that increased limbal hyperaemia, while an associated feature of bulbar hyperaemia, can also be produced separately by conditions associated with corneal stress (Kanski, 1994; Papas *et al.*, 1997; Papas, 1998; Wu *et al.*, 2000; Solomon *et al.*, 2001; Malet *et al.*, 2003; Stapleton *et al.*, 2003; Sweeney, 2003). This suggests that the control of vasodilation may be different between these two ocular areas, and, that under normal conditions in healthy subjects, the baseline redness of the two areas may not necessarily be similar, even when there is no physiological stress. This is supported by our finding of moderate (albeit significant) correlations between the two areas (*Figure 5*). Thus, the difference between redness grades could have an underlying physiological basis.

However, it is more likely that the differences in redness grades are a feature of the CCLRU grading scales. Each grading scale is represented by a series of four labelled, sample images, of progressing severity of condition. Grading scales are typically divided into four or five grades. Nevertheless, interpolating the scales into decimal intervals increases their sensitivity (Bailey *et al.*, 1991; Sparrow *et al.*, 2000). The selection of these images was by expert opinion, but the intervals between successive images may (Papas, 2000) or may not (Wolffsohn, 2004) be equal and the grading scales may not be aligned (i.e. same score for same level of severity as proposed by Woods (1989)). Papas (2000) reported that by decimalising the CCLRU grading scale for bulbar redness, the grading approximated an interval scale. If scales are aligned, a single grading result can be more easily interpreted with respect to normal limits, as the user need not remember different confidence limits for different clinical appearances (Efron *et al.*, 2001). Differences between the appearances of normal healthy eyes for corneal fluorescein staining (Dundas *et al.*, 2001), palpebral conjunctival roughness (Mackinven *et al.*, 2001) and bulbar conjunctival redness (Murphy *et al.*, 2007) shown in *Figure 1*, suggests that the CCLRU scales are not aligned. In other words, each clinical appearance of the CCLRU grading scale has an individual grade level for 'normal' that may not correspond with the 'normal' grade for other clinical appearances (*Figure 1*). A similar situation found in our study (*Figure 4*) suggests that the limbal and bulbar redness scales may not be inter-related (aligned). Also, the slope of the correlations between limbal and bulbar redness (*Figure 5*) may indicate that the two redness scales do not change at the same rate. Overall, this suggests that the authors of the CCLRU grading scale did not create grading scales with a universal scaling (Woods, 1989), despite the use of universal language for naming of the sample images. Our study and previous similar studies (Dundas *et al.*, 2001; Mackinven *et al.*, 2001; Murphy *et al.*, 2007), show that each CCLRU grading scale has the potential to detect changes in clinical appearance, but comparisons between the scales requires some form of calibration. Some of these limitations may not occur with the Efron grading scales which use images painted by a medical illustrator (Wolffsohn, 2004).

When considering the redness scores themselves, the quadrant-average bulbar redness scores compared well with a previous study of normal bulbar hyperaemia which also used the CCLRU grading scale (Murphy *et al.*, 2007). The mean quadrant-average bulbar redness was 1.8 units and the upper 95% confidence limit for normality was 2.6 units, while Murphy *et al.* (2007) found mean quadrant-average score of 1.9 units and an upper 95% confidence limit of 2.6 units (*Figure 1*). Those two distributions were slightly different (Kolomorogov–Smirnov, $z_{240} = 1.39$, $p = 0.042$), eyes tending to be slightly less red in our study (*t*-test, $t_{240} = 1.87$, $p = 0.06$). In a previous study of 40 subjects, an average overall bulbar redness of 0.78 units and an upper 95% confidence limit of 2.3 units, using a six-level grading scale, were found (McMonnies and Chapman-Davies, 1987a). In our study, the mean limbal redness quadrant-average score was 1.6 units and the upper 95% confidence limit was 2.4 units. From our study, we suggest a bulbar redness score of greater than 2.6 units or a limbal redness score of greater than 2.4 units may be considered unusual, when derived from the quadrant average, using the CCLRU scales. Although the time of the day may influence the grade of normal hyperaemia, the time of observation in this study was restricted to office-hours (10:00 hours to 18:00 hours), and, ocular redness is reported to be relatively constant in that period (Duench *et al.*, 2007).

However, the quadrant-average score is not the typical method of achieving a score, more commonly

the clinician makes a single overall judgment of the redness, even though the images used on the CCLRU scale are of the temporal quadrant. For bulbar hyperaemia, this produced a significantly higher average redness score of 2.0 units, with an upper 95% confidence limit of 3.0 units. For limbal hyperaemia, the average overall redness score was again 1.6 units, with an upper 95% confidence limit of 2.5 units. Magnification increases visibility of the conjunctival vasculature, so a person who, when observed without a slit lamp from 1 m appears to have a white eye, will have a higher redness grade when viewed with a slit lamp (Murphy *et al.*, 2007). The CCLRU grading scale is commonly used during a slit-lamp examination (Papas, 2000; Murphy *et al.*, 2007; Sorbara *et al.*, 2007). This difference between quadrant-average and overall redness scores suggests that there was a difference in the grading criteria adopted by the observers. When judging overall bulbar redness, the less red superior and inferior quadrants were not visible, and thus, the overall bulbar redness score may have been based on the redness of the nasal and temporal quadrants only. This hypothesis is supported by the lack of a significant difference between the overall bulbar redness score and the average of the nasal and temporal quadrants (2.04 ± 0.46 units; *post hoc* *t*-test: $t_{119} = 1.47$, $p < 0.14$). This effect was not seen for the overall limbal redness scores, possibly because it was easier to see more of the superior and inferior limbal regions when judging the overall limbus redness.

The third observation was that the temporal and nasal quadrants were redder than the superior and inferior quadrants. This is consistent with the findings of previous studies (McMonnies and Chapman-Davies, 1987b; Papas *et al.*, 1997; Murphy *et al.*, 2007), possibly reflecting the greater exposure of these quadrants to environmental conditions.

The agreement between the quadrant-average redness grades found by our two observers (0.4–0.8 units) was comparable with similar studies that interpolated decimal (0.1 unit) increments of CCLRU grading scales (Dundas *et al.*, 2001; Mackinven *et al.*, 2001; Murphy *et al.*, 2007), and was better than the inter-observer agreement of 1.0 units found when using a similar photographic grading scale that was not interpolated (McMonnies and Chapman-Davies, 1987a). Bailey *et al.* (1991) described the benefits of using increments that are related to the agreement between observations. As noted previously (Dundas *et al.*, 2001; Mackinven *et al.*, 2001), the decimal interpolation of such grading scales can be learnt and applied effectively with only modest training by inexperienced observers (Efron *et al.*, 2003a,b). The improvement in agreement for limbal nasal quadrant and quadrant-average at the end of the study may reflect just such a training effect for the observers in our study.

To our knowledge, our study was the first direct comparison of overall redness grading with the quadrant-average. As the agreement coefficients for overall redness were about twice as large as for quadrant-average redness, the additional effort required to grade each quadrant, then taking the average, may be worthwhile in the clinic and in research studies. This difference may explain some apparent differences in reported inter-observer agreement between studies of real eyes (that have used quadrant-average scores) (Dundas *et al.*, 2001; Mackinven *et al.*, 2001; Murphy *et al.*, 2007) and studies of photographs of eyes (that have used overall scores) (Efron, 1998; Chong *et al.*, 2000; Papas, 2000; Efron and Chaudry, 2007). A difference of 0.3 units or more for quadrant-average redness, more than 0.6 units for limbal overall redness and more than 0.5 units for bulbar overall redness, between two observations by two trained observers is likely to represent a real difference in the hyperaemia of that eye.

In conclusion, normal limbal redness appearance has been described for the first time. Although higher than expected, it has a lower grade than that for normal bulbar redness, which was observed to be similar in appearance to previous studies using the CCLRU grading scales. However, these higher redness grades observed for ocular hyperaemia are not necessarily because of a greater physical redness, but may be due to features of the grading scale used. Bulbar redness and limbal redness were inter-related, although the strength of this relationship is weakened by the poor alignment of the CCLRU grading scales. Further study, perhaps using an alternative grading scale, would indicate whether a real difference in redness between the limbal and bulbar areas is present. There is also a need for the development of a series of inter-related (aligned) grading scales, in which similar grades in the individual scales indicate similar ocular states.

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2.2 Clinical Tests for Successful Contact Lens Wear: Relationship and Predictive Potential of Lid Wiper Epitheliopathy, Conjunctival Folds and Ocular Signs

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ORIGINAL ARTICLE

Clinical Tests for Successful Contact Lens Wear: Relationship and Predictive Potential

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ABSTRACT

Purpose. Although comfort is important for contact lens wearers, common clinical tests can fail to predict patients' symptoms. Lid wiper epitheliopathy (LWE) and lid parallel conjunctival folds (LIPCOF) are related to dry eye symptoms in lens wearers. This study investigates the predictive value of LWE and LIPCOF as objective measures of discomfort, and their relation to the ocular surface in soft contact lens wearers.

Methods. Subjects were classified as symptomatic or asymptomatic, using the Contact Lens Dry Eye Questionnaire (CLDEQ). Pre-lens tear break-up time (PLBUT), limbal and bulbar hyperaemia, corneal staining, LWE and LIPCOF were assessed in the right eyes of 61 (23 M, 38 F; mean age 32.1 years; range = 18 to 55) experienced contact lens wearers. Differences between groups, and relationships between LWE, LIPCOF (nasal, temporal and sum) and objective signs were examined using non-parametric analyses. The positive and negative predictive values for symptoms of each objective measure were calculated.

Results. Thirty eight subjects were classified as asymptomatic, 23 symptomatic. LWE and LIPCOF severity scores were significantly increased in symptomatic patients (U-test, $p < 0.03$), while no significant differences were found between groups for PLBUT, corneal staining or hyperaemia ($0.29 < p < 0.88$). Significant positive correlations were found between LWE and LIPCOF scores (temporal $r = 0.67$, $p < 0.001$; nasal $r = 0.39$, $p < 0.001$), and between LWE and hyperaemia (bulbar, $r = 0.28$, $p < 0.001$; limbal $r = 0.36$, $p < 0.001$). Age and gender were different in the two groups ($p < 0.05$). The predictive value of temporal LIPCOF was positive = 56.9%, negative = 77.1% with a cutoff value of ≥2 (PPV/NPV/cutoff value), of nasal LIPCOF 70.7%/75.0%/≥1, of LIPCOF Sum 79.8%/86.5%/≥2, and of LWE 53.1%/81.1%/≥1.

Conclusions. Contact lens wearers with dryness symptoms exhibit significantly more LWE and LIPCOF, but not increased corneal staining, bulbar hyperaemia or decreased PLBUT. LWE and LIPCOF are significantly correlated: this may reflect their common frictional origin. LIPCOF Sum severity scores appear to be most predictive for symptoms.

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Key Words: lid parallel conjunctival folds, conjunctivochalasis, lid wiper epitheliopathy, symptoms, contact lens

Contact lens wear comfort depends on a number of factors, including the interaction between the tear film and the ocular surface. Fifty-three percent of patient drop-outs from contact lens wear in the United Kingdom and 73% in the United States are caused by discomfort.[1] Although the primary reasons for discontinuing contact lens wear are dryness and discomfort,[2] current clinical tests are barely able to predict these symptoms.[3,4] Lid Wiper Epitheliopathy (LWE) and Lid Parallel Conjunctival Folds (LIPCOF) are related clinical signs in contact lens-induced dry eye.[5,6]

LWE is a clinically observable alteration in the epithelium of the advancing lid margin, the lid wiper. In patients with dry eye, the thickness of the tear film is insufficient to separate the ocular surface and lid wiper.[7] Due to this deficiency, the lid wiper is subjected to trauma during the entire lid movement, as a result of the continual rubbing of the narrow surface area of lid wiper tissue against the corneal surface, including any contact lens.[6,7]

LIPCOF are subclinical folds in the lateral, lower quadrant of the bulbar conjunctiva, parallel to the lower lid margin[5,8,9] (Fig. 1), easily observable by slitlamp. Several causes of bulbar conjunctival folds are hypothesized: conjunctival 'looseness' as a result of inflammatory processes, a decrease of elastic fibers, aging, and lymphatic dilation by mechanical forces between the lower lid and conjunctiva that gradu-

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FIGURE 1.
LIPCOF degree 2, two parallel conjunctival folds at the temporal quadrant of the eye.

ally interfere with lymphatic flow.[10–15] Bulbar conjunctival folds were first described by Hughes[16] and named conjunctivochalasis. Age does not appear to be correlated with subclinical conjunctival folds.[8] Höh et al.[8] described these as LIPCOF, as distinct from conjunctivochalasis, where an age-association was suspected by the authors. To avoid confusion, in this study LIPCOF refers only to subclinical conjunctival folds at a defined location, observed without fluorescein instillation and used as a test for predicting dry eye in non- and contact lens wearers.[5,8,14]

There are clear relations between LWE and LIPCOF, but their nature is still unknown. Moreover, the predictive values of these tests have not been reported in literature.

This study investigates the potential to predict contact lens wear discomfort by assessing the relationship between LIPCOF, LWE, and standard clinical tests in a cohort of symptomatic and asymptomatic contact lens wearers.

METHODS

The right eye of 61 experienced contact lens wearers (male = 23, female = 38; mean age 32.1 years, range = 18 to 55), randomly selected from the contact lens patients of Horst Riede GmbH, Weinheim, Germany, were examined. The subjects were grouped into symptomatic and asymptomatic patients according to their response to the Contact Lens Dry Eye Questionnaire (CLDEQ).[17]

Inclusion and Exclusion Criteria

Subjects were excluded if they had any ocular/systemic pathology or allergy known to affect the conjunctiva, e.g., Sjögren's Syndrome, rheumatoid arthritis, diabetes, infections, hay fever, or if they were taking any medication known to affect the ocular surface or tear film. Subjects were also excluded if they had undergone ocular surgery or were pregnant. All subjects had worn soft monthly disposable lenses (24 to 62% water content) for at least 6 months; high water content lenses were excluded. The lenses must have been worn for 3 weeks before the evaluation visit and used at least 4 times a week in normal wearing modality. Time of examination was between 3:00 p.m. and 6:00 p.m. All procedures were conducted in accordance with the Declaration of Helsinki (1983), and approval for the study was given by the Cardiff School of Optometry and Vision Sciences Ethics Committee. All subjects signed an informed consent form before participating to this study.

Tests and Classification

Limbal and bulbar hyperaemia, and corneal staining were classified into four grades, interpolated in 0.1 increments (CCLRU grading scale, University of New South Wales, Sydney, Australia).[18–20] Pre-lens tear break-up time (PLBUT) was assessed by three repeated measurements using the Tearscope (Keeler, UK) with a fine grid insert. LIPCOF was evaluated in the area perpendicular to the temporal and nasal limbus on the bulbar conjunctiva above the lower lid (temporal and nasal LIPCOF, respectively, Fig. 2) with a slitlamp microscope using 18 to 24× magnification, as necessary. The grading score of Höh et al.[8] adapted by Pult and Sickenberger[5] (Table 1) was employed. A further combined LIPCOF score (LIPCOF Sum) was calculated by adding together the nasal LIPCOF grade and temporal LIPCOF grade. LWE was made visible using a combination of instilled fluorescein and lissamine green, and evaluated for both upper and lower lids. A second instillation of both

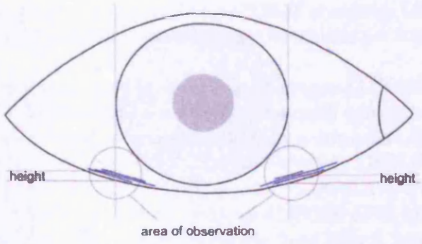

FIGURE 2.
Areas of observation of temporal and nasal LIPCOF. A color version of this figure is available online at wwww.optvissci.com.

TABLE 1.
Grading scale of LIPCOF[5]

	LIPCOF grade
No conjunctival folds **or** disrupted micro-folds in one line	0
One permanent and clear parallel fold **or** one permanent and clear parallel fold plus disrupted micro-folds above	1
Two permanent and clear parallel folds up to a height of 0.2 mm **or** two permanent and clear parallel folds plus disrupted micro-folds above up to a height of 0.2 mm	2
More than two permanent and clear parallel folds higher than 0.2 mm **or** more than two permanent and clear parallel folds plus disrupted micro-folds above higher than 0.2 mm	3

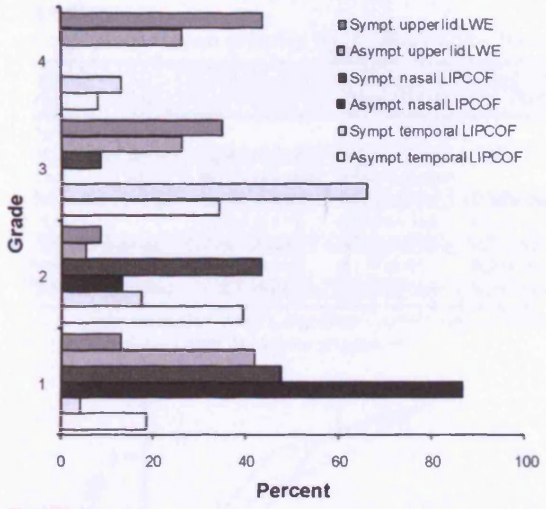

FIGURE 3.
Distribution of LIPCOF and LWE grades in contact lens wearers.

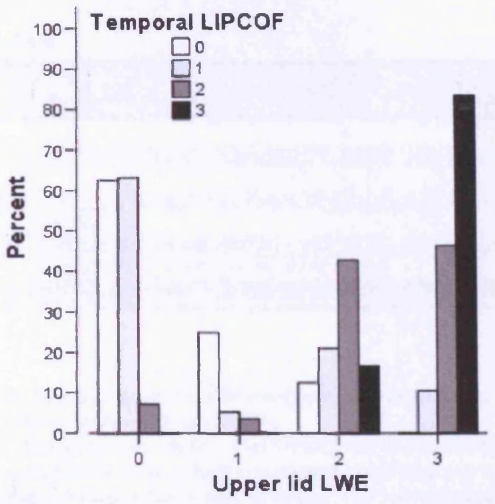

FIGURE 4.
Relation between temporal LIPCOF grade and upper lid LWE.

dyes was carried out after 5 min.[21] LWE was observed using a slitlamp microscope with 18x magnification classified according to Korb et al.[6,7] Care was to taken to differentiate between the fluorescein and lissamine staining associated with Marx's line and that from staining of the lid wiper.[6]

Statistical Analyses

Since the data was ordinal and not normally distributed, non-parametric analyses were used, as appropriate, on WinSTAT 2005.1-Software (R Fitch Software, Bad Krozingen, Germany) and SPSS 14.0 (SPSS, Chicago). Correlations were calculated using Spearman Rank and differences were analyzed by the U-Test (Mann-Whitney). The validity of the Bonferroni correction for data analyzed here is debated in the statistical literature[22–25] and beyond the scope of this paper. We have indicated where significance at 5% is lost after applying the Bonferroni correction. Predictive values were calculated for all significant clinical tests. By plotting the true predictive rate (sensitivity) against the false predictive rate (1-specificity), also known as the receiver operating characteristic curve (ROC), the discrimination of the tests was evaluated by calculating the area under the curve (AUC).

RESULTS

Thirty-eight subjects were classified as asymptomatic, and 23 as symptomatic.

Upper-lid LWE, temporal and nasal LIPCOF, and LIPCOF Sum severity scores were significantly increased in symptomatic patients ($p < 0.03$) (Fig. 3), while no significant differences were found between groups for lower-lid LWE, PLBUT, corneal staining or hyperaemia ($0.29 < p < 0.93$).

Significant positive correlations were found between upper-lid LWE and LIPCOF scores (Fig. 4 and Table 2). Lower-lid LWE was correlated to temporal, but not nasal LIPCOF (Table 2). Upper-lid LWE, but not lower-lid LWE or temporal LIPCOF, were correlated to bulbar and limbal hyperaemia. Temporal LIPCOF was correlated to limbal hyperaemia, but not to bulbar hyperaemia. LWE scores and LIPCOF scores were not correlated to PLBUT or staining. LIPCOF was related to age (temporal $r = 0.36$, $p < 0.002$; nasal $r = 0.45$, $p < 0.001$).

The predictive values for symptoms of temporal LIPCOF were positive = 56.9%, negative = 77.1% and a cutoff value ≥2 (PPV/NPV/cutoff value; for a prevalence of 43% dry eye symptoms, Guillon and Maissa[26]); nasal LIPCOF 70.7%/75.0%/≥1; LIPCOF Sum 79.8%/86.5%/≥2; and LWE (upper lid) 53.1%/81.1%/≥1. The AUC of temporal LIPCOF was 0.685, nasal LIPCOF 0.701, LIPCOF Sum 0.746, and LWE (upper-lid) 0.654 (Fig. 5 and Table 3). Symptomatic patients were significantly older ($p = 0.049$; 35.9 ± 11.8 SD years) than asymptomatics (29.8 ± 10.6 SD years), and there were more females in the symptomatic group ($p = 0.047$).

DISCUSSION

Although PLBUT corneal staining and hyperemia are frequently accepted signs of dry eye, their usefulness as predictors of the development of contact lens-induced dry eye is disputed.[27–29] In this study, no significant differences were found between symptomatic and asymptomatic lens wearers for these clinical signs. The PLBUT probably relates more to the surface properties of the lens than to individual lens wearers.[29–32] As all subjects were experienced, successful, contact lens wearers, it may be assumed that extreme values for redness, staining, etc. would not be seen among such a population and, as such, significant differences and correlations may be less apparent.

In contrast, LIPCOF and LWE were significantly increased in symptomatic contact lens wearers. LWE of the upper-lid appears to correlate well with LIPCOF and hyperemia, but not to corneal staining or PLBUT. No significant correlations were found between LIPCOF and bulbar hyperemia, or staining and PLBUT.

TABLE 2.
Correlation between objective signs, calculated by Spearman Rank

	Temporal LIPCOF	Nasal LIPCOF	Upper-lid LWE	Lower-lid LWE	PLBUT	Corneal staining	Bulbar hyperemia
Temporal LIPCOF							
Nasal LIPCOF	**0.511 (<0.001)**						
Upper-lid LWE	**0.675 (<0.001)**	**0.390 (<0.001)**					
Lower-lid LWE	**0.296 (0.010)[a]**	0.173 (0.092)	**0.315 (0.007)[a]**				
PLBUT	−0.042 (0.375)	0.095 (0.234)	0.074 (0.284)	−0.050 (0.350)			
Corneal staining	0.048 (0.361)	0.171 (0.093)	0.087 (0.251)	0.003 (0.490)	−0.022 (0.433)		
Bulbar hyperemia	0.175 (0.089)	0.174 (0.091)	**0.281 (0.014)[a]**	0.107 (0.205)	−0.030 (0.410)	0.197 (0.064)	
Limbal hyperemia	**0.218 (0.046)[a]**	0.116 (0.186)	**0.361 (0.002)**	0.007 (0.478)	−0.070 (0.295)	0.068 (0.301)	**0.739 (<0.001)**

p-values are in parentheses, significant correlations are bolded.
[a]Not significant after Bonferroni adjustment.

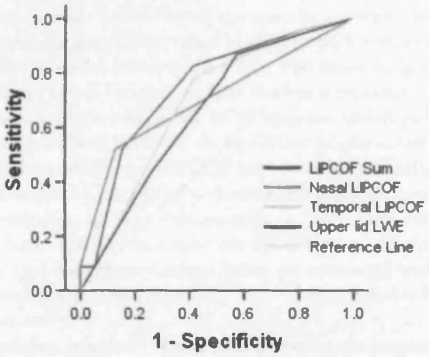

FIGURE 5.
Probability of detecting dry eye symptoms in contact lens wear. The reference line is the line of non-discrimination and represents an AUC of 0.50. A color version of this figure is available online at wwww.optvissci.com.

TABLE 3.
Probability of detecting dry eye symptoms in contact lens wear analyzed by ROC

	AUC	Standard error	Significance	95% Confidence interval
LIPCOF Sum	0.746	0.073	<0.01	0.62–0.87
Nasal LIOCOF	0.701	0.065	<0.01	0.56–0.85
Temporal LIPCOF	0.685	0.069	0.016	0.55–0.82
LWE	0.654	0.071	0.045	0.52–0.80

The significant correlation between LWE and LIPCOF supports our suggestion that both signs have a similar etiology: induced by friction during blinking, as also suggested by Watanabe et al.[15] There is evidence for direct contact of the marginal conjunctiva with the surfaces of the oculus bulbi.[33–35] Stratified squamous epithelium, which is seen in LWE, is a characteristic feature of other body tissues that experience frequent rubbing (e.g., cornea, skin, and oral mucosa)[7] and its presence in the particular region of the lid wiper[34] infers that the marginal conjunctiva is intimately and mechanically associated with the surfaces of the oculus bulbi.

As negative correlations between LIPCOF and non-invasive break up time or tear meniscus height are reported,[5] this friction may result from deficient tear film stability or volume. Tear volume may genuinely be reduced in cases with LIPCOF, or the tear film may be partly bound in the folds. However, improvements in tear film stability have been accompanied by a reduction in LIPCOF when phospholipid liposome eye sprays have been used in dry eye patients,[36,37] suggesting that tear stability is a factor, and certainly inserting a contact lens reduces tear film stability.[38] However, pre-lens tear film stability and LIPCOF were not significantly related in this study. It may also be reasonable to suggest that it is the mechanical influence of the lens edge that produces conjunctival folds in these cases, but no relationships between fitting criteria and different corneo-scleral profiles have previously been found.[39]

Nevertheless, the relationships between LIPCOF and tear film stability, volume and LWE in contact lens wearers strongly points to LIPCOF being a result of mechanical forces during blinking: the bulbar conjunctiva will be stretched, rubbed and massaged during the blink, which, in turn, may result in an over-expansion or/and lymphatic dilation[15] that is visible as bulbar conjunctival folds. The resulting friction may also present as staining of the lid wiper,[6,7] but further investigation is needed to determine which arises first, LWE or LIPCOF. The stronger relationship between LIPCOF and LWE, rather than with nasal LIPCOF, may result from the temporal bulbar surface presenting a larger surface area of exposed epithelium in most subjects, and thus may be more susceptible to drying that will further increase friction.

The results indicate a positive correlation between LWE and hyperemia. Hyperemia is the result of an increase in the volume of blood in the anterior scleral, bulbar conjunctival and limbal vessels, and occurs in response to inflammation, irritation and systemic disease.[19,40–47] It seems likely that irritation can be a factor in soft contact lens wear that may progress to inflammation. The lack of correlation between bulbar hyperemia and LIPCOF suggests a progressive pathogenesis where LWE and redness increase, but LIPCOF is not seen until later in the inflammatory spectrum/processes.

Even though LWE and LIPCOF are significantly increased in symptomatic patients, for the researchers as well as for the clinician, it is important to know how predictable these tests are for contact lens-induced dry eye.[48] This can be analyzed by the predictive values,

which are produced from the sensitivity and specificity of the test; with these depending on the cutoff value of the test and the prevalence of the syndrome itself. For this study, the cutoff values used to discriminate asymptomatics from symptomatics were determined from the data pool, except for temporal LIPCOF which was taken from previous research.[5] As the fine calculation of cutoff values is an iterative process, the predictability of these tests was additionally clarified by the ROC.

While Schirra et al. reported the importance of evaluating LIPCOF at the lateral quadrant of the eye close to the lower lid,[5,9] in this study we found that nasal LIPCOF is also a good predictor for contact lens-induced dry eye. Indeed, the sum of temporal and nasal LIPCOF has a higher predictive value than regional LIPCOF scores, or the other objective signs. Thus we propose that LIPCOF Sum can be included as an improved test to predict contact lens-induced dry eye. In contrast, despite LWE being significantly increased in symptomatic lens wearers, the predictive values indicate that LWE, with lissamine green as a second dye serves better to exclude dry eye symptoms. These outcomes are confirmed by the ROC analyzes, which indicate that while LWE and LIPCOF are significant predictors of contact lens-induced dry eye, LIPCOF Sum is the best, as reflected in the greatest AUC (0.746). Further study is required to extend the investigation of these relationships in high-water content contact lens wearers. Since there are differences of opinion on whether thin high-water content lenses are associated with patient comfort and ocular signs[49–51] vs.[27,52] they were excluded from this study.

Nevertheless, using a set of tests to diagnose dry eye symptoms in contact lens wearers might increase the predictability and should be considered in further studies.

In conclusion, we have shown that symptomatic, experienced, soft contact lens wearers exhibit significantly more LWE and LIPCOF, but not corneal staining, bulbar hyperemia or decreased PLBUT. LWE and LIPCOF are significantly correlated, suggesting that LIPCOF results from friction during blinking. Among contact lens wearers, older women are more likely to present symptoms. LIPCOF Sum appears to be more predictive of symptoms than other clinical tests.

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2.3 Mucins and Ocular Signs in Symptomatic and Asymptomatic Contact Lens Wear

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ORIGINAL ARTICLE

Mucins and Ocular Signs in Symptomatic and Asymptomatic Contact Lens Wear

Monica Berry*, Heiko Pult†, Christine Purslow*, and Paul J. Murphy‡

ABSTRACT

Purpose. Lid wiper epitheliopathy (LWE) and lid parallel conjunctival folds (LIPCOF) are related to dry eye symptoms in contact lens wearers. Both clinical signs are assumed to be related to mechanical forces during blinking. As the mucus layer is a protector of the ocular surface tissue, this study investigates whether any alterations of mucins are detectable comparing symptomatic and asymptomatic soft contact lens wearers.

Methods. Comfort was evaluated using the Contact Lens Dry Eye Questionnaire. Corneal staining, LWE, and LIPCOF were assessed in the right eyes of 50 (19 men, 31 women; mean age, 32.1 ± 11.4 years) experienced lens wearers. The tear film was sampled using Schirmer strips pressed onto the temporal conjunctiva and from harvested contact lenses. Mucins were assessed in dot-blots and Western blots after electrophoresis on 1% agarose or 4 to 12% NuPAGE Gels. Non-parametric analyses were used to study differences between groups and correlations between objective tests, mucins, and symptoms.

Results. Thirty-one subjects were classified asymptomatic and 19 symptomatic by the questionnaire. LWE and LIPCOF were significantly increased in the symptomatic group ($p < 0.035$). MUC5AC reactivity was significantly decreased in symptomatics ($p = 0.050$). MUC4 was correlated to temporal LIPCOF and LWE, ($r = -0.47$ and -0.46; $p < 0.01$). MUC16 and MUC5AC correlated with corneal staining ($0.36 < r < 0.53$; $p < 0.04$).

Conclusions. Symptomatic contact lens wearers exhibit significantly more LWE and LIPCOF, and decreased MUC5AC reactivity. LWE and LIPCOF are significantly correlated; this may reflect their common frictional origin. Increased friction might follow from insufficient mucins, or an altered composition of the resident mucins at the ocular surface. In this study, we show that decreased mucin production is associated with the severity of LWE and LIPCOF.

(Optom Vis Sci 2008;85:E930–E938)

Key Words: contact lens, lid parallel conjunctival folds, lid wiper epitheliopathy, mucins, symptoms

Comfort during contact lens wear strongly influences continuation of use; approximately half of patients who drop-out from contact lens wear in the United Kingdom and three quarters in the United States do so because of lens wear discomfort.[1] Discomfort is thought to be related to a number of factors, including the interaction between the tear film and the ocular surface. Changes in the composition or quantity of the preocular fluid—as a result of excessive evaporation, hyperosmolarity,[2,3] decreased tear clearance, or changes in the morphology of the ocular surface epithelia—might all influence the comfort of wearing contact lenses. Changes in the quantity or quality of mucins are also among the likely causes of ocular discomfort, because the biophysical characteristics of fluids are affected by these components. However, assessing mucin quality in the tear film is only possible in clinical practice in collaboration with laboratory scientists. So, if a clinical test can provide indirect information on this tear film parameter, clinicians may be able to apply it when investigating the source of contact lens wear discomfort in their patients. In this article, we address the relationship among anatomical changes, mucins, and ocular discomfort in contact lens wear.

Lid wiper epitheliopathy (LWE) and lid parallel conjunctival folds (LIPCOF) are predictive clinical signs in contact lens-induced dry eye.[4,5] LWE is an alteration in the epithelium of the advancing lid margin, the lid wiper, caused by friction during lid movement[5,6] (Fig. 1). LIPCOF are subclinical folds in the lateral, lower quadrant of the bulbar conjunctiva, parallel to the lower lid margin[4,7,8] that are easily observable with a slit-lamp. These folds are different from larger conjunctival folds, mainly named conjunctivochalasis, which might be caused by conjunctival "looseness,"[9–11] inflammatory processes, or aging. In this study, LIPCOF refers only to subclinical conjunctival

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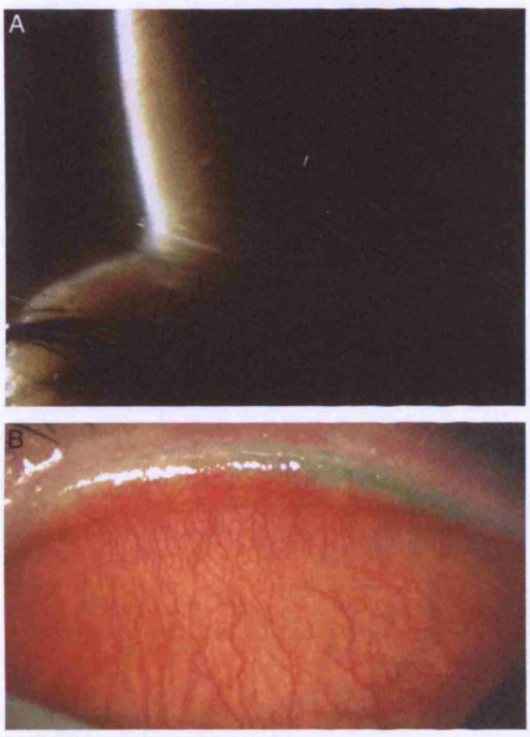

FIGURE 1.

Lid parallel conjunctival folds (LIPCOF) and lid wiper epitheliopathy (LWE). A, LIPCOF grade 3: three parallel conjunctival folds in the temporal quadrant of the eye. Observed with a slit-lamp biomicroscope these delicate folds have the appearance of glass noodles. B, Lissamine Green staining highlights the area affected by epitheliopathy, grade 2.5 (average of grade 3 in length and grade 2 in width). Note the similarity of the 1% Lissamine Green with the beautiful rose bengal-fluorescein staining presented by Korb et al.[5,6]; Fig. 1 and Figs. 4, 5, respectively. The first of these articles also contains an excellent schematic of staining in LWE (Fig. 4).

folds at a defined location observed without fluorescein instillation.[4,7,12] Care has to be taken to differentiate between parallel, permanent conjunctival folds (LIPCOF) and disrupted microfolds or conjunctival flaps.[13] Conjunctival flaps are induced by contact lenses, while LIPCOF are reported in contact lens wearers and non-lens wearers with dry eye symptoms. LIPCOF disappear by lifting the lower lid and are always parallel to the lid. They are more distinct than conjunctival flaps or even disrupted microfolds.

The prevalence of LWE in dry eye patients is between 76 and 80% in contact lens wearers with dry eye symptoms.[5,6] Examining previous studies, about 73% of contact lens wearers show some degree of LIPCOF, and LIPCOF grade 2 or higher can be seen in about 40% of contact lens wearers and are associated with dry eye symptoms.[4,14]

Lubrication of the ocular surface—one of the functions ascribed to mucins[15,16]—is pivotal in contact lens comfort. It is not clear whether mucin (MUC) species composition, or mucin glycosylation or quantity affect comfort. Surface mucins lubricate and anchor the tear film to surface epithelia. Further protection from friction is provided by shear thinning of the tear film, and the prevention of bacterial contamination. Because LWE and LIPCOF are correlated[14] and arise as a result of increased friction,[5,6,17] a relationship with mucin characteristics is to be expected.

To determine any relationship between the composition of surface and lens-adherent mucins and dry eye symptoms in contact lens wearers, mucins on worn soft contact lenses and mucins from the ocular surface of the same individual, were analyzed in respect to the gene products present and mobility on electrophoresis. The latter is determined by the distribution of size and/or size-charge ratios for each MUC species. It has been shown that mucins adherent to contact lenses can be analyzed at the level of gene product and molecular characteristics.[18] In LIPCOF or LWE, there is an assumed failure of tear film protection. In this study, we are evaluating whether this failure is reflected in mucins from the individual ocular surface.

METHODS

All procedures were conducted in accordance with the institution's Ethic Committee and the Helsinki Declaration of 1975, as revised in1983.

Fifty experienced contact lens wearers (m = 19, f = 31, mean age 32.1 ± 11.4 years), randomly selected from the contact lens patients of Horst Riede GmbH, Weinheim, Germany, were examined. The subjects were grouped into symptomatic (n = 19) and asymptomatic (n = 31) patients according to their response to the contact lens dry eye questionnaire (CLDEQ) published by Nichols et al.[19] in 2002.

Acceptance and Exclusion Criteria

All subjects included in the study had worn hydrogel monthly disposable lenses (24 to 62% water content) for at least 6 months, and for 3 weeks before the evaluation visit used these lenses at least four times a week in normal wearing modality. Time of examination was between 3:00 p.m. and 6:00 p.m.

Subjects were excluded if they had any ocular/systemic pathology or allergy known to affect the conjunctiva, e.g., Sjögren's syndrome, rheumatoid arthritis, diabetes, infections, hay fever, or if they were taking any medication known to affect the ocular surface or tear film. Ocular surgery and pregnancy were also exclusion criteria.

All procedures were conducted in accordance with the Declaration of Helsinki (1983), and approval for the study was given by the Cardiff School of Optometry and Vision Sciences Ethics Committee. All subjects signed an informed consent form before participating in this study.

Tests and Classification

Comfort was evaluated using the CLDEQ.[19] LIPCOF was measured in the right eye, in an area perpendicular to the temporal and nasal limbus on the bulbar conjunctiva above the lower lid,[14] using the grading score of Höh et al.,[7] adapted by Pult and Sickenberger.[4] (Fig. 1A). A further combined LIPCOF score (LIPCOF Sum) was calculated by adding the nasal and temporal LIPCOF grades. LWE was visualized with fluorescein and lissamine green, with a second application 5 min after the first[20] (Fig. 1B). This method was chosen to reflect common clinical practice, and because, in evaluating LWE, the frequency of instillation is more important than the volume of dye.[20] LWE was evaluated for upper

and lower lids, using a slit-lamp microscope and classified according to Korb et al.[5,6] Care was taken to differentiate between the fluorescein and lissamine staining associated with Marx's line and that from staining of the lid wiper.[5] Corneal staining was assessed after fluorescein instillation and graded using the Corneal and Contact Lens Research Unit, University of New South Wales, grading scales, with grades interpolated to 0.1 grade units.

Sample Collection and Extraction of Adherent Material

Mucus collected from the normal ocular surface, as well as other mucosal surfaces, contains some mucins that require pretreatment to elute in aqueous buffers[16,21,22]; the latter are necessary for mucin analysis. Our earlier study indicated that mucins adhering to contact lenses require a similar treatment.[18] Although the significance of the "insoluble" fractions is not clear, they are part of the physiological mucin complement. For these reasons, we performed two extractions, as described below, to ensure that we analyze the entire mucin complement of the ocular surface.

Ocular surface fluid samples were obtained by gently pressing Schirmer strips onto the temporal bulbar conjunctiva. Contact lenses were collected from each subject after 4 weeks of daily wear. Strips and contact lenses were individually stored at −20°C until analyzed.

Each lens was extracted with a 3:1 mixture of 4 M guanidinium chloride (Sigma, Poole, UK) with protease inhibitors and radioimmunoprecipitation assay (RIPA) buffer (Sigma). RIPA buffer was used to extract adherent material from Schirmer strips. A second extraction, with the addition of dithiotreitol (DTT), was used to solubilize mucins from any remaining macromolecular assemblies. Reactivity with antibodies against mucin peptide-core epitopes (Table 1) was probed in dot-blots on polyvinylidene difluoride (PVDF) membranes (Immobilon-P, Millipore, Watford, UK), and in Western blots after electrophoresis. After incubation with appropriate secondary antibodies, reactivity was visualized with either a color substrate [3,3'-Diaminobenzidine (DAB) or 5-bromo-4-chloro-3-indolyl phosphate/p-nitroblue tetrazolium chloride (BCIP/NBT) as required, Sigma. Poole, UK] or with a fluorescent substrate (Duo-Lux, Vector Laboratories, Peterborough, UK). Images were acquired on a UVP High Performance Transilluminator (Ultra-Violet Products Ltd., Cambridge, UK) and quantified with LabWorks4 (UVP). For large mucins, electrophoresis was performed on 1% agarose, for 4 h at 60 V, followed by vacuum blotting for 1.5 h on Immobilon. The smaller mucins were evaluated on 4 to 12% NuPAGE Novex Bis-Tris gels (Invitrogen, Paisley, UK), after electrophoresis for 35 min at 200 V^{23} and semidry blotting (Trans-Blot SD, BioRad Laboratories, Hercules, CA) on Immobilon.

Statistical Analyses

Where data were ordinal or not normally distributed, nonparametric analyses were performed, using WinSTAT 2005.1 (R Fitch Software, Bad Krozingen, Germany) and SPSS 14.0 (SPSS Inc., Chicago, IL). Correlations were calculated using

TABLE 1.
Antibodies used to assess mucins in extractions from strips and contact lenses

Antibody	Mucin	Epitope	Source	Control
BC2	MUC1	VNTR	Santa Cruz	Conjunctiva
P-18	MUC2	Near C terminus	Santa Cruz	Blocking peptide
P-20	MUC4	N terminus	Santa Cruz	Blocking peptide
45M1	MUC5AC	Near C terminus	Sigma	Gastric mucin
CLH2	MUC5AC	VNTR	Santa Cruz	
Man5BIII	MUC5B	Non-VNTR	D Thornton, Manchester	Saliva
G-16	MUC5B	N terminus	Santa Cruz	Blocking peptide
EurMUC7a	MUC7	Histatin-like domain	D Swallow, London	Saliva
V-20	MUC7	Internal	Santa Cruz	Blocking peptide
CA125	MUC16	Glyco-epitope	Dako	Saliva
N-20	MUC16	N terminal	Santa Cruz	Blocking peptide

Blocking peptides (all from Santa Cruz) inhibited the reactivity of each antibody at the concentration used. Control saliva was collected from four healthy individuals, centrifuged, and separated from the microbial pellet.

TABLE 2.
Distribution of mucins adherent to lenses

% lenses	MUC16	MUC1	MUC2	MUC4	MUC5AC	MUC5B	MUC7
1st extraction							
CLDEQ-dry	92.31	30.77	38.46	23.08	46.15	84.62	46.15
Asymptomatics	95.65	56.52	56.52	21.74	65.22	91.30	78.26
DTT extraction							
CLDEQ-dry	100	100	92.31	92.31	61.54	100	100
Asymptomatics	100	100	95.83	87.5	79.17	100	100

The table indicates the percentage of contact lenses on which mucin was detected on either of the two extractions. The second extraction, in the presence of dithiothreitol, dissolved material in macromolecular aggregates.

Spearman rank analysis and differences by Mann-Whitney U-tests. When data have been normalized (as proportions or percentages), analysis of variance (ANOVA) and post hoc tests were performed on Prism4 for Macintosh, (Graph Pad, San Diego, CA).

RESULTS

All mucin species described at the ocular surface could be detected adhering to contact lenses and Schirmer strips. These are: MUC1, MUC4, and MUC16 associated with the cell surface, and

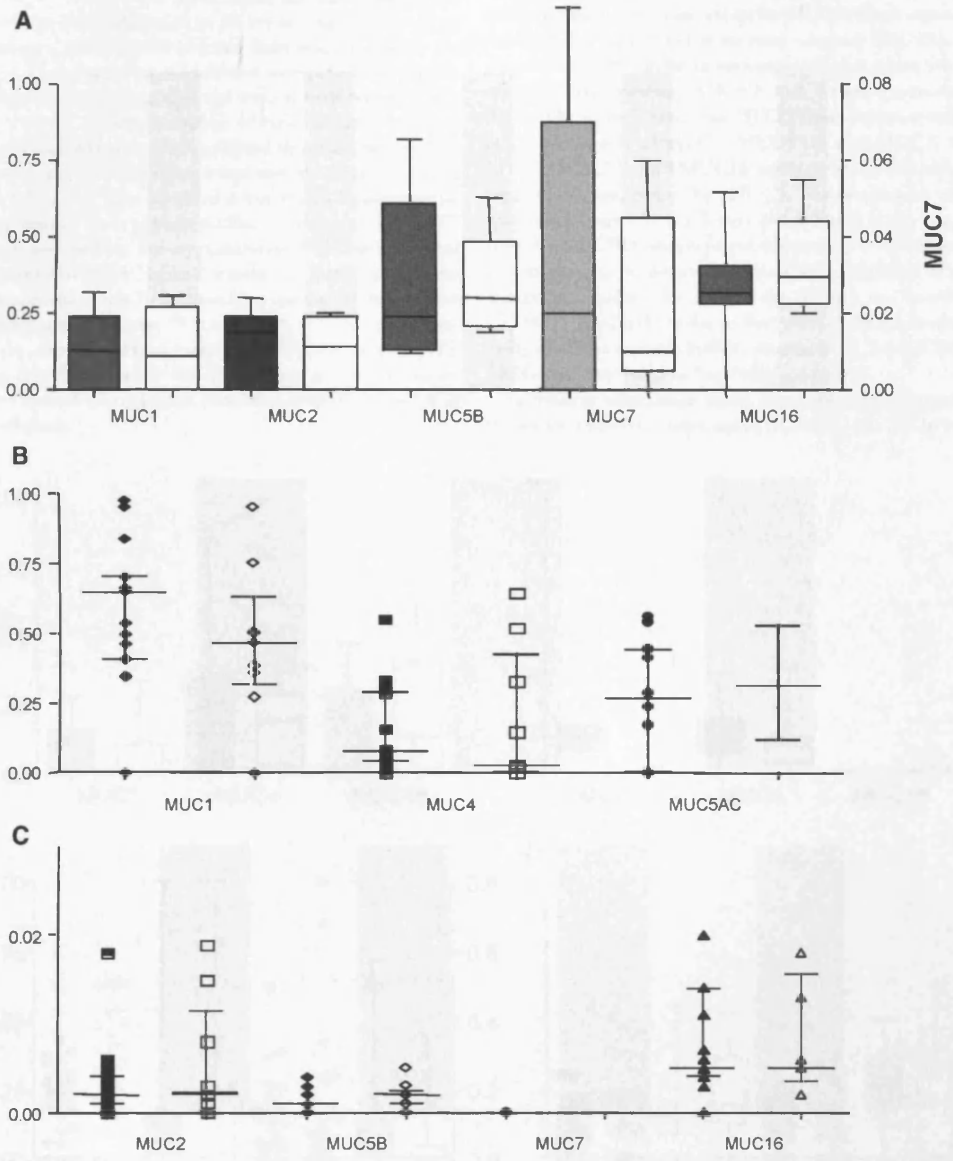

FIGURE 2.

Mucin species at the ocular surface. Proportions of mucin species in extractions from impressed strips. Each strip was extracted and analyzed individually: conditions and reagents were constant in all analyses. A, First extraction: All mucins except MUC7 are expressed in relatively equal proportions (median and interquartile range). MUC7 proportions are shown on the right Y scale. B and C, Extraction with dithiothreitol: MUC1, MUC4, and MUC5AC encompass most of the reactivity (B); whereas MUC2, MUC5B, MUC7, and MUC16 represent a small fraction only (C). Data presented as aligned scatter graphs, lines indicate the median and interquartile range. Solid symbols: asymptomatics; unfilled symbols on light gray background: patients with dry eye symptoms.

MUC2, MUC5AC, MUC5B, and MUC7 that are secreted mucins, with origins in either conjunctiva, cornea, or lacrimal glands (for recent reviews, see Refs. 24, 25). Not all species were detected in every extraction. For example, in the first extraction, MUC7 could not be detected on 23% of impressed strips, and 27% of contact lenses, though not both from the same individual; MUC5AC was below detection in 28.1% of contact lenses from asymptomatics, and in 36.8% of lenses from wearers with dry eye symptoms. The proportions of lenses that were positive for mucins were different for asymptomatics and wearers with dry eye symptoms ($p = 0.047$, Friedman analysis of variance), and there was also a significant difference when analyzed by mucin species ($p = 0.0016$, two-way ANOVA on percentage positive contact lenses), as shown in Table 2. DTT has been used to free mucins from macromolecular aggregates.[26] As in previous studies,[16,18] extraction with DTT has yielded more mucins. For asymptomatics, 17.4% of lenses that were positive for MUC5AC in the first extraction were negative in the second, as opposed to only 7.7% lenses from contact lens wearers with dry eye symptoms. In contrast, 34.9 and 38.5% of lenses (asymptomatics and dry, respectively) were positive for MUC5AC after the DTT extraction though negative in the first, suggesting that this mucin adhered to lenses in a manner that prevents it from eluting native in aqueous solutions.

Distribution of Reactivity with Mucin Antibodies

To compare the proportions of different mucin species in individuals, reactivity with each mucin antibody was expressed as a percentage of the summed reactivities in that extraction. Schirmer strips impressed on the ocular surface yielded first-extraction mucins in similar proportions, except for MUC7, which represented a much smaller proportion of the total reactivity (Fig. 2A). Mucin proportions were similar in asymptomatic and patients with dry eye symptoms (two-way ANOVA with repeated measures, $p = 0.97$). The second, extraction (DTT) from Schirmer strips, was comprised largely of MUC1, MUC5AC, and MUC4, whereas MUC2, MUC5B, and MUC16 represented <0.5% each of the total mucin population (Fig. 2B, C). The proportions of mucin species solubilized by DTT were not different in the two groups (two-way ANOVA with repeated measures, $p = 0.61$), nor could any interaction be detected between the proportion of a given mucin and patient classification by the dry eye questionnaire CLDEQ. Thus, at the ocular surface, some of the mucin species are easily soluble in aqueous buffers, whereas MUC1, MUC5AC, and MUC4 are also found in "insoluble" complexes.

In material adherent to lenses, reactivities with all mucin antibodies were similar, except antiMUC7 (Fig. 3A, B). In contrast,

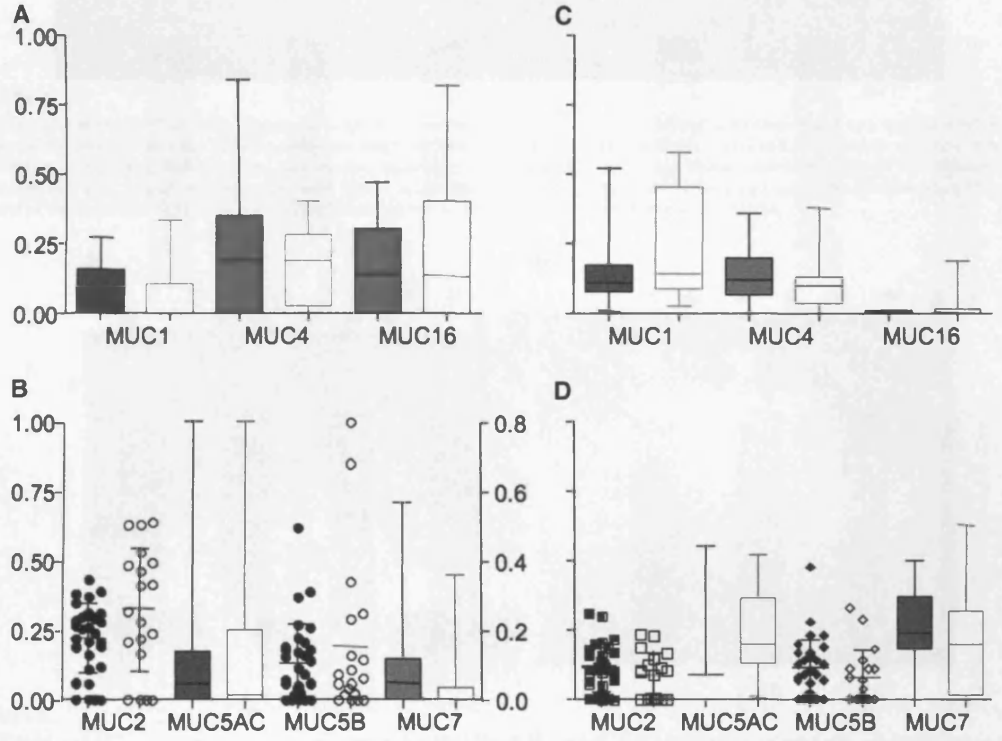

FIGURE 3.

Distribution of mucin species adherent to contact lenses. The same antibodies and conditions were used to probe mucin reactivity after each extraction. The distributions of adherent mucin species are similar in asymptomatic or patients with dry eye symptoms. A and B, First extraction. C and D, Extraction with dithiothreitol. Note the much smaller proportion of MUC16 and increased MUC7 (right y scale) in the DTT extraction. Solid symbols: asymptomatic patients; unfilled symbols: patients with dry eye symptoms. Bars on scatter distributions represent means ± standard deviations. Box and whiskers plots show median and interquartile ranges.

after addition of dithiothreitol, the proportion of MUC16 reactivity was decreased in both groups, and that of MUC5AC increased in symptomatics; neither reached statistical significance (Fig. 3C, D). There were significant interactions between the proportions of mucin reactivty, extraction (i.e., without and with DTT), and patient group ($p < 0.001$). However, the distribution of reactivities was similar, irrespective of whether a lens belonged to an asymptomatic wearer or one with symptoms of dry eye, (two-way ANOVA with repeated measures, $p = 0.077$).

Mucin Characteristics

Mucins on the ocular surface, sampled with strips, and those adherent to contact lenses were characterized by their electro-

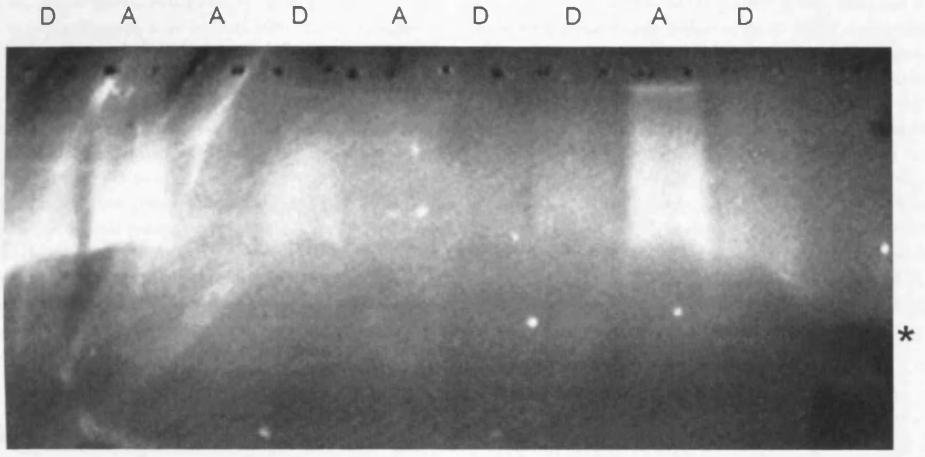

FIGURE 4.

Characteristics of MUC5AC on contact lenses. Electrophoretic mobility of MUC5AC on vacuum bolts after electrophoresis on 1% agarose. All MUC5AC on lenses migrated less than the 250 KDa molecular weight marker (*). The smear, here visualized with Duo-Lux fluorescent substrate, denotes the presence of multiple glycoforms. Agarose gels separate molecules according to their size/charge ratios; most mobile are the most charged mucins relative to their size. Overall mobility ranges were similar in asymptomatics and dry eye patients. The frown across the gel indicates a high protein content of the extraction. Separating mucins from other proteins will have left insufficient material for analysis.

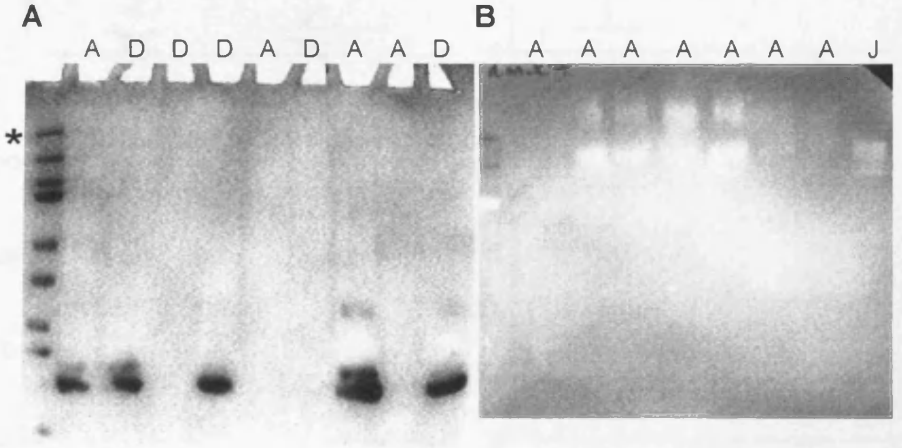

FIGURE 5.

Characteristics of MUC4 and MUC7 on the ocular surface. Semidry blots of MUC4 and MUC7 from impressed strips after electrophoresis on NuPage Bis-Tris gels indicate that the electrophoretic mobility of smaller mucins was not affected by dry eye symptoms. A, MUC4 migrated far into the gel, sometimes as two close bands equivalent to <20 KDa molecular weight marker. A less mobile band was observed only occasionally. Reactivity was visualized with BCIP/NBT. B, MUC7 consistently migrated as two distinct bands, one above and one below 250 KDa in impressed strips. Conjunctival extractions (J) show a single band of similar mobility with the more mobile MUC7 glycoform extracted from strips. Reactivity visualized with Duo-Lux fluorescent substrate. Molecular weight markers in the first lane of both blots are 250 (*), 150, 100, 75, 50, 37, and 25 KDa.

phoretic mobilities. On polyacrylamide gels, such as the NuPage gels used in this study, molecules migrate proportional to their sizes; small molecules migrate further than larger ones. Mobility on agarose electrophoresis depends on both molecular size and charge.

After agarose electrophoresis, MUC5AC from impression strips revealed a range of electrophoretic mobilities, indicative of different size-charge ratios, as expected for this large gel-forming mucin (not shown). MUC5AC from lens extractions migrated less than the 250 KDa molecular weight marker in agarose gels, with glycoforms in the same range in asymptomatics as in patients with dry eye symptoms (Fig. 4). MUC4 mobility on NuPage gels was surprisingly high (Fig. 5A), with either a single band or doublet around 40 KDa, indicating proteolytic cleavage of the molecule. MUC4 in saliva, run on the same gel, shows a single or a doublet of bands of much lower mobility. MUC7 resolved in a doublet of bands of low mobility (around 150 KDa), similar to saliva controls (Fig. 5B).

Having established that gross characteristics of mucins are not different in the two patient groups, we can address the overall levels of mucins in the two groups of patients. More mucin adhered to contact lenses in asymptomatics; however, mucins in aggregates were increased in wearers with dry eye symptoms (Fig. 6), and especially MUC4 and MUC16. This pattern was not observed in the total mucin lifted by the impressed strip.

Ocular Surface

LIPCOF sum scores were significantly different in the two patient groups (Kruskal-Wallis ANOVA, $p < 0.001$), though regional scores for LIPCOF (temporal vs. nasal etc.) were not (Kruskal-Wallis non-parametric ANOVA, and Dunn's post hoc tests, $p > 0.05$). Within either patient group, temporal and nasal scores were significantly different ($p < 0.001$ in asymptomatics and $p < 0.01$ in patients with dry eye symptoms). Upper, but not lower, LWE scores were significantly different in contact lens wearers without and with dry eye symptoms ($p < 0.035$, $p < 0.929$, respectively). In both groups, the sum of nasal and temporal LIPCOF scores is correlated to the upper LWE score ($p = 0.0005$, Spearman's $r = 0.57$ for asymptomatics, and $p = 0.004$, $r = 0.73$ for patients with dry eye symptoms), but not to the LWE score for the lower lid ($p = 0.165$, Spearman's $r = 0,18$ and $p = 0.091$, $r = 0.39$, respectively). MUC4 was correlated to temporal LIPCOF and LWE, ($p < 0.01$, Spearman's $r = -0.47$ and -0.46), whereas MUC16 and MUC5AC correlated with corneal staining (Spear-

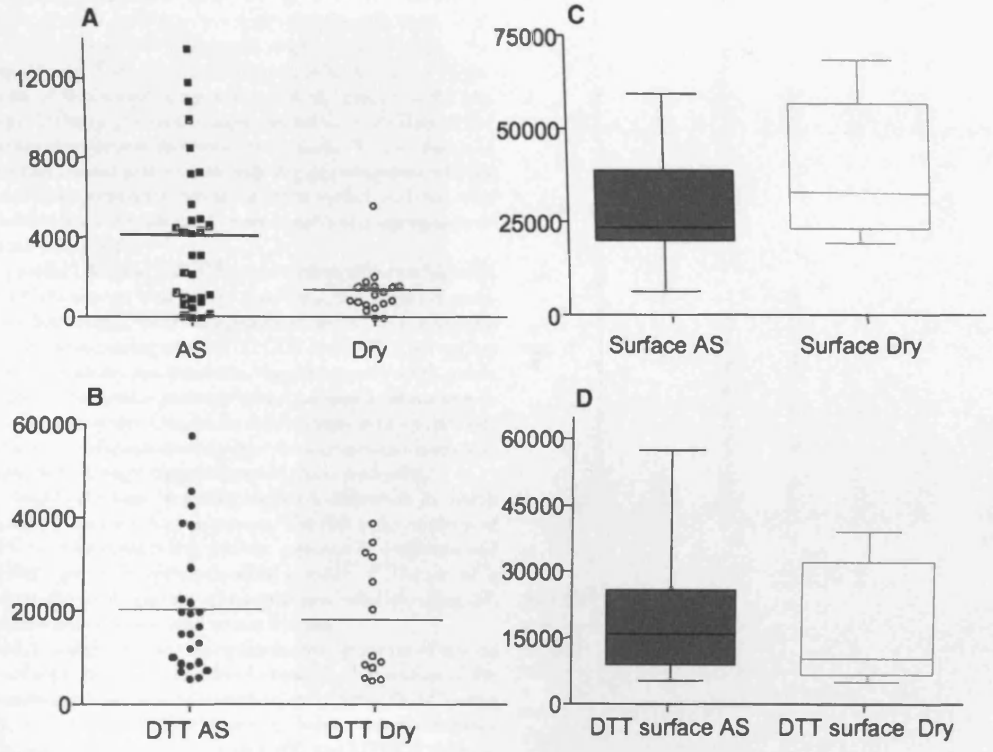

FIGURE 6.

Total mucin adhering to contact lenses. Mucin concentration expressed as intensity of reactivity with antimucin peptide-core antibodies (integrated gray pixels). A and B, Extractions from the same contact lenses without (A) and with (B) DTT solubilization of macromolecular aggregates. C and D, Extraction from the same strips impressed on the conjunctiva without (C) and with (D) DTT solubilization of macromolecular aggregates. Filled symbols: asymptomatics; open symbols: patients with dry eye symptoms.

man's r = −0.36 and −0.53, $p < 0.04$). These correlations are, however, not significant after Bonferroni correction.

When the patients were ordered first by LIPCOF scores and then by LWE scores, a clear relationship emerged between mucin levels and severity of scores. This relationship is also seen in the asymptomatic group, but not in the group of contact lens wearers with dry eye symptoms (Fig. 7).

DISCUSSION

In this study, contact lens wearers were divided into two groups: with and without dry eye symptoms, according to their answers to the CLDEQ dry eye questionnaire. We analyzed mucins collected from the ocular surface with a strip impressed on the conjunctiva, and mucins adhering to the contact lenses of the same individuals, to establish whether any changes in ocular surface mucins could be detected in contact lens wearers with symptoms of dry eye.

A difference was noted in the amount of mucin collected from the two groups of patients, which is most marked in the first extraction from contact lenses. This difference needs to be interpreted with caution: mucin quantification is at best semiquantitative. To conduct meaningful comparisons between individuals, we used the same reagents and methods for all extractions, and the vast majority of strips and lenses were analyzed in a single experiment. Mucin species and their proportions were conserved in the two patient groups. Furthermore, and importantly, mucin size distributions or mucin size/charge ratios were also similar in the two groups. Different glycoforms might restrict the availability of the peptide-core epitope to the antimucin antibody. We therefore conclude that contact lens wearers with dry eye symptoms had decreased mucin concentrations at the ocular surface, and that more of their mucins were contained in macromolecular aggregates (solubilized with DTT).

Upper lid LWE and LIPCOF scores were significantly higher in contact lens wearers with dry eye symptoms, as reported in previous studies. Overall, we noted a pattern of decreasing mucin levels in relation to increasing scores of LIPCOF and LWE. This was lost in patients with dry eye symptoms, suggesting more subtle mucin changes in this group, probably related to specific alterations in their oligosaccharides. Our results show changes in mucin production irrespective of symptoms and suggest that increased stimulation, as in increased friction, might trigger a change in mucin production.

It would have been surprising to detect differences in overall mucin characteristics, for two reasons. The first is the mildness of dry eye in these contact lens wearers: presence of symptoms and reversible signs of dry eye are classified as mild.[27,28] The second is technical: the small quantity of material precluded unveiling differences in a potentially small mucin fraction.

Well balanced, i.e., containing the normal spectrum of mucins and sufficient mucus is considered crucial for lubrication of the conjunctiva and cornea and for contact lens comfort.[15,16,29] In this study, we show that despite all mucins being present, decreased mucin quantitiy is associated with LWE and LIPCOF severity, rather than dry eye symptoms. These results support the concept that LWE and LIPCOF follow from a failure of the tear film[5,6,14] and specify the need for a sufficient quantity of mucins for the maintenance of a healthy ocular surface.

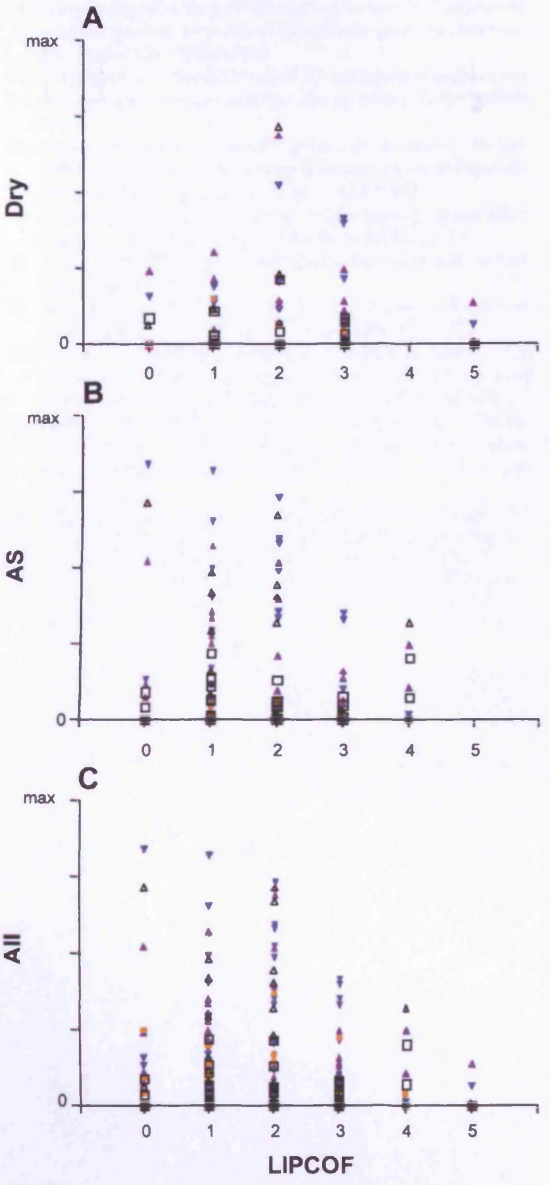

FIGURE 7.
Relation between mucins and lid parallel conjunctival folds (LIPCOF) and lid wiper epitheliopathy (LWE) scores. Patients were ordered first by increasing LIPCOF scores and then by increasing scores of lower lid LWE. A, Patients with dry eye symptoms. B, Asymptomatic patients. C, Distribution irrespective of symptoms. In the entire population, as in asymptomatics, there is a clear decrease in mucin levels with the increase in pathology scores. This is less clear in the group of patients with dry eye symptoms. A color version of this figure is available online at www.optvissci.com.

CONCLUSIONS

Symptomatic soft contact lens wearers exhibit significantly more severe LWE and LIPCOF, while ocular surface mucin composition is conserved. These increased scores of ocular pathology are accompanied by decreased mucins, which might explain the increased friction manifesting in changes to the ocular surface morphology. The contact lens clinician should therefore consider including LWE and LIPCOF as part of their standard clinical examination routine for contact lens wearers, noting the clinical grade of these indicators of mucin insufficiency. Any progressive change in grade can then prompt intervention by the clinician to promote wearing comfort by altering lens type, wearing schedule or providing supplementary tear film lubrication.

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