Longitudinal assessments of corneal and tear film characteristics after LASIK versus continued contact lens wear

Bente Monica Aakre

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To Harald, Marit, Frida and Sivert

’Det som ligger bak deg
og det som ligger foran deg
er for småting å regne
sammenlignet med det som ligger inni deg’
R.W. Emerson

Preface

This thesis is organised into four different parts, each part contains one or two chapters. Chapters (1.0-5.0), sections (i.e. 2.1) and sub-sections (i.e. 2.1.1) are numbered consecutively. Figures and tables are numbered in relation to which section they belong to (i.e. figure 4.1-x). Where cross-referencing between sections occurs, referencing to papers may not have been repeated.

The results from this study have been partially published in:

Doughty MJ, Aakre BM, Ystenaes AE, et al. Short-term adaptation of the human corneal endothelium to continuous wear of silicone hydrogel (lotrafilcon A) contact lenses after daily hydrogel lens wear. Optom Vis Sci 2005; 82:473-480

Doughty MJ, Aakre BM. Central versus paracentral endothelial cell density values in relation to duration of soft contact lens wear. Eye Contact Lens 2007; 33:180-184

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Abstract

Changes in oxygen availability, exerted mechanical force or even surgical manipulation are all factors known to influence corneal characteristics, yet detailed information about the changes such influence may induce is limited in ophthalmic literature. Furthermore, the appearance of the corneal endothelium, which is often used as a presage of structural and functional changes taking place in the cornea, has not been systematically studied over extended periods and information such as endothelial morphometry is hence not available. The purpose of this study was therefore to evaluate the endothelial morphometry over a two-year period in subjects who underwent different types of intervention, either in the form of change in contact lens material, LASIK surgery or by replacing existing contact lenses with a similar type. A group of spectacle wearers served as a control group.

Results obtained through the course of the study revealed that subjects who continued wearing conventional contact lenses had substantial amounts of polymegethism compared with the control group. Those who had undergone LASIK surgery had significantly reduced amount of polymegethism three months after surgery and reached a concentration level that was consistent with the control group after 24 months. A similar tendency was found in the subjects who were refitted with silicone-hydrogel (SiH) lenses although the high number of endothelial cells that was found to reverse back to a six-sided shape after LASIK treatment was not apparent in this group of subjects. This may be associated with the sustained inflammatory response, which is arguably present in the corneas of these subjects.

LASIK surgery seems to have no detrimental effect on the corneal endothelium and the procedure may even reverse some of the contact lens induced changes in the endothelial mosaic. Pre-ocular tear film evaluation revealed no inferior quality or quantity nor did these subjects report any higher dry eye sensation than the other subjects.

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CHAPTER 1: INTRODUCTION

1.0 General introduction

Traditionally, individuals with a myopic refractive error (near sightedness) had the option of wearing spectacles or contact lenses. Individuals respond differently to contact lens wear, with some adapting quickly and having many years of successful wear while others suffering from discomfort and a feeling of dryness. This thesis study started at a time when a completely new type of contact lens (the silicone-hydrogel) entered the Norwegian market. At the same time, myopic individuals also faced the option of having excimer laser surgery to correct their refractive error. When a new contact lens type becomes available, and especially one with a very different material and wearing regime, it is natural that individuals (being seen as patients) and their optometrists seek information to get answers to a range of questions. These include whether this new lens will be more comfortable than their current lenses, or will their vision be better with this lens? In addition, since contact lens wear has been widely reported to cause certain changes to the cornea and conjunctiva, it is also expected that questions will be asked as to whether the new lens will produce similar changes, or even whether any corneal or conjunctival changes caused by previous (long-term) contact lens wear could be reversible. Similarly, if refractive surgery (such as Laser-Assisted In-Situ Keratomileusis; LASIK) is chosen as an option, will this mean that a patient will achieve better vision than if they were wearing spectacles or contact lenses? Many individuals who choose to undergo corneal refractive surgery are contact lens wearers. Patients can thus be expected to ask whether their eyes will feel more comfortable after LASIK, whether the refractive surgery will cause unwanted corneal changes over the long term, or perhaps whether any tissue changes caused by previous (long-term) lens wear may be reversible once the refractive surgery has been undertaken. To answer many of these questions, a long-term study was considered useful and, in particular, to undertake a comparison between four different groups. These would be spectacle wearers, wearers of soft (hydrogel) contact lenses, individuals who switched from hydrogel to silicone-hydrogel contact lenses and soft hydrogel lens wearers who underwent LASIK.

To provide a context of this thesis and the methods of choice in the present study, the next three sections review the anatomy and ultra structure of the normal human cornea, conjunctiva, and tear film, and how others have carried out the assessments of these structures (sections 1.1 to 1.2). To address some of the questions listed above, section 1.4 and 1.5 review how these structures may change with contact lens wear and subsequent excimer laser surgery (LASIK) for myopia. Last, section 1.6 presents a summary of questions, which forms the aims and objectives of this study.

1.1 Cornea

1.1.1 General characteristics

The cornea is a thin transparent piece of tissue at the front of the eye. It plays an important role in refracting incoming light before it enters the lens for then to ultimately reaching the retina (Figure 1.1-1).

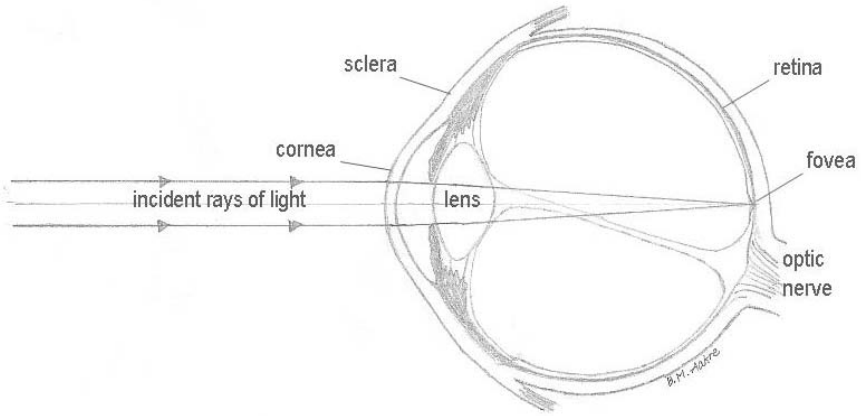


Figure 1.1-1

The quality of transparency is very important to provide a clear retinal image and the surface curvature partly determines the refracting power of the eye. Together with the surrounding opaque sclera, the cornea forms the outer tunic of the eye. This fibrous tunic serves to give shape to the eye and to protect the delicate internal structures. The cornea appears approximately circular, but is actually slightly elliptical in shape with a visible horizontal diameter in adults of about 11 to 12 mm and a vertical diameter of 10 to 11 mm (Muller and Doughty, 2002). The anterior radius of corneal curvature averages around 7.8 mm with a range from 7.0 mm to 8.5 mm (Kinge *et al.*, 1999; Reuland *et al.*, 2007). The mean corneal refractive index is 1.376 and the mean refractive indices of the epithelium, stromal anterior and posterior surfaces were reported to be 1.401, 1.380 and 1.373, respectively (Patel *et al.*, 1995). The shape and refractive index make the cornea highly refractive; it provides about two-thirds of the total optical refractive power of the eye, with the rest provided by the crystalline lens.

1.1.2 Structure and transparency

From anterior to posterior, the corneal layers are the epithelium, the anterior limiting lamina (ALL, also known as Bowman's membrane), the stroma, the posterior limiting lamina (PLL or Descemet's membrane), and the endothelium.

The corneal epithelium is a stratified squamous epithelial layer and while in contact with the surrounding conjunctival epithelium, is distinctly different from it (Doughty, 2002). It usually has five to seven layers of cells including basal column-shaped cells underlying an intermediate layer of cells (often called wing cells) and the layers of cells in the front are the flattened squamous cells.

Underneath the basal cells is an extremely thin basement membrane. The squamous cells form a macroscopically smooth and regular corneal surface with an interface with the pre-corneal tear film. When observed at high magnification with a scanning electron microscope, it can be seen that the surface of these outermost (apical) cells are actually covered with very small ridges (microplicae) which help to form the interface with the tear film (Bergmanson and Doughty, 2005). Part of this interface includes a mucous layer (see section 1.3.1). Overall, the corneal epithelium serves as an important barrier on the anterior side of the cornea. This barrier function is achieved, among other things, by a continuous array of tight junctions between the squamous cells, and these limit the movement of water (e.g. from the tear film) in and out of the corneal tissue. In addition, cell-cell attachment proteins called desmosomes, which are present between all wing and basal cells, produce the structural integrity of the epithelial cell layer. Structures called hemi-desmosomes anchor the basal cells to the basement membrane, meaning that the attachments of the basal cells to the underlying anterior limiting lamina (ALL) are not as strong as compared to the attachments between the cells. This difference in the location of desmosomes and hemi-desmosomes means that the corneal epithelium can be easily removed from the ALL. The barrier function also serves to limit the entrance of pathogens, such as bacteria, into the corneal tissue. The mucus covering on the squamous cells also facilitates the latter function. If there is slight damage to the junctions between the squamous cells, then these will be made visible with the use of fluorescein dye (see section 1.3.3). If there is substantial damage to the epithelial cell layer, then fluorescein will very readily penetrate into the stroma. Even if some damage occurs to the epithelial cells (e.g. as a result of contact lens wear), the corneal epithelium is self-renewing and any epithelial defects will rapidly heal. This occurs because of division of the basal cells, which then migrate both anteriorly, and towards the centre of the cornea (Thoft, 1989). The basal cells undergo differentiation as they migrate and, after they reach the corneal surface as squamous cells, they will eventually desquamate into the tear film. It is generally thought that the shedding rate of the superficial cells equals the sum of cell proliferation from the basal layer and movement of more peripheral cells towards the centre of the cornea (Thoft and Friend, 1983). If damage occurs to the more superficial cells, there will be an increased division of basal cells to compensate for this.

The anterior limiting lamina (Bowman's membrane) is composed of a very fine collagen fibril-meshwork. In reality, the ALL is not a continuous 'membrane' since it contains numerous small pores through which un-myelinated nerve fibres pass from their origins in the sub-epithelial plexus through into the basal layers of the corneal epithelium (Oliveira-Soto and Efron, 2001; Bergmanson and Doughty, 2005). The nerve endings in the corneal epithelium provide the basis for ocular surface sensitivity to any mechanical contact (Lawrenson, 1997; Belmonte *et al.*, 2004). Intact corneal nerves are essential in the process of tear production (reflex tearing) (Lucarelli *et al.*, 2003).

The corneal stroma makes up around 90% of the total thickness and has certain biomechanical properties such as strength, elasticity, and form. The stroma is composed of layers of stacked sheets, and it has been estimated that there are 200 of these in the central region of the human cornea (Bergmanson *et al.*, 2005). These lamellae contain organized arrays of fine collagen fibrils

embedded in an extra-cellular matrix (also called the ground substance) composed of proteoglycans, which will readily absorb water and so result in swelling of the corneal stroma. Adjacent lamellae in the more anterior layers of the stroma run in random directions and are often branched, while those in the more posterior region are more likely to be orthogonal (right angles) to each other (Komai and Ushiki, 1991). These differences are likely to contribute to differences in the biomechanical and swelling properties of the anterior versus the posterior stroma (Müller *et al.*, 2001). Situated between the stromal lamellae, flattened cells with slender processes maintain the extracellular matrix and the collagen fibrils by constant synthetic activity (Bergmanson and Doughty, 2005). The density of these cells, the keratocytes, is greatest in the anterior part of the stroma (Petroll *et al.*, 1995).

The posterior limiting lamina (PLL or Descemet's membrane) is the basement membrane of the corneal endothelium, from which it is synthesized throughout life (Bergmanson and Doughty, 2005). Like the ALL, it is composed of collagen.

The corneal endothelium is a single layer of cells across the posterior side of the cornea. The cells are in contact with each other with the lateral cell-to-cell borders being highly interdigitated (Bergmanson and Doughty, 2005). The parts of the cell-cell borders closest to the surface that is in contact with the aqueous humour inside the eye have incomplete tight junctions between them. This forms a 'leaky' barrier that limits the bulk flow of aqueous humour into the corneal stroma but allows for slow movement of aqueous humour into the cornea (Barry *et al.*, 1995). In addition to its barrier function, the endothelium has properties of ionic pump mechanisms and water transport (Fischbarg and Maurice, 2004). If the corneal endothelial cells are damaged, then they spread out and migrate across the basement membrane to cover the damaged area. This is because the adult human corneal endothelium, unlike the corneal epithelium, is not self-renewing and has little capacity to divide in the living eye (Joyce, 2003), although some recent studies have indicated that endothelial stem cells exists (McGowan *et al.*, 2007). Such cell enlargement, after cell loss by damage or stress, results in a reduction in the cell density as well as (usually) changes in the cell shape and variability in the cell areas (see section 1.1.4).

As noted earlier, the healthy cornea is normally transparent, in contrast to the surrounding sclera. While both the cornea and sclera are composed of collagen fibrils, those in sclera are formed into irregular and often entangled bundles, which vary in width and thickness and often appear to be branched. The scleral fibrils have a wide range of diameters that are much larger than those of the corneal stroma (Doughty and Bergmanson, 2004) with the result that the sclera scatters rather than transmits light. In contrast, the size of a collagen fibrils in the corneal stroma is considered to be too small to significantly scatter light (Benedek, 1971; Vaezy and Clark, 1991), although they have a slightly higher refractive index (1.411) compared to the surrounding matrix of 1.365 (Leonard and Meek, 1997).

The regulation of the overall water content of the stroma determines the corneal thickness because the corneal stroma has a very marked capacity to absorb fluid, at least without the barrier functions

of the epithelial and endothelial cell layers. In situ, this capacity is not obvious unless the epithelial or endothelial cell layer is damaged (Doughty and Bergmanson, 2004). The relatively low hydration state of the corneal stroma in situ can be attributed to the mechanical influence of the intra ocular pressure (Ehlers, 1967) and to a mechanism promoting deturgescence (thinning) of the stroma. Since the late 1940s, three components of the cornea have been considered important in the overall determination of the final corneal hydration, thickness and transparency. These three components are the barrier properties of the corneal epithelium, the water binding capacity of the corneal stroma and the barrier and pump function of the corneal endothelium (Stiemke *et al.*, 1995). Almost all research since the late 1950s on corneal hydration control has been concentrated on the epithelial and endothelial cell layers, with the researchers taking turns to debate whether the epithelium or endothelium was more important. In later years it has become well known that actual damage of the endothelial cell layer would have a more substantial impact on corneal hydration control than equivalent changes to the epithelium (Fischbarg and Maurice, 2004).

1.1.3 Corneal thickness

As described above, corneal transparency is essential for the formation of a clear retinal image, and corneal thickness is a direct parameter representing corneal integrity. This section will review the corneal thickness values obtained when using different pachymetry methods with the aim being to validate the results from the present study.

Pachymetry can accurately measure corneal thickness. Pachymeters are instruments designed to measure the distance between the outer surface of the corneal epithelium and the inner surface of the endothelium. Although giving consistent and reliable results, different instruments yield slightly different corneal thickness values (Doughty and Zaman, 2000). The present study used a non-contact specular microscope (Topcon SP2000P), whereas most studies in the ophthalmic literature have used ultrasound devices to measure central corneal thickness. In a meta-analysis of the literature, Doughty and Zaman (2000) showed that the average central corneal thickness value as measured by ultrasound pachymeters was 544 µm (range 490 to 600 µm, read from Figure 12). An analysis of reports of corneal thickness measured by the non-contact specular microscope Topcon SP2000P yields an average value of 532 µm (range 516 to 548 µm) (Bovelle *et al.*, 1999; Cho and Cheung, 2000; Modis *et al.*, 2001b; Modis *et al.*, 2001a; Doughty *et al.*, 2002b; Modis *et al.*, 2002; Sanchis-Gimeno *et al.*, 2004; Ogbuehi and Almubrad, 2005a; Ogbuehi and Almubrad, 2005b; Sanchis-Gimeno *et al.*, 2006).

In humans, individual central corneal thickness values vary. An average central corneal thickness value of 536 µm was obtained from an analysis of all reports, published over a 30 year period, of corneal thickness on nominally normal human subjects regardless of the method used (Doughty and Zaman, 2000). However, Doughty and Zaman (2000) further demonstrated that the overall thickness for a normal cornea range rather widely from 473 to 595 µm. Central corneal thickness can also vary between different ethnic groups (Doughty and Zaman, 2000). Corneas of individuals of pigmented origins have been reported to be thinner than in white individuals, although significant

variance between different Asian populations may also exist (Aghaian *et al.*, 2004). The present study was conducted in Norway, where the population is predominantly white (Northern) European. There are few Nordic studies of corneal thickness. However, a review of some existing studies (Alsbirk, 1978; Olsen and Olsen, 1993; Eysteinsson *et al.*, 2002; Hjortdal *et al.*, 2005) suggest that the average central corneal thickness in healthy, adult Nordic individuals is 545 µm, ranging from 516 to 623 µm, which is similar to the range reported in the literature (Doughty and Zaman, 2000).

Para-centrally the thickness of the cornea has been reported to be anywhere between 9 and 52% (average 21%) greater than the values at the central region (Doughty and Zaman, 2000). Studies that are more recent indicate that the mid-peripheral (2.0 to 3.0 mm from centre) corneal thickness can be from 2.3 to 19.8% thicker than the central thickness. The variation between studies depends upon the pachymetry method and where on the cornea the measurement was made (Liu *et al.*, 1999; Cho and Cheung, 2000; Gonzalez-Meijome *et al.*, 2003a; Gonzalez-Meijome *et al.*, 2003b; Yeniad *et al.*, 2003; Yeniad *et al.*, 2004; Doughty and Jonuscheit, 2007; Jonuscheit *et al.*, 2007; Khoramnia *et al.*, 2007). Studies using optical pachymetry methods, such as scanning slit techniques (OrbScan or Pentacam) and specular microscopy, report larger differences in central and mid-peripheral corneal thickness measures (from 9.7% to 19.8%) than studies using ultrasound pachymeter devices (from 2.3 to 10.9%).

There are discrepancies between pachymetry studies, which, at least partly, relate to instrumentation and where on the cornea the measurement was made. However, there seems to be limited information in the literature about differences in the mid-peripheral to central corneal thickness relationships.

1.1.4 Corneal endothelial cell morphometry

As described earlier, in section 1.1.2, the corneal endothelium has limited capacity of mitosis and at the same time it plays a significant role in maintaining corneal deturgescence. Thus, any change to this monolayer across the posterior surface of the cornea, which may occur after contact lens wear or other potential stress factors, is important to monitor closely. This section first reviews the different methods of assessing endothelial morphology and morphometry. Then follows the description of the three most commonly used morphometric variables in the literature: *endothelial cell density* (ECD), *variation in cell area* (COV) and the *percentage of six sided cells* (%SIX). Last, a review of the literature on regional differences in ECD, COV and %SIX is presented. The aim of this section is to enable validation of data from the present study and to define gaps in the literature.

Depending on the researcher's aim of study, there are several ways of assessing endothelial morphometry. In an excised cornea, the outlines of the posterior surfaces (edges) of the endothelial cells can be observed by light microscopy especially after the cells have been stained with a combination of trypan blue and alizarin red (Geroski and Edelhauser, 1989). Small areas, usually of the central region of the cell layer, can then be photographed and the number of cells per unit area

counted or the areas of the cells measured. This can provide estimates of endothelial cell density (ECD) or the average cell-area values.

For the living eye, it is possible to view these cell edges in the slit lamp under specular reflection carefully focused at the level of the endothelium. Under high magnification (≥ 40x), a mosaic of cells can be seen in normal eyes, with the cell-to-cell borders appearing darker against a lighter background. As a general clinical method, it can really only be used to check whether there is a uniform specular reflection. Under stress or following damage to the endothelial cell layer, distinctly darker amorphous regions can be present as 'blebs' (Zantos and Holden, 1977) or 'guttae' (Waring *et al.*, 1982). The observer can subjectively grade these structural changes.

Nowadays, it is not only easy to examine the corneal endothelium in clinical practice but also to obtain much higher magnification (> 100 X) images of the cell layer using a specular microscope or a confocal microscope. Only the former instrument, and its output, will be considered here. Non-contact specular microscopes have been developed that automatically align with the endothelium to optimize the specular reflex and record images digitally (Landesz *et al.*, 1995; Ohno *et al.*, 1999). The picture can be subjectively assessed, but it is now commonplace for the morphometry of the cells to be quantitatively assessed, often using automated or semi-automated image analysis software programs. As a result, morphometric variables such as mean cell density, mean cell area and variation in cell size- and shape can be routinely obtained (Vecchi *et al.*, 1996; Cheung and Cho, 1998; Cheung and Cho, 2000). Since the current study will assess the endothelial morphometry of living eyes in detail, a quick, non-contact method of photography is highly preferable.

The most frequent morphometric variable recorded is that of the central endothelial cell density (CECD). It is usually a measure of the number of cells within a defined area of the central region of endothelial cell surface (i.e. close to the optical centre of the cornea) and is presented in terms of cells / mm^2 (even though a much smaller area than this is actually photographed and assessed). CECD values of 6000 cells / mm^2 seem to be common in infants, and values of close to 4000 cells/mm^2 in children (Muller *et al.*, 2000). These substantial reductions in cell density can be accounted for by the growth of the growth of cornea and so stretching the cell layer (endowed with a relatively fixed number of cells at birth) as its diameter increases in the early years of life (Speedwell *et al.*, 1988; Muller and Doughty, 2002). By early adulthood, the cell density typically ranges from about 2500 to 3500 cells / mm^2 (Bourne and Kaufman, 1976; Yee *et al.*, 1985; Bahn *et al.*, 1986; Sherrard *et al.*, 1987; Williams *et al.*, 1992; Stulting *et al.*, 1996; Bourne *et al.*, 1997; Doughty *et al.*, 2000) with there being a clear tendency for a slow age-related decline over the adult years. This further reduction of CECD in adults is attributed to the fact that if cells are damaged, or die because of stress, then they are not replaced. For the elderly, this rate of cell loss has been estimated to be about 0.6% per year (Bourne *et al.*, 1997). Based on evaluation of CECD values in diseased corneas or following corneal transplant (where some damage is inevitable), a minimum value of around 700 cells/mm^2 is considered necessary for a monolayer with some degree of

barrier function to form. This is required for the cornea to sustain a healthy appearance and have a reasonable transparency for clear vision (Edelhauser, 2006).

After cell density, the most frequently reported measurements are the actual cell area values and the variance in these. The average values for endothelial cell area in healthy adult corneas is typically between 300 to 350 µm and the distribution of the cell area values is close to Gaussian in most cases (Doughty, 1998a; Doughty *et al.*, 2000). However, as the cornea ages and some cells are lost, there can be enlargement of some cells in particular and this is evident in a skewing of the cell area distribution (Yee *et al.*, 1985; Sherrard *et al.*, 1987). This phenomenon of irregularity in the area in the endothelial cell layer has been termed polymegethism and literally stands for "many sizes" derived from Greek polys = many and megethos = size. The degree or extent of polymegethism has usually been reported by calculating the Coefficient of Variation of the average cell area (COV). This COV is a parameter derived from the standard deviation (S.D.) of mean cell area where COV is equal to 1 S.D. of the average cell area divided by the average cell area. It can be expressed as a fraction (e.g. 0.25) or as a percentage (i.e. 25% if the value is multiplied by 100). Doughty et al. (1993) have shown that estimates of COV are very dependent on the number of cells for which the areas have been measured, and so there have been a range of different values reported even for healthy young adults. A review of the literature shows that typical central COV values (CCOV) for healthy adults (without contact lens wear or surgery) appear to be between 22 and 31% (Schoessler *et al.*, 1984; MacRae *et al.*, 1985; Stocker and Schoessler, 1985; MacRae *et al.*, 1986; Carlson and Bourne, 1988; Carlson *et al.*, 1988; Lass *et al.*, 1988; Orsborn and Schoessler, 1988; Nieuwendaal *et al.*, 1991; Sibug *et al.*, 1991; MacRae *et al.*, 1994; Nieuwendaal *et al.*, 1994; McMahon *et al.*, 1996; Setala *et al.*, 1998; Bourne *et al.*, 1999a; Bourne *et al.*, 1999b; Wiffen *et al.*, 2000).

The last endothelial cell morphometry index that has received considerable attention is that of the 'hexagonality', since the appearance of the cell mosaic has been likened to the appearance of a honeycomb. Cell sizing grids are included in many specular microscopes and the cell array is depicted as hexagonal. It has been noted that the cells only sometimes have a geometric shape and symmetry that conforms to a symmetrical hexagon (Doughty, 1992). Notwithstanding, a reporting on the hexagonality or the '% hexagons' can be done (Sibug *et al.*, 1991; Landesz *et al.*, 1995; Perez-Santonja *et al.*, 1997) to indicate the percentage of '6-sided cells' (Doughty, 1992; Doughty, 1998a). In even the healthiest appearing human corneas, the proportion of 6-sided cells (hereafter called %SIX) is not 100%, but usually somewhat less than this. If there are other cells that are not 6-sided (e.g. 5-sided and 7-sided), then it is considered that the cell layer shows some degree of pleomorphism, i.e. variation in cell shape. Typical estimates of the central %SIX (C%SIX) range from 59 to 74% in (presumably) white adult individuals (MacRae *et al.*, 1985; MacRae *et al.*, 1986; Carlson and Bourne, 1988; Carlson *et al.*, 1988; Orsborn and Schoessler, 1988; Nieuwendaal *et al.*, 1991; Sibug *et al.*, 1991; MacRae *et al.*, 1994; Nieuwendaal *et al.*, 1994; Bourne *et al.*, 1999b; Wiffen *et al.*, 2000; Esgin and Erda, 2002). As the extent of pleomorphism increases, it is likely that there is an increase in the polymegethism, i.e. the COV value. This increase in COV is to be expected since, for example, 5-sided cells tend to be smaller than 6-sided

cells and the 7-sided cells tend to be larger than 6-sided cells. In the extreme, there can be extremely small 4-sided cells and it is not uncommon to find very large 8- or even 9-sided cells (Doughty, 1998a).

As noted earlier, most evaluations of endothelial cell morphology have used a sample of cells photographed within the central region of this cell layer. With such evaluations, it is generally assumed that the data obtained for the central region of the endothelium is representative of the rest of the cell layer. However, if the degree of polymegethism is very high, then even immediate adjacent regions from a specular microscope image may be substantially different in terms of ECD and COV values (Hirst *et al.*, 1989; Doughty *et al.*, 1993). Moreover, there is also some evidence that, even in endotheliae with low polymegethism (i.e. where the cell density is likely to be uniform), there can be distinct regional differences when comparing central regions with either paracentral regions or peripheral regions. The paracentral (mid-peripheral) region can be considered as being half way to the edge of the cornea and modern day specular microscopes allow for images to be taken at a location 3 mm from the centre (Cho and Cheung, 2000). The peripheral region is that which is as close to the edge of the cornea from where an image can be captured with a specular microscope, i.e. approximately 1 mm from limbus (Wiffen *et al.*, 2000). To observe further peripheral endothelial cells in human corneas, microscopy studies of donor corneas are necessary. In such studies, the endothelial cell density near Schwalbe's line have been reported to be 31%, 49.5% and 22.9% higher than the CECD (Schimmelpfennig, 1984; Daus *et al.*, 1990; Amann *et al.*, 2003). In clinical studies, using either contact or non-contact specular microscopy, the *mid-peripheral* endothelial cell density (MPECD) has been found to be either similar to (Schanzlin, 1999; Azar *et al.*, 2001; Muller *et al.*, 2004) or up to 6% lower (Azen *et al.*, 1981; Roszkowska *et al.*, 2004) than the CECD. However, most studies have reported the MPECD to be *higher* than the CECD (from 1.4% to 7.1% with an average of 3.9%) (Azen *et al.*, 1981; Yee *et al.*, 1985; MacRae *et al.*, 1994; Stulting *et al.*, 1996; Trocme *et al.*, 1996; Schanzlin, 1999; Cheung and Cho, 2000; Azar *et al.*, 2001; Amann *et al.*, 2003; Wirbelauer *et al.*, 2005). Similarly, *peripheral* ECD have been reported to be from 3.9% to 10.2% higher than CECD (Schultz *et al.*, 1986; Trocme *et al.*, 1996; Amann *et al.*, 2003). On the other hand, Wiffen et al. (2000) reported the peripheral cell density to be similar to, or a little lower than, the CECD. The discrepancies between studies have been attributed to age, contact lens wear and inter-endothelial differences. For example, several studies have reported that the ECD is higher superiorly and lower (or similar) inferiorly compared to CECD (Azen *et al.*, 1981; Schultz *et al.*, 1986; Schanzlin, 1999).

Regional differences have also been reported for polymegethism and pleomorphism. In some studies on contact lens wearers, the COV has been reported to be larger centrally than peripherally (Wiffen *et al.*, 2000), the difference being especially pronounced in PMMA lens wearers (MacRae and Matsuda, 1987; MacRae *et al.*, 1994). However, in non-lens wearers (and in studies not being specific on the number of contact lens wearers) the mid-peripheral COV have been reported to be similar to central COV or up to 3% higher (Yee *et al.*, 1985; MacRae *et al.*, 1994; Stulting *et al.*, 1996; Trocme *et al.*, 1996; Cheung and Cho, 2000; Azar *et al.*, 2001; Amann *et al.*, 2003).

Similarly, the %SIX has been found to be significantly larger in the mid-peripheral and peripheral corneal endothelium of contact lens wearers (MacRae and Matsuda, 1987; MacRae *et al.*, 1994; Wiffen *et al.*, 2000). In non-lens wearers, up to 4% increase in the peripheral %6-sided cells have been reported (Wiffen *et al.*, 2000). However, most studies have reported a close to even distribution of the %SIX in the central and mid-peripheral corneal endothelium (MacRae *et al.*, 1994; Stulting *et al.*, 1996; Trocme *et al.*, 1996; MacRae and Rich, 1998; Cheung and Cho, 2000; Azar *et al.*, 2001; Amann *et al.*, 2003).

There are clear indications in the literature that regional differences in ECD, COV and %SIX exist. However, there are discrepancies between the studies, and it is not clear why eventual regional differences form. Since the current study has the possibility to address differences between contact lens wearers and non-lens wearers, an attempt will be made to evaluate the effect of lens wear on the distribution of endothelial cells and cell morphometry. Furthermore, the current study aims to evaluate how consistent any of these regional differences might be especially in relation to change in contact lens wear or following refractive surgery.

1.2 Limbus and conjunctiva

1.2.1 Anatomy and ultrastructure of the limbus

A soft contact lens does not only cover the cornea but also the corneoscleral junction (limbus) and partially the conjunctiva. Limbus can be defined as the 1-2 mm circular area between cornea and the surrounding conjunctiva and sclera. Although the limbus is commonly considered to comprise the outer portion (i.e. corneal and conjunctival epithelium and stroma), the anatomy of all the corneal layers changes gradually over the limbal zone.
At the inner corneal surface, the single endothelial cell layer extends over a transition zone called Schwalbe's line thereafter covering a network of connective tissue. This network of connective tissue, the trabecular meshwork, is connected to Descemet's membrane, or the posterior limiting layer (PLL) of the cornea, which terminates at the limbus. The corneal stromal fibrils and lamellae lose their regular organisation, as this layer becomes sclera. The ultrastructural characteristics of Bowman's layer, the anterior limiting layer (ALL), also terminates at the limbus where the structure changes into Tenon's capsule and the conjunctival tissue. The 5-7 layer thick corneal epithelium increases to 7-15 layers in the limbal zone. The limbal epithelial thickness varies because of the underlying wrinkles or folds known as the palisades of Vogt. Apart from the basal cells, the epithelial cells in the limbus region are similar to corneal epithelial cells. These basal cells of the limbal epithelium are smaller and less columnar than corneal epithelial cells and their basal surface have undulating extensions into the underlying matrix, which probably serves to anchor the cells to the basement membrane since the basal cells in the limbal area have smaller amounts of hemidesmosomes than the corneal epithelial basal cells (Bergmanson and Doughty, 2005). Some of the basal cells in the limbal area comprise the stem cells of the corneal epithelium. Also in contrast to the cornea, the limbal epithelium contains some Langerhans cells and even

melanocytes. Beneath the limbal epithelium is a layer of connective tissue, which is more loosely and irregularly arranged, compared to the corneal stroma. It contains fibroblasts, melanocytes, macrophages, mast cells, lymphocytes and plasmacells, blood vessels, lymphatic vessels and nerves. This connective tissue form large radial ridges that form the palisades of Vogt. These folds, which can be viewed by biomicroscopy, house small blood vessels, lymphatic vessels and nerves. The limbal epithelium reaches down into the grooves and the corneal epithelial stem cells are thought to be situated here.

1.2.2 Anatomy and ultrastructure of the conjunctiva

The conjunctival tissue lies on top of the sclera that surrounds the cornea and extends across the surface of connective and muscular tissue that forms the eyelids (Doughty, 2002; Bergmanson and Doughty, 2005). The entire conjunctival surface is generally divided into two main zones, namely one that is visible around the cornea and between the eyelids (which is referred to as the bulbar conjunctiva) and one that which is not normally visible, which covers the inner surface of the eyelids (and referred to as the palpebral or tarsal conjunctiva). The bulbar conjunctiva extends backwards to the limits of the eyelids where, at the fornix, it reflects to become the palpebral conjunctiva that extends over the underside of the eyelids right out to the eyelid margin. The region under the eyelids, formed between the bulbar and palpebral surfaces, forms the conjunctival sac (Figure 1.2-1), which may be regarded as a third zone (Gipson *et al.*, 2005).

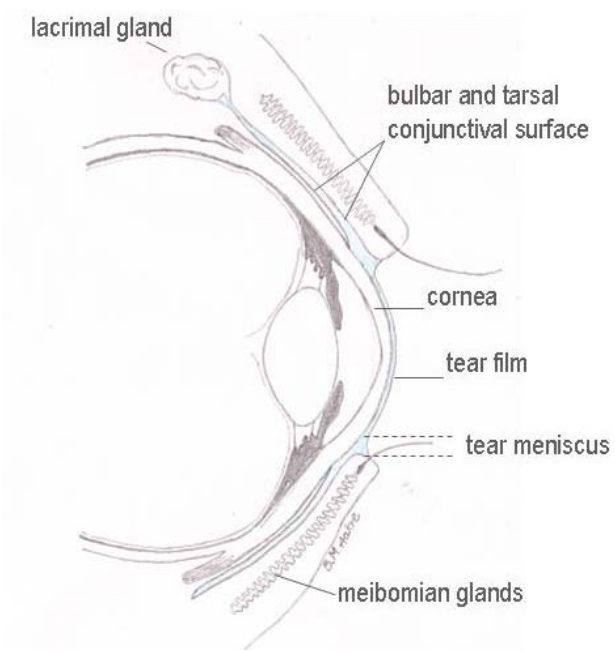


Figure 1.2-1

Layers of non-squamous epithelial cells comprise the conjunctival epithelium. Between them are numerous mucus producing cells, usually referred to as goblet cells. The number of cell layers varies from two to four across the bulbar part and around six at the sclerolimbal junction to ten to twelve at the lid margin. Compared to corneal epithelial cells, the conjunctival epithelial cells contain more organelles (mitochondria), the basal cells undulate along with the underlying

connective tissue and the apical cells do not have the same flat form as the corneal epithelial cells. Scanning electron micrographs reveal numerous intracellular vesicles, which suggest that conjunctival cells may have phagocytising capacities or that these cells may also produce mucin (Gipson *et al.*, 2005). Within the middle portions of the upper eyelid tissue and close to the fornix, the accessory lachrymal glands (of Wolfring and Krause respectively) are located, while ducts from the main lachrymal glands emerge into the fornix region. In the anterior portions of the eyelid tissue, the main tarsal glands (the Meibomian glands) are located, which have ducts that emerge close to the eyelid margin just anterior to the junction between the mucus membrane of the conjunctiva and the eyelid skin. The Meibomian gland orifices are arranged in a distinct line just along the inner edge of the marginal tissue of the eyelids (Bergmanson and Doughty, 2005).

Beneath the conjunctival epithelium is a greatly vascularised loose connective tissue that is rich in immune cells (fibroblasts, lymphocytes, mast cells, plasma cells and neutrophils), the presence of which reflects the conjunctiva's large capacity of managing infectious agents (Gipson *et al.*, 2005). One outer adenoid layer and one inner fibrous layer comprise the tissue, which is commonly termed conjunctival stroma or substantia propria. The deep fibrous layer contains small and large blood vessels and nerves. It is organised with dense groups of collagen fibres as opposed to the lamellar organization found in the corneal stroma. Some deeper bundles contain many fibrils of larger diameter. The collagen of the tarsal portion of the upper palpebral conjunctiva forms a fibrous plate, making it possible to evert the upper eyelid during clinical examination (Bergmanson and Doughty, 2005). Exposure to vasodilatation agents, such as histamines and prostaglandins, results both in dilation of the vessels and recruitment of white blood cells (eosinophils and granulocytes). The conjunctival appearance changes from a quiet "white" state to hyperaemia and injection. As with mast cells, in response to stimulation, inflammatory mediators are released into the blood vessels and surrounding tissue (Bergmanson and Doughty, 2005).

The nerve supply to the limbus and conjunctiva serves to support sensory and secretory functions as well as vasculature. Conjunctival sensory nerve branches and nerve terminals are extensively dispersed within both conjunctival epithelium and stroma. Neural distribution to the vasculature has a sympathetic supply and a parasympathetic supply. This may explain the local control of blood flow often seen in the irritated or inflamed eye (Bergmanson and Doughty, 2005).

Both within the outer adenoid layer and in the deeper fibrous layer are lymphatic vessels, which often follow the route of the blood vessels. When neovascularisation of the cornea develops, lymphatic vessels may extend into the cornea. In response to allergens or mixtures of histamine and prostaglandins, the conjunctiva can become dilated and swollen (chemosis), presumably due to a combination of dilated blood vessels and lymphatic vessels and an increased fluid of the adenoid layer. The fluid must come from the blood vessels. A slightly different effect of tissue oedema occurs across the palpebral conjunctiva. The oedema leads to formation of a pattern rather than general swelling of the tissue. These lines of circular elevations are known as papillae, which comprise a combination of tissue oedema, aggregates of white blood cells and scar tissue. Each papillae diameter is 0.025 mm^2 to 1.0 mm^2 and can be viewed by biomicroscopy

(Bergmanson and Doughty, 2005). The development of papillae is associated with an allergic reaction and there seems to be a mechanical component involved (e.g. the presence of a contact lens, see also section 1.4.3, page 38).

1.2.3 Clinical assessment of the limbus and conjunctiva

In the past, the conjunctiva and its related structures have been assessed clinically by using a slit lamp. Subjective written descriptions by the observer of redness, roughness, and amount of fluorescein staining of the conjunctival tissue have been common. However, to enable detection of subtle changes, most studies in recent years have used some form of grading tool. Descriptive scales from 0 to 4 have been frequent and in the contact lens community the usage of printed or photographic scales have become the clinical standard (Terry *et al.*, 1993; Efron, 1998; CCLRU, 2002). Independent of which scale being chosen, Bailey et al. (1991) suggested to use units of 0.1, which improve the sensitivity of such scales. However, it is not feasible to directly compare the results of ocular surface grading with studies that have used other types of grading scales (Efron *et al.*, 2001; Wolffsohn, 2004).The current study chose to use Efron's printed scale, since this is standard in the university clinic and the observers had most experience with this scale (Efron *et al.*, 2003). The scale allows grading of meibomian gland openings (meibomian gland dysfunction, MGD), tarsal conjunctival roughness and redness, bulbar conjunctival redness and limbal redness. The intra-observer reliability of the printed Efron scale is reported to be between $\pm$ 0.34 to $\pm$ 0.56, which makes it possible to confidently detect changes of 1 unit (Efron, 1998; Efron *et al.*, 2001; Efron *et al.*, 2002; Efron *et al.*, 2003)

More sophisticated grading tools have been used in the recent years. In the era of digital photography, many researchers have developed software, which can detect smaller changes than does a printed or photographic scale. For example, Papas (2000) used colour images of bulbar conjunctiva which were 'digitally analysed to extract…vessel width, number of vessels, proportion of area occupied by vessels, relative redness both in vessels and in the whole image'. However, the review of further studies using such techniques have been omitted from this thesis since a clinical approach of assessing the conjunctiva was chosen and it was considered beyond the scope of this study to use advanced methods for grading.

1.3 Tear film and ocular comfort

1.3.1 Composition and structure of the pre-corneal tear film

The tear film covers the cornea, the bulbar conjunctiva, and the palpebral conjunctiva (Figure 1.2-1). It serves to keep the corneal and conjunctival epitheliae moist and lubricated to facilitate frequent eyelid movement across the exposed ocular surface. Since oxygen is readily soluble in an aqueous solution, the tear film also serves as the primary source of O_2 for the cornea and

conjunctiva (Larke, 1997). The tear film covering the cornea contributes to there being a uniform and smooth optical interface with the air, while the entire tear film (including that under the eyelids) serves to protect the eye against stress and surface infection. The latter functions are attributed to the presence of various chemicals, peptides and proteins with anti-oxidant and antibacterial activity (Larke, 1997).

The tear film consists of an *aqueous phase* embedded between an inner *mucus phase* and an outer (anterior) *lipid phase* (Holly and Lemp, 1977; Farris, 1985). The mucus is formed into a mucin layer that is mainly derived from the goblet cells of the conjunctiva, but there is a secondary source derived from mucin-secreting vesicles present in at least some of the most superficial conjunctival epithelial cells (Inatomi *et al.*, 1995; Gipson, 2004). Thereafter, the mucin is probably interspersed with the aqueous layer because of different degrees of hydration of the mucous components (Tiffany, 1988). There can also be both very fine strands of mucus-like material as well as a porous gel-like matrix overlying the epithelial cell surface (Doughty, 2003). The aqueous phase of the tear film, which is produced by the main and accessory lachrymal glands, contains inorganic electrolytes and low and high molecular organic substances including a small amount of glucose (Farris, 1985; Larke, 1997). The pH (open eye) ranges from 7.1 to 8.6 and the temperature (open eye) between 30 to 35°C (Doughty, 1991). The electrolytes contribute towards the osmotic pressure exerted by the tear film (Doughty, 1985; Larke, 1997). In humans the osmotic pressure (osmolality) has a certain value that is currently considered to be less that 316 mOsm/kg in normal eyes and higher than this in individuals with ‘dry eyes’ (Tomlinson *et al.*, 2006). The lipid phase consists of polar and non-polar lipids, secreted from the Meibomian glands, which usually have the capacity to spread well over the aqueous phase of the tear film to delay tear evaporation and stabilize the tear film (Craig and Tomlinson, 1997).

1.3.2 The tear film and its relationship to ocular comfort

The tear film changes during both contact lens wear and following refractive surgery. The tear film is dynamic, changing with each blink and it is affected by general health, certain diseases, and the environment and by the use of medications. In the extreme, tear film disorders can lead to desiccation of the ocular surface cells leading to a condition called keratoconjunctivitis sicca (KCS). KCS is considered to arise from either a deficiency in the secretion of tears (an aqueous tear deficiency) or a higher than normal evaporation of the tears (referred to as an evaporative tear deficiency) (Lemp, 1995), although both can occur together, especially when there is severe dry eye associated with systemic diseases such as rheumatoid arthritis. In contact lens wearers and those having had refractive surgery, it is most unlikely that severe dry eye will develop, but the symptoms of ocular discomfort that can accompany tear film changes can be very annoying and uncomfortable (see sections 1.4.2 and 1.5.2). One of the intentions of the current study was to make longitudinal assessments of dry eye symptoms in soft (hydrogel and SiH) lens wearers and in pre- and post-LASIK subjects. The following sections summarize how others have assessed ocular comfort with the aim being to develop and validate the questionnaire used in the present study.

Dry eye symptoms are common. Many people will respond positively when asked if they think they suffer from dry eyes. In a large Canadian survey distributed to optometric practices, 28.7% answered 'yes', and amongst the contact lens wearers 50.1% responded positively to the dry eye question. Smaller scale surveys have reported similar results: Two optometry-based surveys found that approximately 30% of the respondents had symptoms of dryness and discomfort (Begley *et al.*, 2001; Nichols *et al.*, 2005). A Japanese ophthalmology-based survey discovered that 33% believed they had dry eyes (Shimmura *et al.*, 1999). It can thus be expected that up to one-third of the general population seeing an ophthalmologist or optometrist will report symptoms of dry eyes. However, individuals may describe dry eye symptoms differently.

In addition to 'dryness', related symptoms can include 'foreign body sensation' or mild 'scratchiness' or intermittent 'grittiness'. As the condition worsens, it can be expected that the symptom intensity will increase to a 'burning' or 'irritative' sensation (Holly and Lemp, 1977). Varieties of descriptors of symptoms have been used. These have included the sensation of 'sand/gravel', 'dryness', 'soreness', 'itching', 'foreign body sensation', 'ocular fatigue', 'light sensitivity', 'crust of eye lashes', 'sticky eyes', 'need to keep eyes shut', 'redness', 'blurred vision', 'tiredness', 'watering', 'pain', 'aching' and 'excessive blinking' (McMonnies, 1986; Toda *et al.*, 1993; Bjerrum, 1996; Bandeen-Roche *et al.*, 1997; Doughty *et al.*, 1997; Rolando *et al.*, 1998; Nichols *et al.*, 1999; Shimmura *et al.*, 1999; Vajdic *et al.*, 1999). In contact lens wearers the most common symptom of dry eyes is 'dryness' (McMonnies and Ho, 1986; Little and Bruce, 1994a; Vajdic *et al.*, 1999; Begley *et al.*, 2000), but 'redness' (Vajdic *et al.*, 1999; Nichols *et al.*, 2004b), 'discomfort' (Begley *et al.*, 2001), and 'visual changes' (Begley *et al.*, 2001) are also symptoms reported by many contact lens wearers. In recent years, questionnaires specifically designed to diagnose moderate dry eyes (Narayanan *et al.*, 2005) and contact lens related dry eyes have been designed (Begley *et al.*, 2000; Nichols *et al.*, 2002a; Nichols *et al.*, 2005). However, there is limited agreement as to what constitutes the most appropriate set of questions to be asked from those suffering from 'dry eye'. Since individuals describe symptoms of dry eyes differently, it is reasonable that a questionnaire include more than one descriptor. In addition, the severity and / or frequency of the symptom are relevant.

The criteria for determining an effective method for summarizing symptoms is likely to be different according the type of patient. For the purpose of detecting dry eye disease (associated with ocular surface desiccation), it is reasonable to identify if a symptom is experienced persistently (Bjerrum, 1996). However, for the purpose of identifying risk factors for the possible development of dry eye, the most common strategy has been to create a symptom score of the severity and / or frequency. These scoring systems have used either an ordinal 4 or 5 point scale (McMonnies, 1986; Bandeen-Roche *et al.*, 1997; Oden *et al.*, 1998; Vajdic *et al.*, 1999; Macri *et al.*, 2000; Begley *et al.*, 2001; Begley *et al.*, 2002; Doughty *et al.*, 2002a; Nichols *et al.*, 2002a; Begley *et al.*, 2003; Nichols *et al.*, 2004a; Nichols *et al.*, 2004c; Nichols *et al.*, 2005). However, more useful for these types of symptoms is the use of a visual analogue scale (VAS) (Miller and Ferris, 1993; Kildeso *et al.*, 1999), which have also been used in studies of the severity and / or frequency of dry eye symptoms (Little and Bruce, 1994a; Rolando *et al.*, 1998; Nichols *et al.*, 1999; Begley *et al.*, 2000).

In addition, the pattern of symptoms may be a more useful indicator, and weighting of symptoms (McMonnies *et al.*, 1998), the combination of symptoms (Oden *et al.*, 1998), the number of symptoms (Bandeen-Roche *et al.*, 1997) or the combination of symptom frequency and intensity (Nichols *et al.*, 2002a) can improve a questionnaire's ability for discriminating dry eye subjects from non-dry eye subjects. To identify risk factors for dry eyes, almost all questionnaires have included requests for information on allergies, medication use, environmental factors such as air conditioning, central heating, smoke and hours of TV-watching / VDU work.

The current study has used elements from several established questionnaires and can be found in full in the appendix, section 5.1.1, p216. For the grading of severity of symptoms, visual analogue scales were chosen.

A substantial knowledge of the type and extent of ocular symptoms is largely the result of research over the last 20 years. Traditionally, a number of assessments of the tear film have been undertaken without much attention being given to symptoms. The next section summarizes the most common clinical assessments of the tear film.

1.3.3 Clinical assessment of the tear film and ocular surface

Lemp (1995) suggested that sufficient tear volume and tear film stability is needed to avoid dry eye symptoms and subsequent desiccation of the ocular surface. In more recent years, there has been considerable scrutiny of both old and new tear film tests and, a strong agreement between tear film tests and dry eye symptoms has not been found. Indeed, some correlations between tear film tests and symptoms of dry eyes have been found to be low or absent by several authors (Begley *et al.*, 2003; Nichols *et al.*, 2004c; Lemp, 2005). Therefore, it can be concluded that dry eye cannot be diagnosed based on a single clinical test. Lemp (1995) and Korb (2002) recommended a battery of tests being used; these should include a questionnaire and tests for the appearance and volume of tears. Today, new tear film tests have shown promising results in the diagnosis of dry eyes, such as tear osmolality (Tomlinson *et al.*, 2006). However, when the present studies commenced, these tests were not clinically established. Therefore, studies regarding this and other newer tests have been omitted from this thesis. Outlined below are evaluations of tests frequently used by clinical researchers and of which some were used in this study. The purpose of this review was the selection and validation of tear film tests for the current study.

Assessments of tear volume from tear meniscus height

A 'dry eye' has traditionally been considered as synonymous with a reduced volume of tears. The aqueous volume of the tears is usually visible, especially at higher magnification with a slit lamp, at the location where the eyelid margin is in contact with the corneal and bulbar conjunctival surfaces. The presence of such a strip of tears and its regularity are part of the evaluation of a dry eye (Doughty *et al.*, 2002c), but its quantitative assessment must be considered superior to subjective impressions. The total tear volume is considered to be around 7.0 µl Mishima et al. (1966). Port and Asaria (1990) divided this into the exposed tear volume (covering the cornea, conjunctiva and

the lid margins) and the unexposed tear volume (that resides in the conjunctival sac). The meniscus of tears at the cornea and lid margin boundary is part of the exposed volume and is considered to comprise almost 50% of the total tear volume (Mishima *et al.*, 1966). Because of gravity, the greatest volume of the exposed tears is presumably in the lower lachrymal tear meniscus (Doughty *et al.*, 2001) but there is also a distinct meniscus at the upper eyelid margin (Wang *et al.*, 2006). According to Guillon (2002), up to 90% of the tear volume is found in the superior and inferior menisci. It has long been suggested that a qualitative assessment of the marginal tear strip is a useful guide in the diagnosis of dry eye (Holly and Lemp, 1977; Guillon, 1998). The quantitative measurement of the tear meniscus has been considered to be linked to the total tear volume by several investigators (Port and Asaria, 1990; Mainstone *et al.*, 1996; Doughty *et al.*, 2002c).

The tear meniscus, usually the inferior one, is usually assessed in a frontal (perpendicular) view. With the meniscus in focus in the slit lamp, its vertical height (from its base, along the eyelid margin, to the contact point with the corneal surface) can be estimated by adjusting the vertical height of the slit beam on the slit lamp linked to a rule (Kinney, 1999). A more accurate method is to incorporate a graticule (likely to be to 0.05 mm resolution) into the objective of the slit lamp (Miller *et al.*, 2004), or a photo-slit lamp used to take photographs or video-recordings of the tear meniscus (Doughty *et al.*, 2002b; Doughty *et al.*, 2002c). The average values from different contemporary studies have ranged from 0.16 to 0.35 mm, with an average of 0.21 mm (Doughty *et al.*, 2002c). Two recent studies reported only limited agreement between two TMH measurements taken on different occasions (Kinney, 1999; Nichols *et al.*, 2004b). However, Doughty et al. (2002c) argued that the analysis of different literature reported values should still be valid, with a lower cut-off limit for normal subjects being 0.1 mm, and a value of >0.25 mm indicating either reflex tearing or sub-optimal tear drainage.

In summary, the TMH measure is a non-invasive test, which is widely used in by the optometric profession and which is likely to give an estimate of the tear volume. Therefore, this test was chosen for the present study as one of several tests to assessing the tear film.

Assessment of tear volume using a Schirmer test or a phenol red thread test

Most of the exposed tear volume is situated in the superior and inferior menisci, and a Schirmer test has been, and still is, the most common test of trying to measure this volume (Korb, 2000; Nichols *et al.*, 2000). The test uses a 35 mm x 5 mm long strip of filter paper with a bent end that is usually placed in the lower conjunctival sac over the lower eyelid margin. The eyes may be open or closed as the test strip is left in place for 5 minutes, and then the length wetted by the tears is read after its immediate removal from the eye. This is the Schirmer 1 test, from which a wetting length of > 10 mm is considered normal, while 10 mm or less is often taken as an indicator of tear volume deficiency and thus a dry eye (Farrell *et al.*, 1992; Nichols *et al.*, 2004b). Despite its common use, the Schirmer test has long since been criticized as stimulating reflex tearing in addition to the resting volume of tears (Jordan and Baum, 1980) and some consider that it is useful to carry out the test after the use of a topical ocular anaesthetic. The result is the Schirmer 2 or basal secretion

test flow, which is hopefully less affected by reflex lacrimation (Pflugfelder *et al.*, 2000), However Jordan et al. (1980) and Baum et al (1986) demonstrated that reflex tearing could still occur in response to the anesthetic eye drops in some individuals. The test has also been criticized for the long test time that is required although a one-minute Schirmer test has been found to correlate highly with those from a 5 minute test, either with or without topical anaesthesia (Bawazeer and Hodge, 2003). However, even a 1-minute test has been suggested to predominantly measure reflex tears (and not tear volume) in normal subjects (Yokoi *et al.*, 2000).

As the distinction between basal and reflex tears became more widely accepted a quicker and less invasive test, which uses a cotton thread rather than a filter paper strip, was developed in the late seventies by Katsuaki Kurihashi (1986). Another Japanese researcher, Hikaru Hamano, further elaborated the test in 1982 and presented it as a basal tear volume test (Hamano *et al.*, 1983). Such a thread, usually impregnated with a vermilion red coloured dye (phenol red), also has a bent over end that is placed over the inferior eyelid margin to be in contact with the tear meniscus. The visibility of the wetted length is enhanced by the phenol red coloration, which changes to yellow in response to absorption of tears. The test is currently known as the phenol red thread or PRT test. As with a Schirmer test, the PRT test is sometimes carried out with the patient's eyelids open and sometimes closed. Recent studies indicate no overall difference between the test results according to whether the test is conducted open eye or closed eye (Doughty *et al.*, 2007). Moreover, the thread is hardly noticeable by the patient and it is left in place for only 15 sec before being removed.

It has been claimed that the PRT test results show 'fair' inter-visit repeatability (Little and Bruce, 1994b; Cho and Chan, 2003; Nichols *et al.*, 2004b) and 'moderate' inter-examiner repeatability (Cho and Chan, 2003). However, the reported average PRT values in Caucasian adults show a wide range (approximately from 17 to 27 mm) (Sakamoto *et al.*, 1993; Little and Bruce, 1994b; Mainstone *et al.*, 1996; Tomlinson *et al.*, 2001; Miller *et al.*, 2004), and different opinions have been offered for interpretation of a lower cut off that would indicate tear volume deficiency. While Hamano and co-workers (1983) stated that 9 mm or less is indicative of dry eye symptoms, and that 6 mm or less defines dry eye (at least for Asian individuals), Little and Bruce (1994b) suggested that 16 mm indicates borderline tear secretion and less than 11 mm is interpreted as low. The manufacturer (Menicon) of the commercially available PRT test (Zone-Quick) suggests that less than 10 mm wetting length is "considered small".

As with a Schirmer test, there continue to be differences of opinion as to what the PRT test measures and as to how the results relate to a Schirmer test or any other dry eye tests and the severity of any ocular symptoms. Nichols et al. (2003) found a moderate to strong correlation between Schirmer and PRT, while Saleh et al. (Saleh *et al.*, 2006) did not find any significant relationship between the two tests. Hamano (1982, in Japanese, cited in (Hamano *et al.*, 1983) initially suggested that the PRT test measure basal tear production rates, since it is less invasive than the Schirmer test. Blades and Patel (1996) showed that the mode of action of a similar self-prepared cotton thread test was achieved through absorption of the lachrymal lake in the lower

fornix. Because of the short test time Sakamoto (1993) theorized that this test gives an indication of the amount of residual tears located in the inferior conjunctival sac of the eye, which is a statement repeated by many others (Cho and Yap, 1994; Kwong and Cho, 1998; Yokoi *et al.*, 2000). It was also argued that PRT-wetting length estimates tear volume, since the insertion of the thread was shown not to interfere with tear film stability (Cho *et al.*, 1996). In contrast, Tomlinson and Blades (2001) concluded that the PRT is unlikely to measure tear volume of the eye or residual tears in the lower conjunctiva, since they found no correlation between tear meniscus height (TMH) and the PRT wetting lengths. However, other studies have shown that PRT measurements positively correlate to TMH measurements, which have generally been considered a measure of tear volume, (Mainstone *et al.*, 1996; Miller *et al.*, 2004).

In the present study, the PRT test was preferred before the Schirmer test, since the PRT test is a quicker and less invasive test that is more likely to give a measure of the amount of tears situated in the eye and not merely reflex tearing.

Assessment of tear film surface lipid layer and tear stability using non-invasive methods

Examination of the tear film in the slit lamp can show signs of small particles, dust, debris or even cells floating in the tear film. The cellular and particulate material may be higher in those with 'dry eyes' (Guillon, 2002) and the eyelid margins may also show signs of inflammation in even borderline dry eye. In a more advanced dry eye, along with the tear film deficiency, the eyelid margins are more likely to show signs of erythema associated with partial or complete blockade of the Meibomian gland orifices. When even slight changes are present, it is also likely that the tear film will be less stable or even unstable. Such changes can be assessed clinically.

With the correct angle of illumination, the surface of the tear film can be viewed with the slit lamp, especially that of the lipid layer. When illuminated with a white light source, an interference phenomenon can be observed in the 'mirror zone' formed by the light reflected from the cornea (the first Purkinje image). The interference forms because the refractive index of the lipid layer is higher than that of the aqueous layer of the tear film (McDonald, 1968). The spectrum of colours seen in the interference pattern indicate the thickness of the lipid layer and can be observed with a biomicroscope (or specular microscope) using the specular reflection technique (Norn, 1979; Forst, 1992; Guillon and Guillon, 1994; Hamano, 1997; Norn, 1997; Guillon, 2002). An even, grey colour indicates a regular lipid layer; coloured fringes indicate a thick layer of uneven thickness, while scant or nearly invisible bluish fringes indicate a thin or nearly absent lipid layer. An alternative is to use a diffuse cold cathode light source as incorporated into a Tearscope^{TM} (Guillon, 1986).

In addition to using a slitlamp or Tearscope^{TM} to assess the lipid layer, the same techniques can be used to assess how long the appearance of the tear film stays the same; this is a measure of the tear thinning or tear break-up time. If the illumination source also includes a grid pattern, then changes in this under specular reflection can be used to assess tear film stability (Hirji *et al.*, 1989; Loveridge, 1993; Cho and Douthwaite, 1995; Craig *et al.*, 1995; Blades *et al.*, 1999). Alternatively, the set of circular (ring) patterns from a keratometer can be used (Patel *et al.*, 1985). As the individual looks at the light source with their eyes open, and without blinking, any distortion of the

grid line or mires represents local thinning, which is believed to occur just before tear break up (Loveridge, 1993). The time in which this phenomenon occurs is the tear thinning time or TTT. Average (or median) TTT values in normal adults have been reported to be between 16 sec and 60 sec (Patel *et al.*, 1985; Mengher *et al.*, 1986; Tonge *et al.*, 1991; Loveridge, 1993; Blades *et al.*, 1999), but individual TTT values can be from as short as 4 to as long as 214 sec. An extension of the TTT method is to wait for streaks or spots to appear in the tear film, as viewed in reflected light, which is considered a non-invasive measure of the tear film break up (or NIBUT). As with TTT assessments, a wide range of average values have been reported, some of which are actually shorter than reported TTT times (Guillon and Guillon, 1994; Migliardi *et al.*, 2000; Nichols *et al.*, 2002b). A NIBUT of less than 20 seconds has been suggested as the lower normal value for adults, and a minimum value to ensure problem free contact lens fitting (Guillon and Guillon, 1994).

The present study chose to measure the non-invasive tear break-up time (NIBUT) using a Tearscope™ because it was readily available at the university clinic. Furthermore, the Tearscope™ was an obvious choice for the current study because it is portable and measurements were to be taken at two different locations (Oslo and Kongsberg).

Assessment of tear film stability with the aid of fluorescein

An alternative, and much more widely used, method to assess tear film stability is to use fluorescein (Korb, 2000; Nichols *et al.*, 2000). Norn (1969) referred to it as the 'corneal wetting time'. The method involves adding a small amount of fluorescein dye to the tear film, usually by briefly touching a saline moistened fluorescein strip to the bulbar conjunctival surface. The fluorescein mixes with the tears, and after a few blinks the dye is evenly spread and the corneal surface is examined with a slit-lamp using a cobalt blue illumination. A blue-yellow or yellow-green faintly coloured tear film can be seen, depending on whether a yellow filter is also used, and the appearance of a darker zone is looked for. This is considered to represent a complete rupture of the tear film layer (since there is no longer any fluorescein in the dark region) and the time in which it occurs after a blink is recorded as the break-up time (BUT) or fluorescein-tear break-up time (f-TBUT). The average f-TBUT in adults was once considered to be between 28 to 30 seconds, but individual values from 3 seconds to as long as 180 seconds were noted (Norn, 1969; Lemp and Hamill, 1973; Vanley *et al.*, 1977). Some consider that the use of the fluorescein will, in itself, reduce tear film stability and so give shorter break up time values (Mengher *et al.*, 1985; Patel *et al.*, 1985), yet there is no evidence that the f-TBUT values are routinely shorter than NIBUT values (see previous section). Furthermore, contemporary studies indicate that an abnormal f-TBUT value was the most common objective finding in a study of different tear film tests in dry eye patients (Nichols *et al.*, 2003). Fluorescein-TBUT values of < 10 sec are considered abnormal, 5-9 sec suggest borderline dry eye and values of < 5 sec are indicative of dry eye disorder, at least in Caucasian populations (Cho and Douthwaite, 1995; Korb, 2002).

Since the installation of fluorescein was needed to assess the ocular surface (see next sub-section) and f-TBUT is so widely used clinically, it was decided to include the test in the present study.

Assessments of the ocular surface by use of dyes and stains

In even moderate cases of dry eye, present over an extended period, tear film deficiency can be expected to result in a desiccation of the corneal and conjunctival epithelia. This is often referred to as 'damage' to the ocular surface (Lemp, 1995), and can occur with or without there being ocular symptoms, although ocular symptoms are likely to precede the initial ocular surface damage.

In both borderline dry eye assessments, including those in contact lens wearers, another non-fluorescent stain called lissamine green has been used (Guillon and Maissa, 2005). Some practitioners consider this a better option than using rose bengal, which has been traditionally used to identify regions of the more severely damaged ocular surface. These vital dyes readily penetrate any compromised, dying or dead cells, and stain them either bright green or crimson red. The rose bengal test has been incorporated into the international standards for the diagnosis of dry eye and is considered to be particularly useful when diagnosing severe aqueous tear deficiencies (Mainstone *et al.*, 1996; Pflugfelder *et al.*, 1998; Pflugfelder *et al.*, 2000). Lissamine green is considered an option to rose bengal partly because it is more readily tolerated (Doughty *et al.*, 2004). However, since the aim of the present study was to assess successful soft contact lens wearers and severe aqueous deficiencies were highly unlikely to occur, assessments using lissamine green or rose bengal were considered superfluous. On the other hand, another dye, which is routinely used, namely fluorescein, was considered to provide sufficient and important information about the integrity of the ocular epithelium.

Fluorescein sodium is the most commonly used dye in the assessment for any ocular surface damage (Lemp, 1995; Korb, 2000). In this assessment, rather than examining the tear film for uniform fluorescence of the dye (under cobalt blue illumination) and its subsequent disappearance when the tear break up occurs, the phenomenon looked for is positive staining. If there is any notable alteration in the tight junctions between the squamous cells, fluorescein will very readily diffuse into and briefly accumulate in any intercellular spaces (Doughty, 2002). If individual cells were desiccated, then the dye will also permeate into these compromised cells (Wilson *et al.*, 1995). Any staining of the ocular surface with fluorescein appears more likely to occur if there is a low Schirmer test or low PRT value (Pflugfelder *et al.*, 1998; Pflugfelder *et al.*, 2000; Nichols *et al.*, 2003).

The intensity and extent of the fluorescein 'staining' can be assessed and graded. Various grading systems have been developed over many years. Rather than attempting to assess the entire exposed ocular surface, it is usually dived up into several regions (Van Bijsterveld, 1969; Lemp, 1995; Xu *et al.*, 1995; Xu *et al.*, 1996; Macri *et al.*, 2000; Battat *et al.*, 2001; Toda *et al.*, 2001; Nichols *et al.*, 2003; Michaeli *et al.*, 2004; Nichols *et al.*, 2004b; Nichols *et al.*, 2004c). Within each region, the overall density of any hyper fluorescent spots can be assigned to different grades with different practitioners choosing different numbers of grading points (Lemp, 1995; Xu *et al.*, 1995; Xu *et al.*, 1996; Macri *et al.*, 2000; Battat *et al.*, 2001; Toda *et al.*, 2001; Albietz *et al.*, 2002; Nichols *et al.*, 2003; De Paiva and Pflugfelder, 2004; Michaeli *et al.*, 2004; Nichols *et al.*, 2004b; Nichols *et*

al., 2004c; Albietz *et al.*, 2005; De Paiva *et al.*, 2006). In contact lens practice, the use of photographic (CCLRU) or schematic (Efron) grading scales from 0 to 4 is commonly used (Terry *et al.*, 1993; Efron, 1998; CCLRU, 2002). The CCLRU scale grade both the *type* (micropunctate, macropunctate, coalescent, patch), *depth* (superficial, delayed stromal glow, immediate local stromal glow, immediate diffuse stromal glow) and *extent* (% of the area within each of the 5 corneal sections) of staining, whereas Efron's scale have incorporated type and depth into one scale and have no scale on the extent. Both scales suggest grading staining in five zones in 0.1 steps. Begley (1996) have further suggested that if staining is present in several zones, a gestalt maximising method can be used to give an overall score. It is difficult to grade corneal staining and there has been considerable debate, and ongoing debate, as to how repeatable the grading of fluorescein staining might be either between examiners or on different occasions (Nichols *et al.*, 2004b).

The CCLRU scale was chosen for the present study, since the observers were most experienced in using this scale.

1.4 Soft contact lens wear and its effects on ocular comfort, the tear film and cornea

1.4.1 A historical overview of contact lens materials and usage

Over a 40-year period, the characteristics of contact lenses have changed substantially (Barr, 2005). Contact lens types range from hard lenses made of PMMA (used in the 1960s), to older generation soft hydrogel lenses developed in the 1970s, to newer generation soft lens materials developed in the early 1990s alongside rigid (gas permeable) contact lenses, to the development of the silicone-hydrogel lens materials in the late 1990's.

Studies over many years have demonstrated that some contact lenses are better tolerated than others (i.e. the comfort levels are different) and that contact lens wear can have substantial effects on the tear film, ocular surface and the cornea manly depending on the oxygen permeability of the lens material (Bruce and Brennan, 1990). As the corneal epithelium relies on the tear film for an adequate supply of oxygen, the placement of many different soft contact lenses over the corneal surface will limit this oxygen availability (Fatt and Weissman, 1992). This oxygen availability can be predicted based on the oxygen transmissibility of the contact lens material, which can be expected to be 10 to 30 x 10^{-9} mL O_2/s mL mm Hg (Yeung and Weissman, 2005) for modern day soft lenses. The silicone-hydrogel material for contact lenses was developed to remove the problem of oxygen availability (Alvord *et al.*, 1998), with the oxygen transmissibility being at least 87 x 10^{-9} mL O_2/s mL mm Hg and so not limiting the oxygen availability to the cornea (Holden and Mertz, 1984).

The development of better contact lens materials in combination with more cost-effective production techniques has directed how a contact lens is used and worn. Initially, soft lens wearers would usually wear their lenses during the day and then remove them overnight. During the overnight period, the lenses would be cleaned and disinfected, ready for re-use the following morning. Some contact lens wearers would re-use a contact lens for as many months, or even years, as they could without replacing them. However, since the late 1980s, it has been common practice to limit the re-use of a particular contact lens by throwing it away and starting with a new pair of lenses. For Norwegian contact lens wearers, such planned replacement schedules would normally vary from as short as 1 month to as long as 9 months. Frequency of lens replacement would depend on patient preferences (including the cost of new lenses) and what was felt optimum by their optometrist. In the mid-1990s, this planned replacement of soft lenses was developed to being on a daily basis, i.e. a new pair of contact lenses would be used each day and would (usually) not be re-used. This method of contact lens wear is usually referred to as daily-disposable soft lens wear. In the late 1980s, considerable interest developed in the possibility of wearing a soft contact lens both day and night. For a few years at least, such extended wear of soft lenses was tried on a 6-day and 6-night basis (with a break from contact lens wear on the 7^{th} day). For the soft lens materials available in the late 1980's, the practice of extended wear was largely abandoned as posing serious risks to the cornea (Brennan and Coles, 1997). However, development of the silicone-hydrogel (SiH) lens re-introduced the concept of being able to wear a contact lens through the day and nighttime period, i.e. continuous wear. The period of continuous wear is dependent on patient choice, but can be up to 30 days and nights, before the lens is removed and replaced with a new lens. When the current study commenced, 36% of Norwegian soft lens wearers (which constitutes 95% new fits / refits) disposed their lenses daily, whereas 60% disposed their lenses monthly or every 1-2 weeks. Extended wear was prescribed in 11% of the refits, and over 95% of extended wear fits were with silicone hydrogel lenses (Morgan *et al.*, 2002).

The subjects recruited for the current study were chosen to reflect the general Norwegian soft contact lens population. In the following sections, attention will mainly be given to the effects of modern soft (hydrogel) lens wear, with inclusion of material on silicone hydrogel lenses where available, on ocular dryness symptoms (1.4.2), ocular surface (1.4.3), the tear film (1.4.4), corneal thickness (1.4.5) and last the corneal endothelium (1.4.6).

1.4.2 Ocular comfort and dryness symptoms associated with contact lens wear

Dry eye symptoms, which can be very annoying and uncomfortable, have frequently been associated with soft contact lens wear. From the literature, it can be estimated that the proportion of soft lens wearers experiencing some lens related ocular dryness is around 50% (44 to 77%) (Little and Bruce, 1994a; Doughty *et al.*, 1997; Guillon *et al.*, 1997; Vajdic *et al.*, 1999; Begley *et al.*, 2000; Begley *et al.*, 2001; Nichols *et al.*, 2005; Chalmers and Begley, 2006). For selected populations of *successful* soft lens wearers the corresponding numbers are lower ranging from 13% and 11% (Vajdic *et al.*, 1999; Chalmers *et al.*, 2005) to around 18-20% (Begley *et al.*, 2000;

Bergenske *et al.*, 2007). Near 30% reported dry eye symptoms to occur 'often' or 'always' in two studies of large unselected populations of contact lens wearers (Begley *et al.*, 2001; Chalmers and Begley, 2006). The overall mean VAS symptom severity (0 to 100 scale) of contact lens wear related dryness in successful full-time soft contact lens wearers have ranged from 18 (Begley *et al.*, 2000) to around 40 (Lakkis and Brennan, 1996; Dumbleton *et al.*, 2006).

The true cause of contact lens-induced dry eye is unknown and probably multi-factorial (Fonn, 2007). Naturally, the dryness sensation can be due to inadequate or marginal tear film quality or quantity (see section 1.3.3). However, the mere presence of a lens increases the risk of experiencing dry eye symptoms, because the lens alters the tear film in several ways (see section 1.4.4). Material properties of the contact lens itself can also influence the tear film: Low lens surface wettability is associated with shorter pre-lens tear thinning times (Thai *et al.*, 2002); lens-surface deposits lower the surface wettability and are associated with contact lens related dry eyes (Lowther, 1997); dehydration of soft lenses during wear can cause dryness symptoms (Little and Bruce, 1995). Therefore, many researchers over the last 20 years have concentrated on which lens material best interacts with the tear film to enhance ocular comfort and minimizing dry eye sensation.

The effects of dehydration and low surface wettability may depend on the type of hydrogel used. Some studies have suggested that low-water hydrogel lenses cause less dry eye symptoms, since they dehydrate and deposit less than high-water content hydrogel lenses (Efron and Brennan, 1988; Nichols and Sinnott, 2006). Similarly, hydrogel lenses that contain phosphorylcholine dehydrate less and have been reported to improve comfort (Hall *et al.*, 1999; Lemp *et al.*, 1999). However, other studies have found dry-eye symptoms to be independent of the dehydration of hydrogel soft lenses (Pritchard and Fonn, 1995; Fonn and Dumbleton, 2003; Morgan *et al.*, 2004). The surface wetting properties of the lens may be more important to enhance comfort. Lately, the incorporation of monomers into the lens materials has been shown to better comfort (Peterson *et al.*, 2006). Rewetting agents (comfort drops) (Subbaraman *et al.*, 2006) and incorporation of these into the disinfection solutions has also been shown to improve tear function (Thai *et al.*, 2002) and enhance comfort (Stiegemeier *et al.*, 2006). Although there is a continuing debate as to whether dehydration or surface properties of the lens material is most important, there is no doubt that the type of lens material influences the tear film and ocular comfort differently.
During the last decade, many contact lens researchers have focused on how the new SiH lens material affects contact lens-induced dry eye sensation and ocular comfort. Preliminary studies at the CCLRU found similar levels of good comfort for both daily soft hydrogel lens wear and SiH continuous wear up to 30 nights. However, the end of day comfort was better in SiH lens wearers and a significant reduction in dryness symptoms was found in previous wearers of soft lenses after refit with SiH lenses. The wettability of this first generation SiH lenses (Focus Night & Day, as in the present study) were similar to that observed with daily soft contact lenses when observed over a period of 12 months (Sweeney *et al.*, 2000). The researchers suggested that the reason for the increased comfort is that SiH lenses dehydrate less than conventional soft hydrogel lenses (as evidenced by less inferior surface staining). It has later been confirmed that SiH lenses dehydrate

less than conventional hydrogel lenses (Jones *et al.*, 2002; Morgan and Efron, 2003). Moreover, preliminary studies by McKenney et al (cited by Sweeney et al. (2000)) suggested that the SiH lenses accumulate less protein deposits that hydrogel lenses, a result which was later reinforced by several research groups (Santos *et al.*, 2007; Suwala *et al.*, 2007). The preliminary results of decreased dry eye symptoms in SiH wearers initiated the current study. The question being asked was if any reduction of dry eye sensation was lasting and stable when assessed prospectively.

Recently, many studies on the new SiH material's effect on ocular comfort have been conducted, and slightly different results have been reported. Adapted soft contact lens wearers who were refitted into SiH lenses for continuous wear reported slightly lower levels of dryness shortly (2 weeks to 2 months) after lens change (Malet *et al.*, 2003a; Dumbleton *et al.*, 2006; Long and McNally, 2006; Riley *et al.*, 2006). While most studies have also reported lasting reduction of dryness symptoms up to 12 (Brennan *et al.*, 2002; Chalmers *et al.*, 2005) and 36 months of SiH wear (Bergenske *et al.*, 2007), the 'new' comfort level was not sustained in one study (Maldonado-Codina *et al.*, 2005). Apart from the study of Riley et al (2006), which deliberately assessed subjects who had problems with their lens wear, studies assessing symptoms in adapted soft lens wearers before and after refitting into SiH lenses have included all soft lens wearers and not specifying whether they had problems with their lens wear. The present study aimed to assess successful soft lens wearers.

1.4.3 Changes in the ocular surface in contact lens wearers

All contact lens wear induces changes in the anterior segment of the eye, including the conjunctiva, the limbal area and the different layers in the cornea. Such changes should always be closely monitored since they signal if the tissues are under stress and some of the changes even signal increased risk of infections. When a lens type and wearing modality is changed, the monitoring of tissue changes will assist in the judgement of the new regime. The following sub-sections summarize the changes that may occur in soft (hydrogel) lens wearers and SiH lens wearers. For changes in corneal thickness and in the corneal endothelium, see sections 1.4.5 and 1.4.6.

Eyelids

Soft lens wear generally does not change the tissue of the external parts of the eye-lid. However, even if it is not a result of contact lens wear, conditions like meibomian gland dysfunction can reduce contact lens comfort substantially (Driver and Lemp, 1996), because of the consequent reduction in the tear film stability (Craig *et al.*, 1995). Mild forms of meibomian gland dysfunction (MGD) have been shown to be present amongst 21% to 40% of contact lens wearers (Ong and Larke, 1990; Ong, 1996; Molinari and Stanek, 2000). Similarly, the degree of blepharitis, which is associated with the presence of gram-positive bacteriae, is undesirable when an individual wears contact lenses because lens comfort will most likely be reduced and the risk of infection increases (Efron, 2004c). The present study sought to investigate successful lens wearers, and subjects with severe MGD or blepharitis were excluded from participation.

Conjunctival and limbal redness

Increased conjunctival and limbal redness (eye redness) occur to a smaller or larger extent in all soft (hydrogel) lens wearers. Increased eye redness is undesirable both for cosmetic reasons and it indicates that the ocular tissues involved are under stress, increasing the risks for more serious contact lens complications such as corneal neovascularisation (Efron, 2004b). Chalmers et al. (2005) found that 35 to 41% of the daily soft lens wearers had limbal and conjunctival redness ≥ grade 1, using Efron's grading scales. Also using Efron's scale, previous studies have reported mean grades of limbal redness to be around 0.4 to 0.65 and conjunctival redness to be 0.7 to 0.87 (all values are measured from graphs in the respective papers) (Morgan and Efron, 2002; Maldonado-Codina *et al.*, 2005).

Many factors are associated with the increased conjunctival redness, such as preservative agents from storing solutions, mechanical influence of the lens itself and depositions of for example lipids and proteins and bacterial toxins on the lens surface giving an inflammatory response (Efron, 2004d). The main aetiology of the vasodilatory process in the limbal region seen in soft contact lens wearers is probably decreased oxygen supply through the peripheral parts of minus powered lenses (Papas *et al.*, 1997).

SiH lenses largely increase limbal and corneal oxygen availability and a reduction in limbal redness in subjects originally wearing conventional soft lenses would not be surprising. Many studies have assessed conjunctival- and limbal redness in subjects wearing SiH lenses, however with varying results. For *new* wearers, two studies have shown that limbal redness did not differ from non-wearers in subjects wearing SiH lenses on a 30 day continuous wear basis (Papas, 2000; Covey *et al.*, 2001). On the other hand, two prospective studies of 12 and 18 month's length have shown that a statistically significant *increase* in conjunctival and limbal redness occurred in new wearers of SiH lenses (Maldonado-Codina *et al.*, 2005; Santodomingo-Rubido *et al.*, 2006). For *experienced*, successful daily soft lens wearers, who were refitted with SiH lenses for continuous wear, several studies report no statistically significant prospective changes of conjunctival (Sweeney *et al.*, 2000; Montero Iruzubieta *et al.*, 2001) or limbal redness (Dumbleton *et al.*, 2001; Stern *et al.*, 2004). However, several other prospective studies (both short-term and up to 3 years), some of which included both extended and daily soft lens wearers, have alleged a significant reduction of the prevalence and mean grading of conjunctival/limbal redness in subjects being refitted with SiH lenses (Nilsson, 2001; Morgan and Efron, 2002; Long *et al.*, 2005; Dumbleton *et al.*, 2006; Bergenske *et al.*, 2007). Statistically significant differences in conjunctival/limbal redness have also been demonstrated in groups of SiH wearers compared to (daily or extended) wearers of conventional soft lenses (Dumbleton *et al.*, 2001; Fonn *et al.*, 2002). The reduction in redness has also been shown to persist: Two prospective studies showed that bulbar and limbal redness decreased shortly after refitting and the effect lasted throughout the study periods of 12 and 36 months, respectively (Nilsson, 2001; Bergenske *et al.*, 2007).

Most studies have reported less eye redness in SiH wearers when compared to soft lens wear. However, there are some discrepancies between the studies. The reason for these may be

differences in the usage of the soft lenses worn in the different studies, which is not always stated. If a great proportion of subjects wore re-usable lenses, eye redness would be expected to be higher in that group compared to a group who wore daily disposable lenses due to lens surface deposits and preservative agents from storing solutions (Hamano *et al.*, 1994). Similarly, a greater difference would be expected if the subjects initially were extended soft lens wearers. Dumbleton et al. (2001) reported that especially the subjects with an *initial* high grading of eye redness experienced a reduction when refitted with SiH lenses. The present study sought to evaluate conjunctival and limbal redness in successful wearers of (predominantly) daily or monthly disposable soft lenses before and after refitting with SiH lenses.

Corneal and conjunctival staining

Corneal fluorescein staining is a common finding in contact lens wearers, and the number of published reports on this phenomenon has been over 100 per year the last decade (Ward, 2008). There are various causes of the staining, which is often multifactorial (Jones and Jones, 2001; Nichols *et al.*, 2002c; Efron, 2004f). These causes range from dryness, hypoxia, allergy, or hypersensitivity to contact lens care solutions (or the interaction between lens material and solutions), inflammation associated with allergy, or to mechanical reasons (with the contact lens itself damaging the ocular surface). The contact lens wear related ocular dryness can be caused by incomplete blinking, tear film deficiencies (in volume or stability), changes in lens matrix hydration, or due to inadequate lens movement or cell debris (Guillon *et al.*, 1990; Little and Bruce, 1995; Riley *et al.*, 2005; Collins *et al.*, 2006). The corneal epithelial cells constitute an important part of the ocular immune system because the tight junctions and intercellular spaces are generally too small for microorganisms to penetrate. If epithelial cells are shed, it is possible for microorganisms to enter the cornea. A recent study found that epithelial staining predicted infiltrative events in continuous wearers of SiH lenses (Szczotka-Flynn *et al.*, 2007). Therefore, it is important to monitor the existence and grade of staining and identify the causes to be able to reduce or eliminate the abrasion.

Due to different aetiologies, it is difficult to grade corneal staining and available literature has described numerous methods. Mean corneal staining can be expected to be around 0.5 when using a 0-4 grading scale. Studies have reported mean staining grades from 0.1 to 0.85 with a mean and median value of 0.44 and 0.42 in experienced soft contact lens wearers, using a 0-4 scale with decimal units of 0.5 or 0.1 (Lowther, 1993; Begley *et al.*, 1996; Jalbert *et al.*, 1999; Sweeney *et al.*, 2000; Morgan and Efron, 2002; Malet *et al.*, 2003b; Maldonado-Codina *et al.*, 2005).

The prevalence of *any* staining in soft contact lens populations has been reported to be just over 50% (Begley *et al.*, 1996; Nichols *et al.*, 2002c; Jalbert *et al.*, 2004), which is not higher than the prevalence in non-wearers (Schwallie *et al.*, 1997; Dundas *et al.*, 2001). However, the prevalence of staining ≥ 1.0 is higher in lens wearers and has been reported to be from 13% to 50% (Begley *et al.*, 1996; Jalbert *et al.*, 2004; Chalmers and Begley, 2006).

It is likely that the use of different grading scales has caused the discrepancies between the studies. The Cornea and Contact Lens Research Unit, at the University of New South Wales, Sydney, Australia, have developed a grading scale for corneal staining from 0-4 in steps of 0.1 (CCLRU, 2002). The maximum zone score of type, extent, and depth of the staining in the central, and temporal, nasal, superior and inferior quadrants are to be noted by the observer. Begley et al. (1996) suggested that if staining was present in several zones, a gestalt maximising method could be used to give an overall score. In their study, 54% of the contact lens wearers had corneal epithelial staining, 20% had staining ≥ grade 1 and 3.2% had staining ≥ grade 2 (Begley *et al.*, 1996). Studies on SiH lenses similarly gave variable results. Covey et al. (2001) reported the mean level of staining in new SiH lens wearers to be similar to non-lens wearers. However, for experienced soft lens wearers, no differences between the mean staining grade in soft lens wearers and SiH lens wearers was reported (Malet *et al.*, 2003b), although SiH wearers occasionally had significantly less staining than the soft lens wearers due to increased staining in the latter group (Sweeney *et al.*, 2000). Prospective studies of new SiH wearers or SiH wearers who were refitted from conventional soft lenses showed increased mean staining over the first 3 to 6 months (Morgan and Efron, 2002; Maldonado-Codina *et al.*, 2005; Santodomingo-Rubido *et al.*, 2006), whereas in longer term studies, no change from baseline values have been demonstrated (Stern *et al.*, 2004; Long *et al.*, 2005; Bergenske *et al.*, 2007). On the other hand, two studies found the prevalence of staining grade ≥ 1 (or equivalent) to be halved shortly after refitting into SiH lenses (Chalmers *et al.*, 2005; Riley *et al.*, 2006).

Many contemporary studies have assessed differences in staining when switching lens types (Morgan and Efron, 2002; Chalmers *et al.*, 2005; Riley *et al.*, 2005; Riley *et al.*, 2006), or when comparing symptomatic versus asymptomatic lens wearers (Lowther, 1993; Nichols and Sinnott, 2006). Significant surface staining needs to be attended to by change of contact lens or lens care systems. Any association between symptoms (of dryness) and ocular surface staining is likely to depend on the assessment methods and grading systems used. Moreover, there is limited agreement as to what constitutes 'clinically significant' corneal staining.

Some degree of *conjunctival* staining is normal, and it has been noted in 71% of ocular evaluations with a mean grade of 0.5 (Schwallie *et al.*, 1998). Diffuse or confluent conjunctival punctuate staining is even more frequent in soft contact lens wearers, where some staining is present in almost all (98%) of the subjects (Lakkis and Brennan, 1996). The group mean grading of conjunctival staining in soft contact lens wearers ranges from 0.4 to 1.37 (Sweeney *et al.*, 2000; Coles *et al.*, 2004; Maldonado-Codina *et al.*, 2005). The aetiology of most conjunctival staining in soft lens wearers is probably mechanical and not due to hypoxia (Lakkis and Brennan, 1996). Therefore it is perhaps not surprising that no significant differences in conjunctival staining grades in soft lens wearers compared to SiH lens wearers have been reported (Sweeney *et al.*, 2000; Chalmers *et al.*, 2005; Maldonado-Codina *et al.*, 2005). One study even reported a continuous increase in mean conjunctival staining grade in soft lens wearers who were refitted with SiH lenses (Maldonado-Codina *et al.*, 2005). However, the changes were small and within the day-to-day range of conjunctival staining reported for non-lens wearers (Schwallie *et al.*, 1998).

Adverse events

The modulus of elasticity of the material in the silicone hydrogel contact lenses is higher than in conventional hydrogel contact lenses. It has been suggested that this increased rigidity, in combination with the continuous wear modality, may lead to a higher frequency of (partly) mechanically mediated adverse events such as contact lens induced peripheral ulcers (CLPU), superior epithelial arcuate lesions (SEAL), epithelial erosions and contact lens induced papillary conjunctivitis (CLPC) (Sweeney *et al.*, 2000; Dumbleton, 2002). Contact lens wear should be completely safe and such adverse events, although non-infectious, are not desirable and should always be recorded.

Infiltrates seen in extended lens wear often occur in the superior region of the cornea covered by the upper eyelid (Gordon and Kracher, 1985). When the eyelids are closed, as during sleep, contact lens wear may change the number of bacteria and immune components in the tear film, leaving the eye more open to infection or inflammation (Dumbleton, 2002). Infiltrates seen in extended lens wear often occur in the superior region of the cornea covered by the upper eyelid. Well defined, round and small infiltrates with overlying epithelial staining are often termed contact lens induced peripheral ulcers (CLPU). CLPU is an inflammatory response, which has been suggested to occur because of exposure to Gram-positive bacteria, in particular Staphylococcus spp. (Holden *et al.*, 1999). Descriptions of CLPU in the literature have suggested that they can be non-symptomatic and they can resolve without any treatment, leaving behind a small scar (Holden *et al.*, 1999). The incidence of CLPU (or non infectious infiltrates) in adapted lens wearers refitted into SiH lenses for continuous wear have been reported to be 3.9% (Nilsson, 2001), 16% (Fonn *et al.*, 2002), 5.4% (Dumbleton, 2002), 3.7% (Malet *et al.*, 2003b) and 8.5% (Stern *et al.*, 2004).

CLPC (contact lens induced papillary conjunctivitis) is thought to be an immunologic (type I hypersensitivity) response to deposits on the contact lens surface and it is more common amongst persons with a history of allergy (Begley *et al.*, 1990; Efron, 2004a). The tarsal conjunctiva also responds in a similar manner when in contact with a foreign object, which suggests aetiology of mechanical trauma (Efron, 2004a). The hallmark of CLPC is the presence of injection of the superior tarsal conjunctiva in addition to papillary hypertrophy. These enlarged papillae are collections of lymphocytes and plasma cells. Papillae can range from small uniform cobblestone appearance to irregular cobblestone appearance to clusters of giant lesions with white centres that may stain with fluorescein. CLPC may be local or general, unilateral or bilateral, which indicates that the pathogenesis is multifactorial (Skotnitsky *et al.*, 2006). Typical signs of CLPC include decreased contact lens tolerance, increased lens movement, ocular itching, and mucous discharge in the tears. These signs and symptoms increase directly with the severity of papillary conjunctivitis (Holden, 1989). Donshik et al. (1999) reported an incidence of CLPC for frequent replacement (less than 4 weeks) of 4.5%. For the continuous wear SiH lenses the incidences of CLPC have been reported to be 4% (Morgan *et al.*, 2005) 11% (Fonn *et al.*, 2002) and over 20% (Stern *et al.*, 2004). Several researchers have suggested that the higher modulus of elasticity of the material in the SiH contact lenses may lead to a higher frequency of mechanically mediated tarsal lid roughness, or

contact lens induced papillary conjunctivitis (CLPC) (Sweeney *et al.*, 2000; Dumbleton, 2002). Some studies have reported an increase of CLPC in SiH lens wearers (Maldonado-Codina *et al.*, 2005; Santodomingo-Rubido *et al.*, 2006). However, Chalmers et al. (2005) found a reduction in the frequency of higher CLPC grades in previous hydrogel lens wearers 12 months after refitting with SiH lenses. Other studies have also found no significant change in the mean CLPC grade in SiH wearers (Montero Iruzubieta *et al.*, 2001; Morgan and Efron, 2002; Bergenske *et al.*, 2007).

The first generation of SiH-lenses has also been associated with the formation of superior epithelial arcuate lesions (SEAL) (Holden *et al.*, 2001). Up to 4.5% of eyes per year of lens wear have been reported to experience SEAL with continuous wear Focus Night & Day lenses (Dumbleton, 2003; Stern *et al.*, 2004).

1.4.4 Changes in tear film in contact lens wearers

Studies concerning contact lens-related tear film properties differ with respect to lens wear. Some studies have evaluated the tear film while the subjects were not wearing their lenses, mainly to assess whether the tear film characteristics differed between subjects having symptoms and those who did not (Hamano *et al.*, 1990; Lowther, 1993; Guillon *et al.*, 1997; Glasson *et al.*, 2003; Nichols and Sinnott, 2006). Inserting a contact lens on the eye immediately alters the tear film partly because the contact lens surface does not have the same wetting properties as that of the corneal and conjunctival surfaces. The tears usually cover the contact lens within a few blinks, and the wettability of the lens is likely to improve as it becomes covered with a layer of mucus originating from the tear film (Cheng *et al.*, 2004; Hori *et al.*, 2006). However, over time, some of the mucus (and other protein material found in the normal tear film) will dry out and form deposits on the surface of the lens and the wettability of the surface will decline. Regular (frequent) replacement of contact lenses may overcome the problem (Lowther, 1997). With a contact lens in the eye, the tear film lipid is also altered, and tends to be thinner over a soft hydrogel contact lens (Guillon and Guillon, 1994) so that the pre-lens tear film stability is much lower. While thicker lipid layers are more likely to be associated with increased tear film stability, they are also more likely to form a continuous lipid layer over the surface of a lens. As these dry out and degrade, fatty deposits or spoilage of the lens surface can occur (Guillon and Guillon, 1994). This in turn may lead to the development of contact lens wear intolerance as symptoms (including those of dry eye). Using a Tearscope^{TM}, the most common lipid layer observed was a wave (flow) pattern, but with no difference seen between the symptomatic and asymptomatic lens wearers (Guillon *et al.*, 1997; Glasson *et al.*, 2003). The initial presence of a contact lens on the eye, especially for a new lens wearer, can also change the composition of the aqueous phase, as some reflex tearing is likely to be present (Tomlinson, 1992 311). The extent and type of compositional changes probably depend on the oxygen permeability of the contact lens (Craig, 2002; Glasson *et al.*, 2002; Hori *et al.*, 2006). As the eye adapts to the presence of the contact lens, there is likely to be a change in ocular surface sensation and sensitivity (Gilbard *et al.*, 1986; Murphy *et al.*, 2001; Müller *et al.*, 2003) and the proportion of reflex tears and the amount of secreted mucus would be expected to change. During soft lens wear, some mucus may become trapped between the lens surface and the corneal

epithelial to form immobile spherical 'mucin balls' (Millar *et al.*, 2003). This phenomena may be more frequent when silicone hydrogel lenses are worn (Pritchard *et al.*, 2000; Dumbleton, 2003).

Since the tear film characteristics in soft contact lens wearers may differ from non-lens wearers, a review of studies assessing the tear film in soft contact lens wearers was undertaken to establish normal values.

Assessments of tear volume in contact lens wearers

Tear volume has been examined in contact lens wearers, from the perspective that if a patient had a lesser volume of tears, then this volume may not be enough to wet the contact lens. Logically, if there were a significant difference, then there would be a difference in symptoms, i.e. patients with inadequate tear volume would be more likely to have symptoms. Such assessments can be done with a contact lens in place, or shortly after lens removal. Both the perpendicular and side (tangential) method of assessments has been reported for tear meniscus height (TMH) (Guillon *et al.*, 1997; Glasson *et al.*, 2003; Miller *et al.*, 2004; Glasson *et al.*, 2006). Using the perpendicular view, some TMH values have been higher than those found in non-contact lens wearers with averages of over 0.3 mm (Guillon *et al.*, 1997; Glasson *et al.*, 2003) while other studies report averages closer to 0.25 mm (Nichols and Sinnott, 2006). Slightly greater values for TMH may be found for asymptomatic (tolerant) contact lens wearers compared to symptomatic wearers (Lowther, 1993; Guillon *et al.*, 1997; Glasson *et al.*, 2003), but with differences in the methods used, it is not possible to make substantial comparisons with non lens wearers. Assessments of TMH with a contact lens in place indicate that it can decrease slightly during the initial period of soft lens wear (Little and Bruce, 1994c), and a slightly smaller TMH (with the lens in place) was noted in adapted soft lens wearers compared to non lens wearers (Miller *et al.*, 2004). TMH values have also previously been reported to be very similar in soft lens wearers and SiH-lens wearers (Miller *et al.*, 2004), and fitting new subjects with SiH-lenses did not influence the TMH values when assessed over 18 months (Santodomingo-Rubido *et al.*, 2006).

Schirmer test assessments have not been routinely reported for contact lens wearers. The test performs better with more advanced disease (Nichols *et al.*, 2004b), and a low Schirmer test value (at the initial assessment for contact lens wear) would be likely to mean that the patient (or subject) would not be considered as a suitable candidate for contact lens wear. However, an alternative assessment of tear volume, using the phenol red thread test, has been used in several recent studies. A higher incidence of fluorescein staining was noted in contact lens wearers who had PRT wetting lengths of less than 9 mm (Hamano *et al.*, 1990). PRT wetting lengths made with the soft lens in place were noted to be shorter than in non lens wearers, and PRT wetting lengths have been noted to be marginally lower in symptomatic (intolerant) symptomatic lens wearers compared to asymptomatic subjects (Glasson *et al.*, 2003; Glasson *et al.*, 2006; Nichols and Sinnott, 2006). Studies assessing tear volume using the PRT test in tolerant or non-symptomatic contact lens wearers have reported group average values from 16.7 to 20.5 mm (Glasson *et al.*, 2003; Miller *et al.*, 2004; Nichols and Sinnott, 2006). Miller et al. (2004) reported no difference in the PRT wetting lengths in soft contact lens wearers and wearers of SiH lenses. However, a tendency towards

lower PRT values in contact lens wearers compared to spectacle wearers was noted (Miller *et al.*, 2004).

Assessment of tear stability in contact lens wearers

A tear film can cover the surface of a soft contact lens if the surface is wettable, and different studies have used different methods to assess this pre-lens tear film. These include using a slit lamp without the use fluorescein (Elliott *et al.*, 1998), a keratometer (Patel, 1991; Little and Bruce, 1995), (video)keratoscope (Faber *et al.*, 1991; Elliott *et al.*, 1998), a Tearscope^{TM} (Guillon and Guillon, 1994; Guillon *et al.*, 1997; Elliott *et al.*, 1998; Glasson *et al.*, 2003) or interferometry methods (Nichols and Sinnott, 2006). A range of average pre-lens TBUT values have been reported from 3 to 13.7 seconds (Guillon and Guillon, 1994; Little and Bruce, 1995), with slightly longer values observed in subjects that had worn the lenses for longer periods (6 h) or who were wearing their habitual lenses (Glasson *et al.*, 2006; Nichols and Sinnott, 2006). Shorter pre-lens TBUTs have been measured after 30-120 minutes of wear (Faber *et al.*, 1991; Patel, 1991; Little and Bruce, 1995; Elliott *et al.*, 1998), with the lenses being new (and thus uncoated) in some studies. A wide range of average NIBUT values *without* a contact lens in place have been reported for contact lens wearers, from 13.2 sec to 33.5 sec (Faber *et al.*, 1991; Guillon *et al.*, 1997; Elliott *et al.*, 1998; Glasson *et al.*, 2003). This range appears to be no different from that on non-contact lens wearers (see section 1.3.3), but it should be noted that in a number of studies, the NIBUT measurements were interrupted after 45 sec or 60s so the values could be arbitrarily low (Guillon *et al.*, 1997; Glasson *et al.*, 2003). Some subjects have extraordinarily long TBUTs which can also be associated with large differences between the measures (Cho and Douthwaite, 1995; Elliott *et al.*, 1998), and it can be argued that further data is needed on those who have stable tear films rather than ignoring them as outliers. Studies that have used “blink-measurements” (i.e. when the subjects blinks before any disruption of the tear film is identified, this time is used) generally have recorded longer NIBUTs (Elliott *et al.*, 1998; Glasson *et al.*, 2003). Longer f-TBUTs can also be expected when a clear interruption of the tear film is used as an end-point and not small black spots or distortions, which often resolve without leading to a break (Faber *et al.*, 1991). Several studies have reported lower average f-TBUT values for contact lens wearers; from 8.5 sec to 12.7 sec (Lowther, 1993; Elliott *et al.*, 1998; Doughty *et al.*, 2005). Other studies have noted even shorter TBUTs for symptomatic lens wearers than for the asymptomatic lens wearers (Lowther, 1993; Guillon *et al.*, 1997; Glasson *et al.*, 2003). It is yet to be well established how quickly the tear stability in contact lens wearers may recover after lens removal (Faber *et al.*, 1991; Elliott *et al.*, 1998; Doughty *et al.*, 2005), but it is likely that values obtained within a few minutes after lens removal will be lower than those seen 30 minutes later.

A study assessing the tear film after refitting with SiH lenses found no changes in the NIBUT values after refitting (Santodomingo-Rubido *et al.*, 2006).

The present study aimed to measure tear film characteristics in soft lens wearers to yield control data for subjects that underwent LASIK (see section 1.5.4) and to ascertain that no subject had any dry eye disorder at baseline.

1.4.5 Changes in corneal thickness associated with soft contact lens wear

Corneal thickness is a parameter that directly represents corneal integrity and it is well known that corneal thickness will increase during contact lens wear due to oxygen deprivation and subsequent lactate accumulation in the stroma (Dutt *et al.*, 1989; Fonn *et al.*, 1999). During the 1960's, several studies reported observations of central corneal oedema or 'central corneal clouding' in patients wearing (PMMA) contact lenses that were impermeable to oxygen (Boyd, 1965; Ruben, 1967). Daily wear of hydrogel (soft) contact lenses has also been reported to induce some corneal oedema although to a lesser extent than PMMA lens wear (Holden, 1989). A correlation between the swelling rate and the oxygen tension at the corneal surface exists (Harvitt and Bonanno, 1999). Daytime oedema in daily wear of soft hydrogel lenses can be expected to be 1-3% for contemporary soft hydrogel lenses (Nilsson and Wrigstad, 1981; Bonanno *et al.*, 1986; Harvitt and Bonanno, 1999).
The swelling response in contact lens wear is relatively acute and the cornea can be expected to adapt to contact lens wear (Erickson *et al.*, 1998; Kaluzny *et al.*, 2003). Several studies have reported no significant difference between CCT in long-term contact lens wearers and non-lens wearers (Carlson and Bourne, 1988; Carlson *et al.*, 1988; Lass *et al.*, 1988; Sibug *et al.*, 1991; Bourne *et al.*, 1999a; Wiffen *et al.*, 2000; Chang *et al.*, 2001). Some studies have even reported thinner corneas in long-term contact lens wearers when compared to a control group (Braun and Anderson Penno, 2003; Iskeleli *et al.*, 2006). Further thinning may occur after lens cessation or refitting into lenses with higher Dk/t (Holden *et al.*, 1985b; Bourne *et al.*, 1999b; Liu and Pflugfelder, 2000; Nourouzi *et al.*, 2006). Corneal thinning has also been shown retrospectively two weeks after lens cessation when compared to a control group (Liu and Pflugfelder, 2000; Braun and Anderson Penno, 2003). The theory is that long-term corneal oedema causes a loss of stromal matrix, and the thinning is not apparent before the oedema is eliminated, which occurs when the O_2 supply increases to normal levels (Holden *et al.*, 1985b).

Para-centrally (3 mm off-centre) the cornea is about 21% thicker than centrally (Doughty and Zaman, 2000). In contact lens wear, this proportion may change. The cornea may not swell homogenously during soft hydrogel soft lens wear. When assessed 2-4 hours after lens insertion, or overnight, it has been reported that the acute swelling response is larger centrally than peripherally (Bonanno and Polse, 1985; Bonanno *et al.*, 1986; Erickson *et al.*, 1996; Moezzi *et al.*, 2004).

SiH lenses have been an option to contact lens wearers over the last decade. These lenses are comprised of a highly permeable siloxane based polymeric phase coupled with a hydrophilic phase, offering Dk/t values > 175 x 10^{-9} Fatt units (Alvord *et al.*, 1998). It has been demonstrated that these lenses induce only marginal hypoxia related changes in the ocular surface tissue (Covey *et al.*, 2001). In subjects who were adapted soft lens wearers (but who did not wear their habitual lenses on the day of testing), no statistical difference was found in the amount of overnight central swelling in SiH wearers when compared to non-lens wear (Steffen and Schnider, 2007). However,

some increase in corneal thickness has been found to occur with overnight wear of SiH lenses in non-adapted lens wearers (Yeniad *et al.*, 2004; Moezzi *et al.*, 2006). No changes have been noted in adapted wearers of conventional soft hydrogel lens wearers who were refitted with SiH lenses (Dumbleton *et al.*, 2006) and a slight thinning response was found for a group of new (non-adapted) SiH wearers over 12 months of wear (Gonzalez-Meijome *et al.*, 2003b). In light of previous studies of adapted soft lens-wearers that were refitted with lenses with higher Dk/t a corneal thinning response would not be unexpected when adapted soft lens wearers are refitted with SiH lenses.

Para-central corneal thickness in SiH lens wearers has not been frequently assessed. González-Méijome et al. (2003b) found a progressive thinning effect in the central cornea in six non-adapted subjects after 3 to 12 months of SiH lens wear. The peripheral areas did not display such an effect, and since only marginal levels of oedema can be expected to occur with SiH lenses and since this lens material has a higher modulus of elasticity than “traditional” soft hydrogel lenses, the authors suggested that the lenses might possess a shaping effect on the cornea. The central corneal thickness increased again 3 months after cessation of SiH lens wear.

Only a few studies have assessed corneal thickness changes in soft lens wearers after refitting with SiH lenses, and only one study (of new wearers) could be identified that considered the mid-peripheral corneal thickness. Neither could a study be identified that assessed corneal thickness at other locations than centrally in adapted soft lens wearers after refitting with SiH lenses. This study attempted to increase the current knowledge of how both central and local (mid-peripheral) corneal thickness in adapted daily soft lens wearers vary over time and how they responds to a lens change, which increases the oxygen availability but also possess a possible shaping effect.

1.4.6 Changes in the corneal endothelium associated with contact lens wear

Brief historical review of morphometry assessments in contact lens wearers

Attention to the endothelial morphometry commenced in the mid-seventies. With access to slit lamps, changes in the endothelial specular reflection were noted in early contact lens studies, e.g. the ‘bleb’ response (Zantos and Holden, 1977; Barr and Schoessler, 1980), although images of the corneal endothelium were difficult to obtain. However, continuing observation led to what was considered to be the extraordinary observation of an increase in the variance in cell areas, i.e. polymegethism (COV) in PMMA lens wearers (Schoessler *et al.*, 1984; Stocker and Schoessler, 1985). It was noted at the same time that pleomorphism accompanied the polymegethism (Schoessler and Woloschak, 1981), i.e. the percentage of ‘hexagons’ (% of 6-sided cells) decreased. The interest in polymegethism has been sustained by miscellaneous reports of impaired control of central corneal thickness when the condition was present (Rao *et al.*, 1979; Sweeney *et al.*, 1985; Nieuwendaal *et al.*, 1994). Others have found no effects (Bourne *et al.*, 1999a) or only in individuals with a substantially reduced deswellling rate (McMahon *et al.*, 1996).

No correlation between the percentage of six-sided cells and deswellling rate was found (Bourne *et al.*, 1999a). Nevertheless, these two morphometric characteristics (COV and %6-sided cells), in addition to estimates of the central cell density (CECD), have been most widely reported in the contact lens literature, as they likely reflects that the corneal endothelium is under stress. The next two sections first review how the appearance of the corneal endothelial cell morphometry can be expected to be in wearers of different soft contact lens materials and second how the morphometry may change after lens change or cessation when an increase in the oxygen availability has taken place.

Endothelial cell morphometry in soft contact lens wearers

In the literature, endothelial cell density (ECD) values have been reported for daily wear soft contact lens wearers either using older materials (MacRae *et al.*, 1986; Carlson *et al.*, 1988; Lass *et al.*, 1988) or newer materials (Erickson *et al.*, 1998; Setala *et al.*, 1998; Bourne *et al.*, 1999a; Sanchis-Gimeno *et al.*, 2003; Odenthal *et al.*, 2005). In adults, the average ECD values found have ranged from 2467 to 3448 cells / mm^2, with an overall mean of 2933 cells / mm^2. These are only marginally less than the values expected from non-contact lens-wearing individuals. COV values have however been reported to be higher than normal, with average values reported being from 29 to 36% (MacRae *et al.*, 1986; Carlson *et al.*, 1988; Lass *et al.*, 1988; Erickson *et al.*, 1998; Setala *et al.*, 1998; Bourne *et al.*, 1999a; Odenthal *et al.*, 2005) for an overall mean COV of 33%. The corresponding value for non-lens wearers is 27% (see 1.1.4). Similarly, pleomorphic changes have been consistently noted for soft contact lens wearers with average values for the %6-sided cells being from 50 to 64% (MacRae *et al.*, 1986) (Carlson *et al.*, 1988) (Erickson *et al.*, 1998) (Bourne *et al.*, 1999a; Odenthal *et al.*, 2005). The overall mean %SIX of 58% is less than the expected values for non-contact lens wearers of 67% (see 1.1.4). In short, central cell density is generally not different in soft contact lens wearers when compared to non-lens wearers, whereas the grade of polymegethism and pleomorphism are clearly higher. It is generally assumed that either change in the endothelium is linked to the oxygen availability to the cornea, and that the lens type (in terms of oxygen transmissibility) years of wear and the wear schedule (e.g. daily wear versus extended wear or continuous wear) have a very important role (Bourne and McLaren, 2004).

Changes in endothelial cell morphometry in soft contact lens wearers

According to some early studies, changes in endothelial cell shapes and sizes occur within 2 weeks (Holden *et al.*, 1985a) to 3 months (Orsborn and Schoessler, 1988) after soft or rigid contact lens wear has commenced. However, it is unclear to which extent morphometric changes of the endothelium are reversible. A few studies have monitored morphometric endothelial changes in previous wearers of contact lenses with no / low oxygen transmissibility after lens cessation (Holden *et al.*, 1985b; Sibug *et al.*, 1991; Odenthal *et al.*, 2005) or after refitting with soft hydrogel contact lenses of higher oxygen transmissibility (McLaughlin and Schoessler, 1990; Nieuwendaal *et al.*, 1991; Bourne *et al.*, 1999b; Barr *et al.*, 2003; Odenthal *et al.*, 2005). Most of them are inconclusive due to small numbers of subjects. However, a review of such studies can nevertheless

represent trends of any changes that may take place and is thus presented here. Some studies found no changes of the polymegethism (Nieuwendaal *et al.*, 1991; Bourne *et al.*, 1999b; Barr *et al.*, 2003) while others propose a possible reduction of COV after refitting into materials of higher oxygen transmissibility (Barr *et al.*, 2003) or after the cessation of lens wear (Holden *et al.*, 1985b; Sibug *et al.*, 1991; Odenthal *et al.*, 2005). Some of the studies included a control group of non-lens wearers illustrating that the observed reduction of polymegethism was not total (Holden *et al.*, 1985b; Sibug *et al.*, 1991). For example, in the study of Sibug et al. (1991) the mean COV in the control group was 30.8, whereas in subjects who had stopped wearing lenses 2 years before the mean COV was still substantial at 42.0. However, one study (Odenthal *et al.*, 2005) reported a COV value of 27.2 after cessation of lens wear, which is similar to that reported for non lens-wearers in the literature (see section 1.1.4). Similar to polymegethism, increased pleomorphism also takes place relatively shortly after lens wear have commenced (Bourne *et al.*, 1999b; Esgin and Erda, 2002). After refitting with lenses of higher oxygen transmissibility, or cessation of lens wear, only small changes were observed in most studies (Nieuwendaal *et al.*, 1991; Sibug *et al.*, 1991; Bourne *et al.*, 1999b; Barr *et al.*, 2003). On the other hand, two studies reported significant increases in the percentage of six-sided cells (Barr *et al.*, 2003; Odenthal *et al.*, 2005), although not to the levels observed in normal non-contact lens wearing individuals. The reviewed studies also showed a tendency of reduced central cell densities after cessation of (low Dk/t) lens wear (Sibug *et al.*, 1991; Odenthal *et al.*, 2005) or after refitting into lenses with higher Dk/t (McLaughlin and Schoessler, 1990; Bourne *et al.*, 1999b; Odenthal *et al.*, 2005). The reduction seems to be largest when the follow-up period was long (more than a year).

Overall, increased oxygen availability to the cornea seems to halt any progression of polymegethism and there is at least a tendency of partial recovery of this morphometric parameter. Since morphometric changes in the endothelium do not appear with silicone elastomer lenses, which are highly permeable to oxygen (Schoessler *et al.*, 1984; Carlson *et al.*, 1990), it is of interest to evaluate whether the new generation SiH lenses would alleviate some of the changes arising from soft hydrogel lens wear. Up to date, no studies could be identified offering a detailed assessment of the endothelial morphometry in adapted daily soft lens wearers after refitting with SiH lenses.

1.5 LASIK and its effect on ocular comfort, the tear film and cornea

1.5.1 LASIK and its subsequent injury to corneal innervation

The principles of Laser-Assisted In-Situ Keratomileusis - LASIK

As noted earlier (see section 1.1.1), a substantial contribution to the refractive power of the human eye is provided by the cornea. If the eye is myopic, the myopia can be reduced by flattening the central cornea and thus reduce the total refractive power of the eye. This is the main principle in

corneal refractive surgery for myopia, of which LASIK is just one example. In LASIK, the standard procedure is essentially as follows: The ocular surface is anaesthetized with several drops of a topical ocular anaesthetic such as oxybuprocaine. The eyelids are opened, and prevented from moving with a speculum, and a support ring is placed around the periphery of the cornea (in contact with the bulbar conjunctiva). The optical regions of the cornea are marked with a special pen so that centre of the cornea is identified with respect to the centre of the pupil and the edges of the cornea. The support ring allows for a mechanical device called a microkeratome to be placed at one edge of the cornea and this is moved across almost the entire cornea surface to cut through the tissue to a certain depth by motorized means. The diameter of the cutting zone and the depth (thickness) of the cut depend on the preference of the surgeon and the desired flattening that is aimed for. The microkeratome produces a flap of tissue, composed of the epithelium and some of the anterior stromal tissue. After the passage of the microkeratome, the flap is still attached to one edge of the cornea (usually on the nasal side). The microkeratome is lifted clear of the corneal surface and the flap is then carefully lifted and folded over to one side. With the eye held in place, the corneal (stroma) surface is then exposed to collimated 193 nm excimer laser light, which wavelength is fully absorbed by the corneal tissue. Using a series of very high frequency pulses of the laser, part of the stroma is then vaporised (ablated) with the high energy of the laser beam over a period of several seconds. The period of laser-induced tissue ablation determines how deep the tissue removal is. The desired ablation depth is decided upon by the corneal thickness, the optical zone diameter and the amount of refractive error correction that is required. For example, in mild to moderate myopia (-2.00 to -8.00 D), the predicted removal of central tissue is between 18 and 71 µm when the optical zone diameter (OZ) is 5.0 mm and between 38 and 147 µm when OZ = 7.0 mm (Chang *et al.*, 2003). Thereafter, the ablated surface is irrigated and the tissue flap is carefully laid back in place. During the immediate post-operative period, the patient will be required to regularly administering eye drops containing antibiotics (to reduce the risk of bacterial infection). In addition, depending on the surgeon, a patient may also be required to administer other eye drops. These can include topical analgesic and anti-inflammatory eye drops (non-steroidal anti-inflammatory drugs such as diclofenac) that help to reduce any sensation of pain after the operation and may be continued for a day or so. Artificial tears to reduce any possible symptoms of ‘dry eye’ are also administered to the patients on the day of surgery (see section 1.5.2 below). The change in refractive power of the cornea is immediate (e.g. from -5 D to 0D) but is likely to slowly change over the subsequent weeks and months (Kozak *et al.*, 2003; Avunduk *et al.*, 2004; Kawana *et al.*, 2004; Hjortdal *et al.*, 2005). In most patients, this change will likely be small and may be either a slight steepening of the cornea (meaning that there is a slight regression of the refractive error change) or a further flattening (meaning that there was an over-correction of the refractive error). In the former situation, this progressive time-related steepening of the post-operative cornea can, in rare cases, be associated with a progressive thinning of the central corneal tissue, a condition referred to as corneal ectasia (Pallikaris *et al.*, 2001; Binder, 2003; Twa *et al.*, 2004) and which results in a failed operation (and sometimes loss of the cornea, requiring a transplant operation).

LASIK, corneal sensation and innervation

There are two immediate effects of LASIK surgery on corneal sensation and comfort, and then the risk of a longer lasting problem of at least some discomfort. In the immediate post-operative period, after the topical anaesthetic has worn off, the patient is likely to experience some degree of ocular pain. This pain originates from the surgery related trauma to the sensory nerves of the cornea and surrounding conjunctiva. Any such pain can be offset with the use of topical analgesics (Non-steroidal anti-inflammatory drugs) and systemic (oral) analgesics. Despite some degree of discomfort, specific tests show that the actual sensitivity of the cornea to a mechanical stimulus is very substantially reduced after LASIK surgery. With the action of the microkeratome, many nerve trunks leading from the central region of the cornea to the peripheral regions will be severed, i.e. epithelial, basal epithelial / subepithelial and some anterior stromal nerves (Linna *et al.*, 1998; Erie *et al.*, 2005; Lee *et al.*, 2006). In the LASIK procedure, some of the deepest stromal nerves are maintained but, overall, the initial destruction of the nerves is associated with a decrease in corneal sensitivity (Kim and Kim, 1999; Linna *et al.*, 2000; Battat *et al.*, 2001; Benitez-del-Castillo *et al.*, 2001; Toda *et al.*, 2001; De Paiva *et al.*, 2006; Lee *et al.*, 2006). Over a period of months to many years, the epithelial and anterior stromal innervation will be re-established (by growths from the peripheral trunks and / or those remaining through the flap edge) (Linna *et al.*, 2000; Erie *et al.*, 2005; Lee *et al.*, 2006). In addition to actually affecting the sensitivity of the cornea to a touch stimulus, ocular surface sensation (comfort) and the tear film will be changed for the period during which the regeneration occurs (Collins *et al.*, 1989; Gilbard and Rossi, 1990; Heigle and Pflugfelder, 1996; Bonini *et al.*, 2003).

It is not fully understood why some subjects experience severe dry symptoms after LASIK, sometimes accompanied with signs of dry eyes as well. However, Toda (2007) has summarized some possible mechanisms that may be involved: The loss of innervation affects the reflex loops of the corneal-lachrymal gland and corneal-blinking leading to diminished aqueous production and lipid expression. The suction ring may also damage the conjunctival goblet cells and thus affecting mucus expression. Altered corneal shape may also change the tear stability. Some of these mechanisms should be possible to assess clinically, and studies, which have measured tear volume and stability, are summarized in section 1.5.4.

1.5.2 Ocular comfort and dryness symptoms after LASIK surgery

The prevalence, frequency and severity of dry eye symptoms after LASIK

Dry eye symptoms are a frequent complication occurring shortly after LASIK surgery. Many studies have reported significant increase of both frequency and severity of dryness symptoms when compared to pre-operative values (Yu *et al.*, 2000; Battat *et al.*, 2001; Toda *et al.*, 2001; Donnenfeld *et al.*, 2003; Cosar *et al.*, 2004; Albietz *et al.*, 2005; Vroman *et al.*, 2005; De Paiva *et al.*, 2006; Tuisku *et al.*, 2007). Even on a longer term (up to five years) after LASIK, around 50% can be expected to report one or more dry eye symptom (Hovanesian *et al.*, 2001; Albietz *et al.*, 2005; Tuisku *et al.*, 2007). Slightly different methods have been used to assess dry eye symptoms. Questionnaires have been used to ask if such symptoms occurred (Toda *et al.*, 2001) or to ask for

details of all symptoms that might be associated with dry eye (Lenton and Albietz, 1999; Yu *et al.*, 2000; Hovanesian *et al.*, 2001). A few studies have included assessments of the frequency and / or the severity of the dry eye symptoms using either an ordinal scale of 4 to 5 levels (e.g. never-rarely-sometimes-often-always) (Battat *et al.*, 2001; De Paiva *et al.*, 2006) or a modified visual analogue scale (VAS) (Hovanesian *et al.*, 2001). Overall, the various studies have noted that the prevalence of dry eye symptoms was greatest in the immediate post-operative period (1 day to 1 month), but the reported estimates have been rather variable, perhaps depending on whether the patients have used artificial tears after the surgery. The reported prevalence of symptoms have ranged from a high of 95% on the first day after the operation (Yu *et al.*, 2000) to a claim being made that these symptoms were no longer present at one month after the operation (Lenton and Albietz, 1999). Some patients would have had dry eye symptoms before their LASIK surgery, and this has been reported to range from 16% to 58% (Yu *et al.*, 2000; Toda *et al.*, 2001; Albietz *et al.*, 2005). Overall, an analysis of the various published studies indicates that dry eye symptoms is a persistent problem (Lenton and Albietz, 1999; Yu *et al.*, 2000; Hovanesian *et al.*, 2001; Toda *et al.*, 2001; Albietz *et al.*, 2005). At 1 day post-operatively, the reported prevalence of dry eye has been reported to be between 33% and 95% (average 65%), and to be between 13% and 85% at 1 to 2 weeks after the operation (average 53%). These estimates, overall, show little change thereafter; averaging 40% (range 0 to 82%) at 1 month, 56% (range 38% to 81%) at 3 months, 57% (range 39% to 78%) at 6 months and was still 51% (range 29% to 85%) even 12 months after surgery.

When the current study commenced, few studies had regularly assessed the prevalence of dry eye symptoms in post-LASIK subjects over a prolonged period. Up to date, few studies have assessed the frequency and severity of the dry eye symptoms post-LASIK, and whether these differ from a control group of contact lens wearers.

1.5.3 Changes in the ocular surface after LASIK

Increased staining of the ocular surface could be expected after LASIK for several reasons. First, LASIK surgery will sever large numbers of epithelial nerves and this is thought to result in moderate to severe corneal epithelial staining in some individuals. The condition is referred to as neurotrophic epitheliopathy (Wilson, 2001). Second, and as outlined under section 1.5.4, many studies state that the quantity and quality of the tear film is deteriorated after LASIK, and last the blink-rate have been reported to be longer post-LASIK (Toda *et al.*, 2001). These processes all have the potential of causing exposure ocular surface staining.

Several studies have assessed ocular surface staining using different dyes. Using fluorescein, an increase of the mean grading score of corneal staining was found 1-2 weeks after LASIK, equalling pre-operative levels thereafter and up to 12 months after LASIK (Battat *et al.*, 2001; Toda *et al.*, 2001; Albietz *et al.*, 2005; De Paiva *et al.*, 2006). However, using lissamine green, Donnenfeld and co-workers noted an increased frequency of subjects with any corneal staining 1 to 3 months after LASIK (Donnenfeld *et al.*, 2003; Donnenfeld *et al.*, 2004). No increase in *conjunctival* staining has

been reported after LASIK using lissamine green (Donnenfeld *et al.*, 2003; Donnenfeld *et al.*, 2004) or Rose Bengal (Toda *et al.*, 2001).

Relatively few studies have assessed corneal and conjunctival fluorescein staining scores regularly over a prolonged period after LASIK. The current study attempted to fill this gap.

1.5.4 Changes in tear film after LASIK

With the frequent reporting of dry eye symptoms after LASIK, there have been numerous studies on the tear film of LASIK patients. These studies have included qualitative assessments of the tear lipid layer, tests for tear secretion or volume (Schirmer or phenol red thread) and both non-invasive tear break up (NIBUT) and fluorescein tear break up time (f-TBUT) assessments and will be reviewed in the following sections.

Estimates of tear volume

Most studies evaluating the tear film after refractive surgery and LASIK have used the Schirmer test without (Schirmer I) or with anaesthetics (Schirmer II). There appears to be little difference in the results from either test; most studies reported a significant decrease in Schirmer test values shortly after LASIK (Aras *et al.*, 2000; Lee *et al.*, 2000; Yu *et al.*, 2000; Battat *et al.*, 2001; Toda *et al.*, 2001; Siganos *et al.*, 2002; Albietz *et al.*, 2005; De Paiva *et al.*, 2006; Kalyvianaki *et al.*, 2006; Lee *et al.*, 2006; Tuisku *et al.*, 2007; Konomi *et al.*, 2008). Pre-operative data for Schirmer tests have ranged from average values of 8.0 mm (Schirmer II) (Yu *et al.*, 2000) to 27.1 mm (Schirmer I) (De Paiva *et al.*, 2006). The calculated overall mean for the pre-operative Schirmer tests in (presumably) white individuals is 17.5 mm (Battat *et al.*, 2001; Siganos *et al.*, 2002; Donnenfeld *et al.*, 2003; Donnenfeld *et al.*, 2004; Michaeli *et al.*, 2004; Albietz *et al.*, 2005; Ghoreishi *et al.*, 2005; Vroman *et al.*, 2005; De Paiva *et al.*, 2006; Kalyvianaki *et al.*, 2006; Tuisku *et al.*, 2007; Konomi *et al.*, 2008). At 1 week after the operation, average Schirmer test values have been reported to be 15.1 mm (range 8.9 to 23 mm) and even lower at 1 month (mean, 14.2 mm, range 9.2 to 25 mm); this indicates an overall reduction by 16%. Slight increases in Schirmer test values appear to occur at later post-operative times. The literature-reported values indicate a mean of 15.5 mm at 2 to 6 months post-operatively and 17.4 mm at 9 to 12 months. Some studies have reported very slight progressive increases in Schirmer test results with time after the operation. Another test which measurement is probably associated with the residual amount of tears in the eye is the phenol red thread (PRT) test, which is frequently used by optometrists (see section 1.3.3, page 26). One recent study using the phenol red thread (PRT) test instead of Schirmer reported small, but significant reductions in the PRT wetting lengths in the early post-operative period (Credie *et al.*, 2007). However, in earlier studies any post-operative reductions in PRT values failed to show significance (Patel *et al.*, 2001; Albietz *et al.*, 2005).

Tear film volume can also be assessed by measuring the tear meniscus height (see page 25). The single study that could be identified reporting TMH values in LASIK subjects found no significant changes in the tear meniscus height after LASIK (Patel *et al.*, 2007a).

Although few studies have used TMH measure or the PRT test in subjects undergoing refractive excimer laser surgery, the TMH measure and the PRT test were preferred in the present study for reasons outlined in section 1.3.3 (page 26).

Assessments of tear film quality and stability (f-TBUT)

It has been reported that the most common lipid layer pattern seen 14 weeks after LASIK surgery was that of an "open meshwork", which represents a thinner and unevenly spread layer compared to a control group with "amorphous" lipid layers (Patel *et al.*, 2001). Break up time measurements with the use of fluorescein have been, and still are, the most frequent method of assessing tear film stability after LASIK (Lenton and Albietz, 1999; Aras *et al.*, 2000; Lee *et al.*, 2000; Yu *et al.*, 2000; Toda *et al.*, 2001; Siganos *et al.*, 2002; Donnenfeld *et al.*, 2003; Donnenfeld *et al.*, 2004; Michaeli *et al.*, 2004; Albietz *et al.*, 2005; Ghoreishi *et al.*, 2005; Vroman *et al.*, 2005; De Paiva *et al.*, 2006; Kalyvianaki *et al.*, 2006; Lee *et al.*, 2006; Tuisku *et al.*, 2007; Konomi *et al.*, 2008). Only a single report could be identified where tear thinning was assessed with a Tearscope^{TM} (Patel *et al.*, 2001). Average values for pre-operative f-TBUT have ranged from 4.1 sec in Japanese patients (Toda *et al.*, 2001) to 21.3 sec in Turkish patients (control group) (Aras *et al.*, 2000). The overall mean for pre-operative f-TBUT data for (presumably) white individuals from the various studies is 11.7 sec (range 7.9 to 21.3) (Aras *et al.*, 2000; Siganos *et al.*, 2002; Donnenfeld *et al.*, 2003; Donnenfeld *et al.*, 2004; Albietz *et al.*, 2005; Ghoreishi *et al.*, 2005; Vroman *et al.*, 2005; De Paiva *et al.*, 2006; Kalyvianaki *et al.*, 2006; Konomi *et al.*, 2008). In the early post-operative period of 1 to 2 weeks, average values of between 5.1 to 9.4 sec have been reported (mean 7.6 sec), while those at 1 month post-operatively have been between 6 and 21 sec (mean 9.7 sec); the latter represents a net decline from baseline of 19%. At 3 and 6 months, the mean f-TBUT values from the studies have been 8.9 sec (range 6 to 14.2 sec) and 9.8 sec (range 5.4 to 16.3 sec) indicating that tear stability does not return to pre-operative levels in this period. The few studies that have assessed f-TBUT longer term after LASIK (from 9 months up to 5 years) found no significant difference from pre-operative values or a control group (Albietz *et al.*, 2005; Tuisku *et al.*, 2007; Konomi *et al.*, 2008).

Most studies have used relatively invasive methods (Schirmer and f-TBUT) to study the effect of LASIK on the tear film volume and stability. In the present study, a decision was made to use the PRT test instead of Schirmer, because the PRT test probably estimates the tear volume rather than the amount of reflex tearing (Mainstone *et al.*, 1996; Miller *et al.*, 2004). Likewise, the TMH measure is non-invasive and thought to reflect tear volume. If LASIK predominantly affects the production of reflex tearing, smaller changes would be expected using non-invasive tests. The f-TBUT test have been criticised for adding fluid to the tear film and thus disturbing it. The non-invasive tear break-up time as measured with the Tearsope™ does not suffer from this type of error.

1.5.5 Changes in corneal thickness after LASIK surgery

A fundamental part of the LASIK procedure for myopia is the excimer laser mediated ablation of an aspherical-convex portion of stromal tissue. The amount of tissue that is removed depends upon the magnitude of the intended correction of refractive error (See section **Error! Reference source not found.**). For a correction of -5.00 D and ablation zones of 6.0 mm or 7.0 mm, the estimated ablation depths are 66 µm and 93 µm, respectively (Chang *et al.*, 2003). In many patients, little, or no, change in CCT occurs over 1 to 2 years after surgery (Hjortdal *et al.*, 2005). However, in some patients, a phenomenon called regression occurs, partly because of changes in central corneal thickness (Netto *et al.*, 2005). In such cases, the CCT slightly but steadily increases up to 36 months after surgery, although the increases may not be statistically significant after the first post-operative month. After LASIK, the *epithelium* has been reported *not* to increase or only modestly increase its thickness (Avunduk *et al.*, 2004; Wang *et al.*, 2004), whereas post-surgical increments of both the stromal bed and flap thicknesses have been reported (Wang *et al.*, 2004). Significant corneal thickening may occur within 3 months after surgery (Avunduk *et al.*, 2004; Wang *et al.*, 2004). For mean myopia values from 4.00 to 6.00 DS, previous studies have reported a mean reduction in central corneal thickness of 72 µm (range 50 µm to 106 µm) 1-3 months after LASIK (Kozak *et al.*, 2003; Erie *et al.*, 2004; Sanchis-Gimeno *et al.*, 2004; Hjortdal *et al.*, 2005; Li *et al.*, 2006; Patel *et al.*, 2007b; Zhao *et al.*, 2007). Six months up to seven years after LASIK, the mean reduction (for similar ranges of myopia) ranged from -72 µm to -52 µm, indicating that central corneal thickness gradually increases with time (Kozak *et al.*, 2003; Avunduk *et al.*, 2004; Hjortdal *et al.*, 2005; Svedberg *et al.*, 2005; Ciolino *et al.*, 2007; Patel *et al.*, 2007b).

The reduction in central corneal thickness is closely associated with the reduction in myopia (Feltham and Stapleton, 2000). However, from the reviewed studies, the subsequent increments in CCT values post-LASIK are not automatically associated with regression of myopia (Avunduk *et al.*, 2004). Dupps and Roberts (2001) described how the (unablated) peripheral cornea thickened after phototherapeutic keratectomy (PTK) in donor corneas. Few studies have assessed the mid-peripheral corneal thickness after LASIK.

1.5.6 Changes in the corneal endothelium after LASIK surgery

Potential hazards to the corneal endothelium following LASIK

LASIK is a refractive surgery procedure usually performed on healthy eyes. For LASIK to be clinically acceptable, it must achieve a high standard of efficacy and an even higher standard of safety. Because of the inability of the endothelium to regenerate in-vivo, effects on the endothelium must be absent or transitory, inducing no permanent damage. Any loss of cells or other morphometric signs of stress would lessen the desirability of LASIK as a secure procedure.
The method of LASIK involves three main procedures and each of these has the potential to damage the corneal endothelium. Therefore, studies that involve all three procedures, or only one of these, are reviewed here.

First, LASIK involves elevation of intraocular pressure. To hold the eye still, a suction ring is placed onto the corneal surface and vacuum is applied so that the intraocular pressure is raised for a short period (around 30 seconds) to over 100 mm Hg (Bradley *et al.*, 2007). Artificial elevation of the intra ocular pressure have been shown to change the appearance of the corneal endothelium in monkeys (Svedbergh, 1975; Ollivier *et al.*, 2003) or rabbits (Melamed *et al.*, 1980), and corneal endothelial cell density is reduced in eyes that have had an episode of acute glaucoma compared to the non-affected eye (Setala, 1979; Olsen, 1980). However, the damage is related to the length of pressure elevation. The short exposure times of elevated IOP during a LASIK procedure may be compared to the similarly raised IOP levels simple eye rubbing can produce (McMonnies, 2008), which single occurrence is unlikely to have a significant effect on the long term appearance of endothelial cell layer. Some acute endothelial changes after LASIK surgery was found to be transient (Kim *et al.*, 2001).

Second, LASIK involves a lamellar dissection. A partial corneal flap is created with a microkeratome. Acute endothelial cell loss / changes have been shown to occur beneath deep (40µm from PLL) incisions into the corneal stroma in rabbits (Dehm *et al.*, 1986; Marshall *et al.*, 1986). After a refractive surgery procedure, which involves deep, orthogonal radial corneal incisions, so called radial keratotomy (RK), endothelial cell loss has been reported in clinical studies (Frueh and Bohnke, 1996). However, in the LASIK procedure only one parallel, lamellar incision is made. In a study of so-called manual keratomileusis (where both the parallel incision and the subsequent ablation are performed manually, without a laser), the endothelial cell density was reduced by 6.2% after surgery of eight myopic eyes (Aquavella *et al.*, 1981).

Third, after lifting the flap, the 193 nm excimer laser is used to remove stromal tissue. The laser does not penetrate beyond a few microns, but because ablations with LASIK are significantly closer to the corneal endothelium than those of photorefractive keratectomy (PRK) are, evaluations of the effect is appropriate. Endothelial damage can hypothetically occur by several mechanisms. The photoablative tissue fragments are expelled orthogonal to the corneal surface. During the acceleration of the removed tissue, repulsive forces are generated (so-called shock / stress waves). At the endothelial surface, the stress wave amplitude has been measured to be 40 atm. regardless of laser beam size (fluence being 180 mJ/cm^2) (Krueger *et al.*, 2001). Some scanning slit and scanning spot systems use fluences in excess of 250 mJ/cm^2 which create stress wave amplitudes of 70 to 100 atm., and are thought to be hazardous to the endothelium (Krueger *et al.*, 2001). Furthermore, radiation of the wavelength used to ablate corneal tissue (193 nm) can theoretically cause mutagenic changes at the endothelial level. An increase in temperature can destroy the endothelium. Long-term metabolic effects after refractive procedures, e.g. changes in glucose diffusion, can also alter the endothelial cell layer. A combination of several of these mechanisms may be required to initiate endothelial damage (Frueh and Bohnke, 1996). Similar to studies of diamond knife incisions, endothelial cell loss beneath the incisions made by a 193 nm excimer laser have been observed by SEM in corneas of rabbits (Marshall *et al.*, 1985; Marshall *et al.*, 1986). Dehm et al. (1986) also noted a ridge extending into the anterior chamber beneath the laser excision, in addition to endothelial oedema end cell junction separation. Later, Berns et al.

(1988) revealed that while high-energy laser pulses resulted in denudation of the endothelium, lower densities per pulse resulted in no cell loss. In the first experimental studies of excimer laser ablations, rather than incisions, the appearance of granular material at the basal side of the endothelium, which migrated anteriorly with time, was noted (Gaster *et al.*, 1989; Hanna *et al.*, 1989). Hanna et al. (1989) also noted enlargement of the intercellular spaces, increased visibility of the endoplasmic reticulum both in corneas which had received an excimer laser ablation with a rotating slit delivery system and in corneas which only had received epithelial scrape injury. Özler et al. (1992) used a similar laser delivery system and noted that higher repetition rates (80 Hz) caused damage to the endothelium at the same depth that did not cause damage using repetition rates under 40 Hz. In vitro studies have also indicated that endothelial function may be affected after excimer laser ablation. Kim and Edelhauser (1996) found that endothelial permeability was significantly impaired from control eyes 3 days after surgery when residual stromal thickness only measured from 150 to 175 µm. Shallower excimer laser treatments do not seem to alter the endothelium to the same extents. Endothelial cell counts from PRK or LASIK treated myopic (-10.0 to -25.0 DS) eyeglobe-pairs showed no difference in endothelial cell counts (or dead cell counts) in treated versus untreated eyes (Kent *et al.*, 1997).

Changes in endothelial cell morphometry following PRK or LASIK

Of clinical studies, one study has assessed acute endothelial cell density changes after LASIK surgery (Kim *et al.*, 2001). Non-contact specular microscopy was performed 15 minutes after surgery, and a non-significant central endothelial cell density (CECD) reduction of 1.1% (from 2816 cells/mm^2 to 2785 cells/mm^2) was noted. Several studies of CECD changes after PRK or LASIK have assessed the endothelium pre-operatively and at up to 12 months post-operatively. However, the results from these studies are not consistent. Two to three months after PRK, some studies have noted no or small changes in CECD (Cennamo *et al.*, 1994; Stulting *et al.*, 1996), while others have noted small increments of 1.69%, 0.56% and 6.96%, respectively (Sher *et al.*, 1991; Carones *et al.*, 1994; Trocme *et al.*, 1996). Most of the subjects in the study that found the largest (and statistically significant) increase of cells were contact lens wearers before they underwent PRK (Trocme *et al.*, 1996). Similarly, three months after LASIK, two studies found no or small, not statistically significant changes in CECD of 0% (Dobbel *et al.*, 2008), -0.31% (Jones *et al.*, 1998) and -0.69% (Perez-Santonja *et al.*, 1997), while for previous contact lens wearers a significant increase in CECD of 2.14% was found in one study (Perez-Santonja *et al.*, 1997). Many studies have also assessed CECD changes 6 months post-operatively. Again, with inconsistent results: After PRK, small non-significant net reductions of ECD from baseline (-0.80% to -2.32%) have been observed 6 months post-operativey in some studies (Pallikaris and Siganos, 1994; Isager *et al.*, 1996; Trocme *et al.*, 1996), while another study found a small increase in CECD of 1.42% (NS) (Rosa *et al.*, 1995). Similarly, for LASIK, a net reduction was found in some studies 6 months post-operatively (-0.59% to -3.24%) (Pallikaris and Siganos, 1994; Pallikaris and Siganos, 1997; Perez-Santonja *et al.*, 1997), while for previous contact lens wearers a significant increase in CECD of 2.74% was found in one study (Perez-Santonja *et al.*, 1997). A small non-significant increase of 0.18% was also reported by Kato et al. (2008), but it is unknown whether the subjects in this study

were contact lens wearers. In the longer term, the results are more consistent. Apart from one of the reviewed studies, all reports a net reduction in the CECD 12 months after PRK or LASIK, ranging from as little as +0.05% to -3.93% with an average of -1.98% (Amano and Shimizu, 1993; Carones *et al.*, 1994; Pallikaris and Siganos, 1994; Spadea *et al.*, 1996; Stulting *et al.*, 1996; Pallikaris and Siganos, 1997; Perez-Santonja *et al.*, 1997; Dobbel *et al.*, 2008). Contrary to this, Trocmé et al. (1996) reported increased CECD (+3.43%) 12 months after PRK compared to pre-operative values. The few studies that have assessed CECD two to five years after surgery got similar results: All but one of the reviewed studies reported net reductions in CECD, ranging from -0.26% to -2.67% (Mardelli *et al.*, 1995; Pallikaris and Siganos, 1997; Isager *et al.*, 1998; Collins *et al.*, 2001; Dobbel *et al.*, 2008). On the contrary, Stulting et al. (1996) reported a 4.47% increase in CECD 2 years after PRK, compared to pre-operative values.

Most studies on endothelial changes after refractive surgery procedures have only assessed the cell density. However, to assess small alterations and signs of cellular oedema or stress, it is commonplace in morphometric research of the endothelium to also report on the variation in central cell area (CCOV) and the percentage of six sided cells (C%SIX). Shortly after LASIK (within 15 minutes), Kim et al. (2001) found the CCOV to be 34.4%, which represented a deterioration compared to the pre-operative CCOV at 32.7%. However, the change was temporary and the group mean CCOV was 30.9% the day after surgery. The authors conclude that these acute changes may represent transient cellular oedema. One month to three years after PRK, the reviewed studies reports an overall mean reduction in the CCOV of 3.4% (percentage points), ranging from no change to a reduction of 8% after surgery (Amano and Shimizu, 1993; Carones *et al.*, 1994; Mardelli *et al.*, 1995; Stulting *et al.*, 1996; Trocme *et al.*, 1996; Perez-Santonja *et al.*, 1997). Statistically significant changes occurred in studies where it was clear that all or most subjects wore contact lenses pre-operatively (Stulting *et al.*, 1996; Trocme *et al.*, 1996; Perez-Santonja *et al.*, 1997), although not all studies are specific on this (Amano and Shimizu, 1993; Carones *et al.*, 1994; Mardelli *et al.*, 1995). Significant changes have been reported both relatively shortly after PRK (Carones *et al.*, 1994; Perez-Santonja *et al.*, 1997) and more than 12 months after PRK (Stulting *et al.*, 1996; Trocme *et al.*, 1996; Perez-Santonja *et al.*, 1997) and no special time-related trend could be extracted from the reviewed material. There are very few studies evaluating the corneal endothelial morphometry after LASIK. Jones et al. (1998) found no change in COV three months after LASIK. However in their extended study, reported by Collins et al. (2001), significant reduction in the COV was reported for both previous lens wearers (from 36% to 32%) and spectacle wearers (from 34 to 31%) three years post-LASIK.

Some studies have also reported on the percentage of six-sided cells after PRK or LASIK. Kim et al. (2001) observed a substantial reduction of 15.8% (percentage points) in the number of six-sided cells shortly after LASIK, which was in accordance with the slit lamp observation of what seemed to be oedematous endothelial cells. As for the variation in cell areas (COV), this decrement was temporary and the percentage of six-sided cells increased to 60.9% one day after surgery, which was still significantly different from the pre-operative value of 63.4%. One to three months after PRK or LASIK, a slight non-significant reduction of around 1% in the number of six sided cells has

been reported by several (Cennamo *et al.*, 1994; Stulting *et al.*, 1996; Jones *et al.*, 1998). On the contrary, in studies, which were specific on contact lens wear pre-operatively, the number six-sided of cells was a little higher 3 months post-surgery and a non significant increase of 4.2% (Trocme *et al.*, 1996) and 2.7% (Perez-Santonja *et al.*, 1997) was reported. Similar to COV, most reports on the assessment of six-sided cells 6 months or more after PRK or LASIK noted a slight increase, of 1% to 3%, in the percentage of such cells (Carones *et al.*, 1994; Rosa *et al.*, 1995; Trocme *et al.*, 1996; Perez-Santonja *et al.*, 1997; Collins *et al.*, 2001). In most of studies, the changes were not statistically significant and no time-related trends could be drawn from the reviewed studies.

There are discrepancies between studies that have assessed endothelial cell layer after PRK or LASIK. There are some indications of different results in previous contact lens wearer compared to spectacle wearers, but a few contradictions in the literature exist. In many studies the group of subjects that were assessed was a mix of previous contact lens wearers and spectacle wearers, or details of contact lens wear was not stated. Therefore, the present study aimed to give a detailed assessment on the effects of LASIK in previous soft contact lens wearers and take into consideration any regional differences in the endothelium, which have also had limited evaluation in the literature (see section 1.1.4).

1.6 AIMS AND OBJECTIVES

1.6.1 Introduction

Numerous studies have assessed the effect of refractive excimer laser surgery on dry eye symptoms and ocular surface characteristics, pre-ocular tear film, corneal thickness and organization of the endothelial cell layer. However, and especially for studies on the corneal endothelium, few studies have assessed changes over a prolonged period, most often only the central parts of the endothelium have been analysed and for the studies on the effect of LASIK it has not always been clear whether the subjects were contact lens wearers or not. No studies offering a detailed assessment of the endothelial morphometry in adapted lens wearers after refitting with SiH lenses have been identified. Although there are some indications in the literature that regional differences can develop in endothelial cell morphometry, most studies have only assessed the central endothelium. Furthermore, there are discrepancies between the studies, and it is not clear why eventual regional differences develop.

When the current study commenced, few studies had regularly assessed the prevalence of dry eye symptoms in post-LASIK subjects over a prolonged period. Up to date, few studies have assessed the frequency and severity of the dry eye symptoms and whether these differ from a control group of contact lens wearers post-LASIK. Furthermore, very few studies on the effect of LASIK on the tear film volume and stability have used non-invasive or less invasive methods.

1.6.2 Aims

The **main aim** of this thesis is to yield new knowledge of the effects of soft contact lens wear on the detailed morphometry of the endothelial cell layer and how the characteristics of this cell layer prospectively change over a period of two years after intervention of either LASIK or refitting with SiH lenses.

The **second aim** of this thesis is to contribute to the current knowledge on the longitudinal effect of LASIK on dry eye symptoms and pre-ocular tear film characteristics compared to soft or SiH contact lens wear.

1.6.3 Objectives

To evaluate how LASIK or SiH lens wear change endothelial cell density and the degree of endothelial polymegethism and pleomorphism caused by previous long-term soft contact lens wear.

To evaluate the regional differences in endothelial cell morphometry in long-term successful soft hydrogel contact lens wearers as compared to spectacle wearers, and how these regional differences may change after LASIK or refitting with SiH lenses.

To explore how the central and mid-peripheral variation in endothelial cell size and shape relate to the central and mid-peripheral cell densities in soft contact lens wearers as compared to spectacle wearers and how this relationship may change after LASIK or refitting with SiH lenses.

To evaluate to what extent the prevalence, frequency and severity of dry eye symptoms in post-LASIK subjects are present over a prolonged period, and if these symptoms differ from those reported from a control group of long-term successful soft contact lens wearers.

To compare the tear film volume and stability change after LASIK when using non-invasive or less invasive methods than most previous studies have used.

CHAPTER 2: METHODS

2.0 Overview

This thesis study was planned as a prospective analysis of vision, tear film- and ocular surface characteristics, corneal thickness and endothelial morphology and morphometry in four separate, parallel cohorts of subjects with myopia (see Figure 2.0-1). The author of this thesis (Bente Monica Aakre; BMA) made the study recruitment and the majority of data collection. However, with the long-term nature of the study and with some subjects (patients) being seen at different clinical sites, assistance with assessments and data collection was provided by Ellen Svarverud (M.Sc. Optom.) and Ann E. Ystenæs (M.Sc. Optom.), both working at the Department of Optometry and Visual Science at Buskerud University College. BMA downloaded, assembled, sorted and typed up all of the collected data. All of the data (i.e. determination of the morphometric indices, tear quality measures, vision and statistical) was analysed by BMA.

The subjects were either spectacle wearers or soft contact lens wearers. Two-thirds of the soft contact lens wearers were intervention groups in that some of these had LASIK performed (to correct their myopia) whereas the others were refitted with silicone-hydrogel lenses for up to 30 days of continuous wear. The groups were matched as best as was realistically possible so that comparisons could be made both over time and between subgroups (at any particular time point).

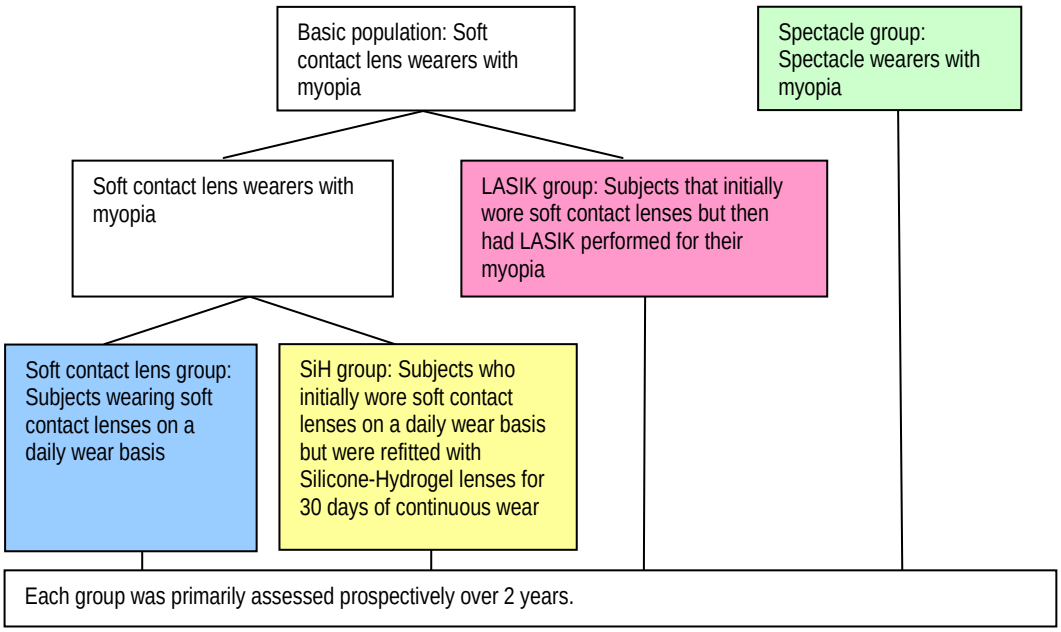


Figure 2.0-1
Study design

The overall plan was to examine each subject six times during 24 months and to obtain a complete set of data. This was not achieved for all subjects that started the study for simple practical reasons (e.g. subjects not being available for examination at all the specified time points during the study). The impact of this will be discussed at the beginning of chapter 3 (Results).

2.1 Subject selection and ongoing management

This study followed the guidelines of the Helsinki Declaration and it was originally approved by the Norwegian Medical Ethics Committee in June 1999. Some revisions were made as the initial recruiting was underway (and again approved by the Norwegian Medical Ethics Committee) and the final protocols were approved by the ethics committees as Buskerud University College and Glasgow-Caledonian University in 2001. A signed informed consent was obtained from all participants, and they could withdraw at any time. All electronic data was linked to an identification number (100-500) and not to personal information.

Subjects were selected based on refractive error (mean spherical equivalent, MSE), ethnic origin, age and soft contact lens experience appropriate to the requirements of the study. The main criterion for eligibility to participate in the present studies was mild-to-moderate myopia. The next most important criterion was that most of the subjects had to be contact lens wearers, apart from a small group of spectacle-wearing myopes. This decision was made because a survey carried out locally indicated that many patients that undergo refractive surgery in the Buskerud University College region were contact lens wearers. Specifically, a printed survey distributed to optometric practices in the Buskerud region, as well as in nearby Vestfold and Telemark regions, yielded a 41% response rate and of these, 70% indicated that the patients referred for surgery were contact lens wearers. The next criterion was to select individuals of Scandinavian origin, rather than a possible mixture of individuals with different ethnic backgrounds. A decision was made to limit the age range of the study participants. The age of a subject is a possible further factor that might influence the overall corneal features and particularly the corneal endothelium (Doughty *et al.*, 2000). Therefore, subjects should not be too young (< 20 years of age) nor too old (> 60 years of age). However, since one of the main interests in the present study was to assess refractive surgery patients, such individuals are typically aged between 20 and 60 years; average ages ranging from 30 to 45 (McGhee *et al.*, 1996; Montes *et al.*, 1999; Salchow *et al.*, 1999; Yo *et al.*, 2000; McDonald *et al.*, 2001). For all of the cohorts in the present study, it was therefore decided that they should have similar ages, namely between 20 and 40 years. However, if a potential participant in the project was just outside these age limits, he or she was not excluded. Nevertheless, if a potential subject were below the legal age of consent (i.e. < 18 years old) or would have likely reached presbyopia before the end of the project (i.e. was > 45 years old), then they were excluded. Finally, in accordance with the literature (Farah *et al.*, 1998), a general exclusion criterion was if a subject had any systemic disease (e.g. diabetes), or any substantial ocular disease (e.g. cataract, glaucoma, keratoconus or corneal dystrophies) that would be expected to have a significant impact on the cornea.

The recruitment of spectacle wearing myopes was initially started through local optometrists who were approached to ask suitable patients if they would like to participate. Folders with information about the study, including an informed consent letter, were distributed to those optometrists who were willing to inform their patients of the proposed study. The response was very low, and abandoned after a few months. An advertisement was then placed in the local newspaper (which

included a brief explanation of the study and asked for participants who had myopia and who never had used contact lenses) after approval by the National Medical Ethics Committee in January 2002. These spectacle-wearing subjects were recruited and examined at the university clinic between March and April 2002. High quality spectacle lenses were dispensed to each subject in the spectacle group. The lenses were kindly provided by Essilor, Norway. Spectacles were dispensed when there had been a change in prescription or when new glasses were otherwise warranted.

For the recruitment of contact lens wearers, the assistance of local optometrists was again sought. However, the response rate was again very low, and after 3 to 4 months, this method of recruiting subjects was rejected. Instead, a few daily soft contact lens wearers known by the author of this thesis were contacted by e-mail and asked if they would like to participate. These subjects were all living in or near Oslo. These subjects were also asked if they knew of other people who also wore soft contact lenses, on a daily wear basis, and who might wish to participate. The new recruitment method proved successful, and all subjects in this group were enrolled and had their first examination in May or June 2002. Subjects in the soft contact lens group continued wearing the same type of lenses as before being recruited. The contact lenses were kindly provided by the suppliers (Ciba Vision; Bausch & Lomb; Vision Care; Johnson & Johnson) or agents (Synsam).

Subjects for the silicone-hydrogel (SiH) group were recruited from the first-year student population at Buskerud University College. These students received a short oral presentation of the project in January 2002, and those who fitted the inclusion criteria were invited to participate. In addition, a description of the study, and an invitation to participate, was sent by e-mail to the department of human resources in the largest local company in Kongsberg. This e-mail also invited interested individuals to volunteer to participate if they fitted the inclusion criteria. The subjects in this group were first examined between February and June 2002. The contact lenses were again kindly provided by the suppliers (Ciba Vision; Bausch & Lomb; Vision Care; Johnson & Johnson) or agents (Synsam).

Recruitment of the LASIK group was made from a surgical practice in Oslo and was achieved with quite some effort. According to the original study protocol, which was approved by the National Medical Ethics Committee in June 1999, recruitment of subjects was meant to be done by the special consultant and not by the author of this thesis. As with the other study groups, local optometrists were contacted for details of those patients whom they had referred for refractive surgery, and to see if any of these patients would like to participate in the proposed study. Folders with information about the study, including an informed consent letter, were distributed to those optometrists who indicated that they were willing to let their patients know about the proposed study. Unfortunately, the response was low, and after 2 to 3 months, this method of recruiting subjects for the study was abandoned and decision made to recruit from the much larger region around Oslo. A surgeon, with an office within an optometric practice in Oslo, was contacted and asked if he might contact his patients directly and to enquire if they would like to participate in the proposed study. This surgeon was chosen because the optometry practice could offer facilities for

testing the patients pre- and postoperatively. Unfortunately, the response rate was again very low, and by May 2000, only four subjects had been tested. These subjects all had PRK done to correct their myopia, but by this time all Norwegian surgeons had converted to LASIK. The lack of subjects caused, amongst other reasons, the study to be temporarily interrupted. On resumption, after considerable further planning and contacts, a decision was made to change the main element of the study protocol to assess post-LASIK patients, rather than post-PRK patients. This required re-submission of ethics documents, but the change was approved by the Medical Ethics Committee in October 2001. A large eye clinic situated in the centre of Oslo also agreed to ask all subjects who fitted the inclusion criteria to participate in the proposed study. Bente Monica Aakre was kindly permitted to contact all the subjects by telephone, and patients, who already had made an appointment for LASIK or a pre-LASIK examination, were contacted. These individuals were asked if they were contact lens wearers and what their prescription was (if not already known). Suitable subjects were thus recruited and appointments for a pre-operative examination were made. Signed informed consent was obtained at the day of the first examination, which took place 1 to 14 days before the LASIK procedure. Since some of the subjects already had been pre-assessed by the surgeon, they had stopped wearing their contact lenses a week or so before the first examination conducted for the present thesis study. Therefore, some of the baseline-data (e.g. visual acuity with lenses in-situ) is missing for this group of subjects. Recruitment and the first examination of the subjects in the LASIK group took place between November 2001 and June 2002.

The LASIK group was under the care of a surgeon (Dr. Willy Pettersen) at the Argus Øyeklinikk in Oslo. Patients were examined by the surgeon pre-operatively, 1 and 14 days post-operatively. These assessments were in addition to the examinations undertaken for the present study. The pre-operative assessments related to the present study mostly preceded the surgeons' assessments. All these patients were asked not to wear their lenses the last 3-7 days prior to surgery, and most ceased lens wear at least a week before surgery. All patients underwent bilateral LASIK in a single refractive surgery centre by one of two surgeons. The Hansatome™ microkeratome (Bausch & Lomb, Rochester, N.Y., USA) with a 160 µm head and 9.5 mm suction ring was used to create a flap 160 µm in thickness and 9.5 mm in diameter. Standard LASIK surgery was performed with a Technolas™ 217z 193 nm excimer laser (Bausch & Lomb), which uses flying spot technology (combining 1mm and 2mm diameter beams) at a repetition rate of 50Hz. Each laser pulse lasts for 18 ns and the laser fluence at the eye surface is 120 mJ/cm^2. The thickness and diameter of the flap was not measured but assumed to be as given by the manufacturer. The ablation zone and ablation depth was determined by the surgeon according to the refractive error, corneal topography and thickness. For the subjects in the present study, the average attempted spherical correction was -4.80 DS and the average attempted cylinder correction was -0.65 DC. The attempted mean spherical equivalent (MSE) averaged 4.93 D (range -2.50 to -8.25 D), the average ablation diameter was 6.10 mm (median 6.0 mm, range 5.5 to 7.0 mm) and the average ablation depth was 93 µm (range 44 to 135 µm). Benoxinate Hydrochloride 0.4% (oxybuprocaine) ophthalmic anaesthetic drops were instilled 3 to 4 times at intervals of 5 minutes before surgery. After the surgery, the patients received Spersadex with chloramphenicol prophylactic ophthalmic drops, to be instilled four times daily for 1 week after

surgery. While none of these patients received any pre-surgical treatment for their tear film (although some presumably had a history of use of contact lens rewetting solutions, for example), all were provided with artificial teardrops (Artelac®) for use in the first week, every 6 h or as needed.

2.2 Clinical evaluations

2.2.1 Facilities and equipment

The LASIK subjects and the soft contact lens-wearing group were examined at the eye surgery clinic in Oslo. Two different examination rooms were used because the clinic was relocated in March 2003. An attempt was made to make the two rooms as similar as possible, although the slit-lamp was changed. Since both rooms were shorter than 6 metres, the visual acuity charts were always placed at 3 metres. The spectacle group and the SiH lens group were examined at the Buskerud University College eye clinic. Despite the fact that many examination rooms in the university clinic are 6 metres in length, a decision was made to assess visual acuity at a distance of 3 metres to match the examination rooms in Oslo as closely as possible. A summary of the instruments used is given in Table 2.2-1.

Table 2.2-1. Instruments used in this study

	Oslo	Kongsberg
Keratometer	Orbscan I or Orbscan II	Javal-Schiötz: Shin-Nippon, Japan (ES)
Slit-lamp	Haag-Streit or Topcon SL 7F	Topcon SL 7F (AEY)
Slit-lamp (tear meniscus height)	Haag-Streit or Topcon SL 7F	CSO (ES)
Specular Microscope	Topcon SP 2000P*	Topcon SP 2000P*
Tonometer	Kowa HA-2*	Kowa HA-2*

* These instruments were portable and thus transported and used at both clinics.

2.2.2 Questionnaire on ocular comfort, health and medical details

All subjects were asked to complete a questionnaire (see the appendix, section 5.1.1 on page 216, which included questions on the presence of typical dry eye symptoms (McMonnies and Ho, 1986; Bandeen-Roche *et al.*, 1997; Doughty *et al.*, 1997). The variables in the questionnaire are listed in the appendix Table 5.1-1. All subjects were asked to indicate the frequency of any particular symptom, with options of ‘never’, ‘sometimes’, ‘often’ or ‘always’. To assess the severity of their symptoms, the subjects were asked to mark a printed 100 mm horizontal visual analogue scale (VAS) where the endpoints representing very mild symptoms and very severe symptoms. Questions on ocular symptoms and medication use were adapted from previous studies on ocular symptoms, including those of office workers and VDU workers (Doughty *et al.*, 1997; Aaras *et al.*,

1998; Aaras *et al.*, 2001; Doughty *et al.*, 2002a; Horgen *et al.*, 2004). Lens wearers were asked about their lens wearing modality, lens material and cleaning/disinfection regime. The subjects who had LASIK done were also asked if they were satisfied with their vision after surgery and all subjects were asked whether they experienced any vision problems during mesopic conditions and while driving a car at night. The severity of visual problems was also graded on a VAS scale. The questionnaire was self-administered, but completed at the clinic, so the patient could ask for help if having problems with completing it. A full examination of any subject lasted for about 50 minutes. No examination lasted for more than 60 minutes.

2.2.3 Evaluation of vision

Visual acuity was assessed with the subject's normal correction (i.e. contact lenses, spectacles or no correction for the post-LASIK group). Habitual high contrast (100%) and low contrast (18% Weber contrast) visual acuity (HCVA and LCVA, respectively) were measured using the Bailie-Lovie LogMAR chart. Optometrists frequently use decimal visual acuity or fraction visual acuity. A conversion table is therefore given in the appendix (Table 5.1-2). The Bailie-Lovie LogMAR chart consists of five British Standard (5x4) letters per line, and the entire chart was constantly visible. Thereafter, those subjects who wore contact lenses removed these. A conventional monocular refraction was then performed, using a retinoscope and a refractor-head. Monocular high- and low contrast visual acuity was measured as described above with best correction in all four groups (BCHCVA and BCLCVA). The outcome measures are detailed in Table 2.2-2. After the evaluation of vision, some of the ocular measures were made (keratometry, specular microscopy, see section 2.2.6 below). The subjects were given a questionnaire (see section 2.2.2 above). This order was chosen to let the tear film stabilize as much as possible before the tear film tests took place.

Table 2.2-2 Variable-list obtained from evaluation of vision

Variable	Abbreviation	Definition	Type	Unit / definition
Vision				
Habitual high contrast visual acuity	HCVA	Resolution property (of high contrast letters) of the eye	Interval	0.02 LogMAR
Habitual low contrast visual acuity	LCVA	Resolution property (of low contrast letters) of the eye	Interval	0.02 LogMAR
Spherical refractive error	SRE	Spherical spectacle refraction for optimum visual acuity	Interval	0.25 DS
Cylinder refractive error	CRE	Cylinder portion of total refraction giving optimum visual acuity	Interval	0.25 DC
Mean Spherical Equivalent	MSE	Theoretical value = |SE| + |CE/2|	Interval	0.25 DS
Best corrected high contrast visual acuity	BCHCVA	Best corrected resolution property (of high contrast letters) of the eye	Interval	0.02 LogMAR
Best corrected low contrast visual acuity	BCLCVA	Best corrected resolution property (of low contrast letters) of the eye	Interval	0.02 LogMAR

2.2.4 Assessment of tear film characteristics

After adaptation to the test room for at least 20 minutes, the tear meniscus height (TMH) was measured using a 0.1 mm graticule gauge attached to one of the ocular eyepieces on the slit lamp biomicroscope. The subjects were seated comfortably and instructed to put their chin on the chin rest and forehead against the forehead rest of the slit-lamp. The canthus mark on the instrument was aligned with the outer canthus of the subject. After adjustments of the slit lamp focus at 10 X magnification, the subject was instructed to look horizontally to the distance (to the clinic wall). A relatively narrow beam of light was focused on the lower fornix of the eye under examination. To avoid reflex tearing due to bright light, care was taken so that the light did not fall on the cornea (Little and Bruce, 1994c). The subject was asked to refrain from blinking and one central measurement was made of the inferior lachrymal tear meniscus height (TMH) in primary gaze.

The pre-corneal tear film was then assessed with the Keeler Tearscope Plus®. The subjects were again asked to look straight ahead but were not specifically asked to undertake any voluntary blink. It was felt appropriate to indicate for some subjects that they should not stare, but blink normally. The overall appearance was noted and categorized into one of seven states (Guillon and Guillon, 1994). The appearances of the surface patterns of the lipid layer (lipid layer thickness; LLT) are described in Table 2.2-3. This assessment of the tear lipid layer was done over a period of 15 to 30 sec after the subject had blinked a couple of times. The non-invasive break-up time (NIBUT) was measured using the Keeler Tearscope Plus®. Subjects were instructed to blink normally a few times and then to refrain from blinking until the appearance of first visible break could be observed. The time between eye opening after a blink and the break was recorded in 0.5 seconds by a built-in stopwatch. Three measurements of NIBUT were made and the average noted. The observations continued for as long as considered necessary to observe a discontinuity, but occasionally a subject blinked before this was observed. In such cases, the time before blink was recorded (Guillon *et al.*, 1997).

A phenol red thread (PRT, “Zone Quick”®) assessment was next undertaken. The 3 mm long, bent end of the fine thread was inserted into the upper part of the inferior conjunctival fornix, approximately one third of the lid length from the outer canthus. The test was carried out following the manufacturer’s instructions except that the subject was asked to look slightly upwards and nasally instead of looking ahead to minimize the possibility of contact between the thread and cornea (Cho and Chan, 2003). The tests were conducted ‘open eye’ with the length wetted after 15 seconds immediately measured with a 0.5 mm ruler on the packaging supplied with the thread. A single assessment was made.

Fluorescein-tear break up time (f-TBUT) was then measured, using fluorescein sodium applied to the temporal palpebral conjunctiva using a Bio-Glo™ Fluorescein Sodium Ophthalmic strip (or Fluorescein Strips Chauvin ™). The strip was wetted with a drop of non-preserved, buffered saline, and excess liquid on the strip was shaken off before application. A Wratten no. 12 barrier filter was

held in front of the slit-lamp objectives and the built-in cobalt blue filter was placed in front of the light source to enhance the appearance of the fluorescein. The patient was instructed to keep eyes open after their next normal blink. It was emphasised that the subjects should not squeeze their eyelids. The time elapsed between the last blink and the first break of the tear film was measured in using a stopwatch to 0.5 seconds. Break was defined as the first appearance of a black line or zone. Three measurements of f-TBUT were made and the average noted.

Table 2.2-3. Variable-list, tear film assessments

Variable	Abbreviation	Definition	Type	Unit / definition
Tear meniscus height	TMH	the height of the tear prism at lower fornix	Interval	0.05 mm
Tear film quality	LLT	estimation of lipid layer thickness using Tearscope	Ordinal	1 = Absent 2 = Open Meshwork 3 = Closed meshwork 4 = Flow 5 = Amorphous 6 = Normal Colour Fringes 7 = Abnormal Colours
Non-invasive break up time	NIBUT	non-invasive tear break-up time as measured with a Tearscope	Interval	0.5 sec
Tear film volume	PRT	a measure of tear quantity by phenol red thread test	Interval	0.5 mm
Fluorescein-tear break up time	f-TBUT	fluorescein break up time	Interval	0.5 sec

2.2.5 Ocular surface characteristics

The ocular surface and adjacent structures were assessed by slit-lamp biomicroscopy before the use of fluorescein and then again after the use of fluorescein.

Any blepharitis and Meibomian gland dysfunction *(MGD)* was graded according to Efron's grading scale from 0 to 4 in steps of 0.1 (Efron, 2004e) for the purpose of exclusion at baseline if exceeding grade 2. Blepharitis and MGD were not included in the analyses but noted for clinical purposes to ensure successful lens wear. Ocular surface characteristics such as bulbar and limbal redness and corneal vascularisation were also graded according to Efron's grading scale from 0 to 4 in steps of 0.1. Bulbar conjunctival and corneal epithelia were evaluated by the use of fluorescein, again with the surface of the eye being viewed in cobalt blue light and a yellow filter (Kodak Wratten no 12). The presence of epithelial staining was graded according to CCLRU's grading scale from 0-4 in steps of 0.1 (CCLRU, 2002). This scale was preferred before Efron's scale since both observers were more experienced in using the CCLRU scale for the grading of corneal staining. The score of type, extent, and depth of the corneal staining were assessed for five zones (central zone plus temporal, nasal, inferior, and superior quadrants). A gestalt maximising method was used to give an overall score; i.e. the observers chose to weight the highest score in one zone more heavily than the other zones using their clinical experience to assign a value rather than just dividing the sum of scores from the five zones by five (Begley *et al.*, 1996). For the statistical analyses, the average grade of extent and depth of staining was used. Conjunctival staining was assessed using

Efron's scale from 0 to 4 in steps of 0.1. Last, the tarsal conjunctiva was examined for redness and roughness (*CLPC*) and graded from 0-4 in steps of 0.1(Efron, 2004e).

Adverse events, both infectious such as microbial keratitis (MK), and non-infectious contact lens induced peripheral ulcers (CLPU), superior epithelial arcuate lesions (SEAL), and contact lens induced papillary conjunctivitis (CLPC) were noted and closely followed up. Subjects who experienced any adverse event were excluded from further participation in the study.

2.2.6 Corneal measurements and photography of the endothelium

Two methods were used to assess the anterior surface curvature of the cornea. For the spectacle wearers and the SiH group, central keratometry readings were taken with a keratometer (Javal-Schiötz principle: Shin-Nippon, Japan). For the soft contact lens wearers and LASIK subjects, the equivalent simulated-K values from the central region of the anterior corneal maps were obtained with an Orbscan II topographer. Corneal thickness values were recorded with a Topcon SP-2000 non-contact specular microscope. Measurements were made, using the instrument in AUTO-focus mode, at the central location (to give central corneal thickness, CCT) and a nasal location 3 mm from the central measurement (to give a mid-peripheral corneal thickness, MPCT). Three measurements were made at each location. At the same time the thickness measurements were made, the central and mid-peripheral region of the endothelium of one eye was photographed with the Topcon SP-2000P non-contact specular microscope. The images were saved as digital files (in both *.TIFF and *JPEG formats.

2.2.7 Endothelial image analysis

The endothelial images containing a scale marker (200 µm) were transferred into the Topcon IMAGEnet software system, which is available as an option to the microscope. As a first step, the automated cell bordering procedure was used to identify approximately 200 cells on each image. In this procedure, a region of interest (ROI) was defined and then all the cell-cell borders were provided as a red overlay by the computer.

As detailed elsewhere (Cheung and Cho, 1998; Cheung and Cho, 2000) the initial outcome was reviewed and, as a manual option with an on-screen editor, corrections were then made to the cell-cell border limits where there appeared to be obvious errors between the cell borders on the digital image and the overlay (see Figure 2.2-1). Usually, some 75 to 100% of the cell borders within the boxed area were adjusted, with these including relocation of cell apices on the overlay and deletion of erroneous "borders". After the corrections were made, 100 contiguous cells were chosen by deleting or blocking out the other traced cells. The software option was then used to calculate the cell areas. The output data consisted of average cell area and its variance (COV), the percentage of each cell type (based on the number of cell sides) and an endothelial cell density estimate (ECD) calculated from the average cell area.

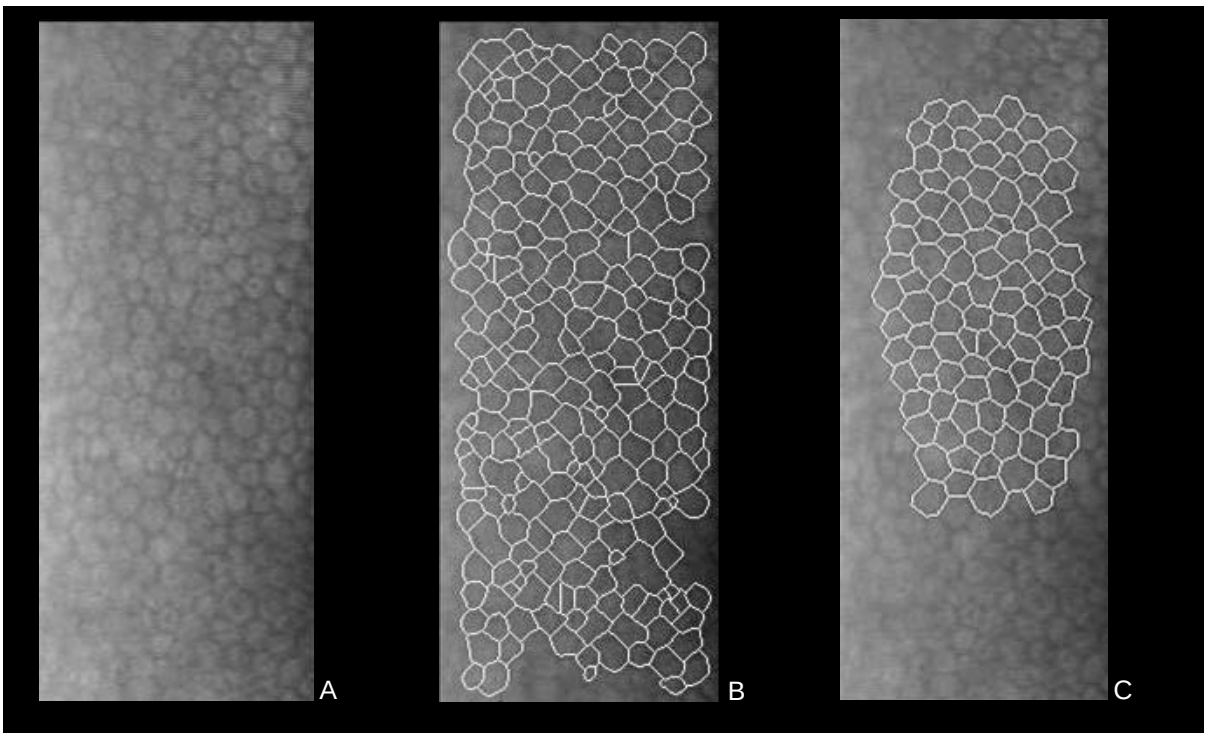


Figure 2.2-1
Endothelial images as provided by Topcon 2000P specular microscopy (A) and after automated (B) or manually edited (C) endothelial cell border tracings (IMAGEnet 2000).

2.3 Data assembly and statistical analysis

All data were collected from one eye only to avoid statistical errors (Ray and O'Day, 1985; Murdoch *et al.*, 1998). The subject's dominant eye was chosen.

For all subjects, data was typed into spreadsheets in SPSS® for Microsoft v. 12.0.1. For all data, average values were calculated and presented in tables as mean values ± 1 S.D., often with the range (minimum to maximum values) in brackets. A decision was made to present mean and standard deviation for all variables although not all were normally distributed (see below) because most other studies have presented their data in this manner and thus comparisons could be made.

For longitudinal comparisons of parametric data, repeated ANOVA tests and appropriate post-hoc tests (Bonferroni correction) were used. Normality was determined by the Shapiro-Wilk test; if $p \geq 0.4$, at the majority of occasions it was considered reasonable to make the assumption of normality. If the data were clearly non-parametric, Friedman's test was used. Since SPSS does not offer any post-hoc test for non-parametric tests of more than 2 related means, the Wilcoxon Signed Rank test was used instead, taking a conservative approach by choosing a significance level of 1% ($\alpha = 0.01$). Between-groups comparisons of parametric variables were made using the One-way ANOVA test and Bonferroni post-hoc test. If the data were non-parametric, Kruskal-Wallis test was used, and similarly to the approach for time-related comparisons, a two-sample test for non-parametric data was used post-hoc to reveal which groups differed (Mann Whitney U test,

α = 0.01). The Chi-squared test was used for counts, and p-values less than 5% were regarded as statistically significant.

For correlation analyses, a simple statistical approach was taken since the aim was not to model in detail eventual relationships, but to simply exploring if any trends were apparent and how they might change after refitting into SiH lenses or after undergoing LASIK for myopia. Assumptions of normality and equal variance of each variable was taken. Linear regression analysis (Pearson's correlation coefficient) was used to explore if any relationships were evident. Partial correlation was used to control for confounders. Scatter plots, with linear regression lines where appropriate, were used to illustrate relationships.

Charts were created using the chart wizard tools in the software SPSS® for Microsoft v. 13.0.1 and v. 16.0 and Microsoft® Office Excel 2003.
Box plots were used to illustrate the median and variation of the data for each group at every occasion. See Figure 2.3-1 for further explanation of this kind of chart.

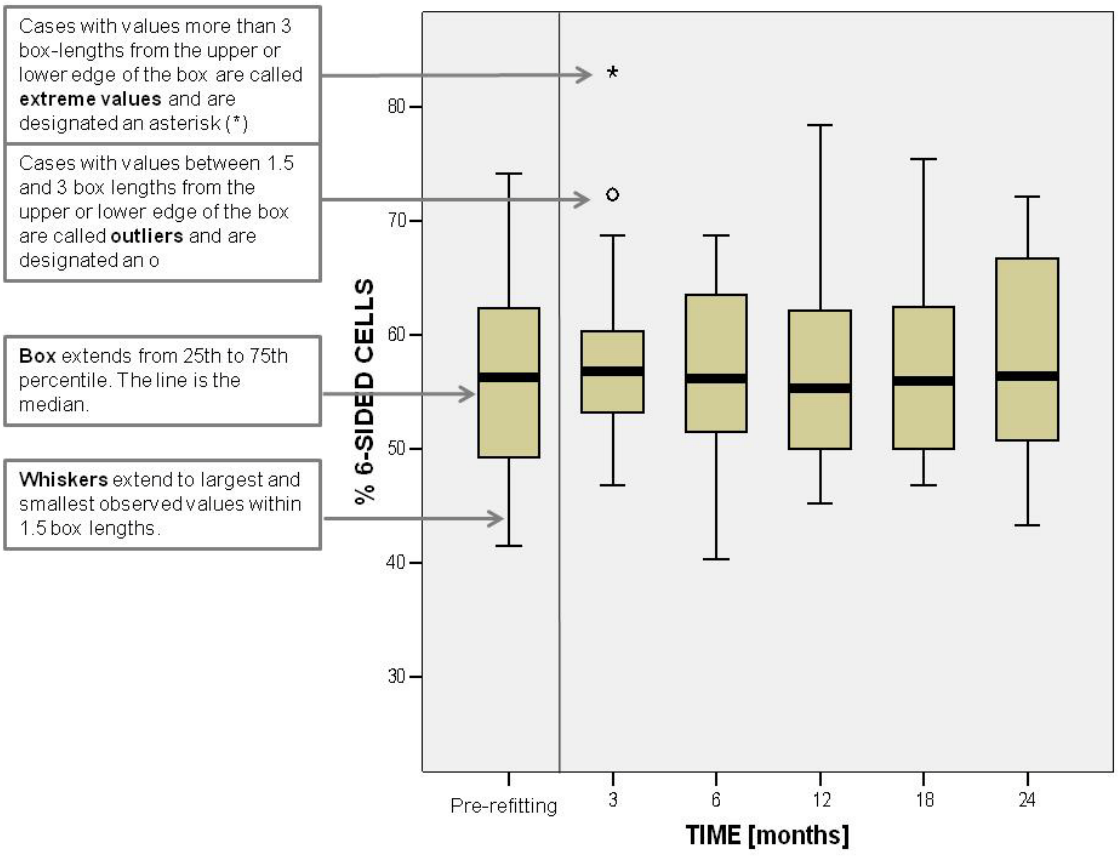


Figure 2.3-1
Explanations of the different elements of a box plot

CHAPTER 3: RESULTS

3.0 General information

Only those subjects who had a complete set of measures (i.e. 6 measurements over a period of 24 months) were included when assessing any time-dependent changes in the data and will be referred to as the *eligible sample (n)*. When any between-group comparisons for a single visit were made, all subjects were included in the analysis (N). For correlation analyses, individuals within each group with three or more measurements were included, excluding the baseline data where appropriate (N).

Normality tests (Shapiro-Wilk test) were run for all variables for each test group. The results of the normality tests are summarized in the appendix from page 214 to 216.

This results chapter is organized in four main results chapters, one for each test group. A fifth section summarises the main results in addition to comparing the groups.

3.1 RESULTS FOR SPECTACLE WEARERS

3.1.1 Group demographics and vision assessments

At the first visit, the average age of the 15 spectacle wearers who agreed to participate was 32.3 ± 5.8 years (mean ± SD) and their mean refractive error was -2.71 ± 1.69 DS (mean spherical equivalent power; MSE). Women comprised 40% of the group. None of the subjects had worn contact lenses on a regular basis before. All 15 attended at all appointments except the 3-month's visit, where three subjects did not attend. Reducing the number of participants from 15 to 12 did not change the subject detail characteristics much (Table 3.1-1). An additional six subjects were assessed after the study was finished. This was done in an attempt to strengthen the power of the study, since differences between the test groups were indicated, especially for the endothelial cell analyses. For details of this extended group of spectacle wearers, the reader is referred to section 0 and the appendix, Table 5.2-2.

Table 3.1-1
Subject details of the spectacle wearers at baseline

	Initial assignment	Completed study
Number of subjects	15	12
Age at first visit (years)	32.27 ± 5.75	32.92 ± 6.32
Age range (years)	23 to 41	23 to 41
Gender (F:M)	6:9	5:7
Refractive error (MSE)[a]	-2.71 ± 1.69	-2.85 ± 1.87

[a] Mean refractive error (MSE) in spherical equivalent power (DS). All other data are mean ± S.D.

All spectacle wearers had myopia within a spherical range of -6.75 to -0.50 DS. Cylinder power did not exceed -1.00 DC for any subject, and the range of refractive errors at baseline for the whole group was -7.25 to -0.875 DS (MSE). The mean values for refractive error remained largely stable throughout the study period (Table 3.1-2, Figure 3.1-1). The box plot in Figure 3.1-1 shows that one high myope (-7.25 DS) was clearly an outlier in the group, but that the refractive errors were stable in all other respects. Statistical comparisons between each set of data revealed no statistically significant differences (p = 0.481, Friedman test).

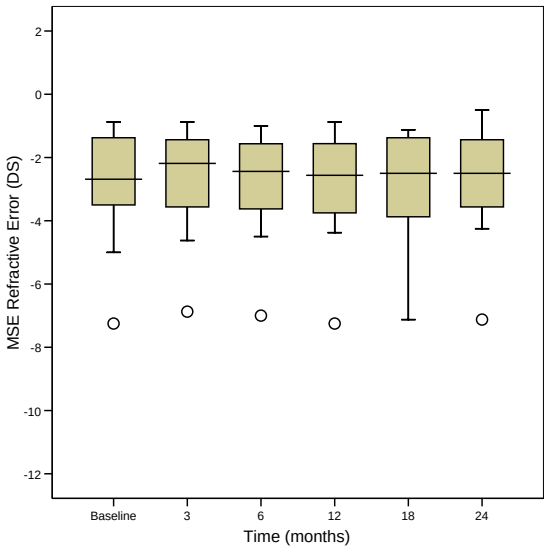


Figure 3.1-1
Box plot to show refractive error (MSE) in spectacle wearers (n = 12) over 2 years

Average high contrast visual acuity with best correction (BCHCVA) was -0.10 ± 0.09 for the entire group at baseline. When just looking at those 12 who completed the study, the baseline value was marginally worse: -0.08 ± 0.09 logMAR units (Table 3.1-2). Low contrast visual acuity was, as expected, a couple of lines worse than high contrast visual acuity: average score at baseline for the entire group was 0.09 ± 0.10 and 0.11 ± 0.09 when only the 12 subjects who finished the study were analysed (Table 3.1-2). Measurements done at later points of time (18 and 24 months) showed a small improvement in group mean visual acuity scores compared to earlier visits. These differences were however not statistically significant (p > 0.282, Friedman test).

Table 3.1-2
Refractive error (MSE) and visual acuity measurements: LogMAR Best Corrected High- and Low Contrast Visual Acuity (BCHCVA and BCLCVA, respectively) of *spectacle wearers* (n = 12) over a period of two years. All values are mean ± 1SD with ranges in brackets.

	Time (months)					
	baseline	3	6	12	18	24
Refractive error[a]	-2.85 ± 1.87 (-7.25 to -0.88)	-2.71 ± 1.77 (-6.88 to -0.88)	-2.83 ± 1.75 (-7.00 to -1.00)	-2.89 ± 1.80 (-7.25 to -0.88)	-2.85 ± 1.78 (-7.13 to -1.13)	-2.74 ± 1.83 (-7.13 to -0.50)
BCHCVA	-0.08 ± 0.09 (-0.20 to 0.10)	-0.07 ± 0.08 (-0.20 to 0.06)	-0.07 ± 0.08 (-0.18 to 0.06)	-0.07 ± 0.09 (-0.20 to 0.08)	-0.13 ± 0.07 (-0.20 to 0.00)	-0.11 ± 0.09 (-0.20 to 0.08)
BCLCVA	0.11 ± 0.09 (0.00 to 0.30)	0.11 ± 0.09 (-0.04 to 0.20)	0.09 ± 0.08 (-0.06 to 0.24)	0.10 ± 0.09 (-0.08 to 0.20)	0.06 ± 0.06 (-0.04 to 0.16)	0.07 ± 0.08 (-0.04 to 0.20)

[a] Mean refractive error (MSE) in spherical equivalent power (DS)

3.1.2 Ocular comfort

Thirteen out of the 15 subjects reported specific ocular symptoms over the study period. They indicated one or more of the symptoms at baseline, namely those of soreness, itchiness, dryness, grittiness or burning. This trend of reporting remained throughout the study. At any one visit, at least nine out of the twelve subjects reported one of the ocular symptoms during the two-year period with there being an apparent, but not significant reduction (Figure 3.1-2).

As can be seen from Table 3.1-3, most (70 to 100%) symptomatic subjects reported that the symptoms occurred “sometimes”. while a few reported having symptoms “often” None of the subjects reported to have symptoms “always”.

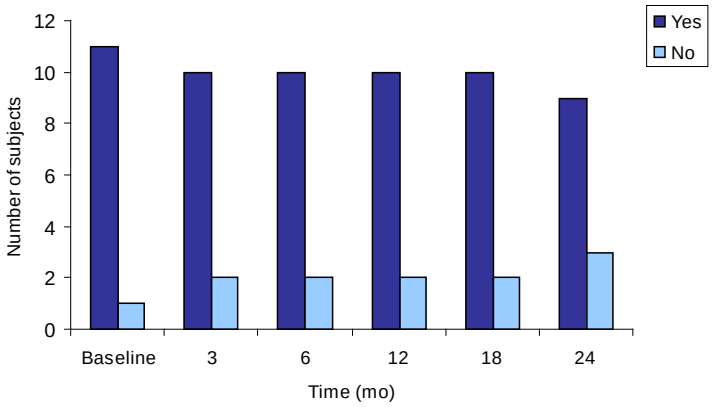


Figure 3.1-2
Frequency reporting one or more ocular symptoms in spectacle wearing subjects (n = 12) over 2 years

Table 3.1-3
Symptom frequency (count) in spectacle wearers (n = 12).

Symptom frequency (n)	Time (months)					
	Baseline	3	6	12	18	24
Never	1	2	2	2	2	3
Sometimes	9	7	7	8	8	9
Often	2	3	3	2	2	0
Always	0	0	0	0	0	0

The group-mean ± SD score using VAS was 30 ± 21 mm at baseline for the 11 subjects who reported symptoms (Table 3.1-4). At successive assessments, the severity of their symptoms fluctuated somewhat with the average values at the follow-up visits ranging between 30 and 44. Stated another way, differences in the average scores of up to 13 (on a 100-point scale) occurred between visits. The range of the reported severity of symptoms at each visit was also very large, being from 59 to 88 (on a 100-point scale). No statistically significant differences were detected between visits for the eligible sample of nine symptomatic subjects (p = 0.757, Friedman test),

although the severity of reported symptoms generally increased over time (as based on average values) (Table 3.1-4). Overall, the severity of ocular symptoms in the spectacle wearers, over a 24 month period, averaged 36 (95% CI = 30 - 42).

Table 3.1-4
Symptom severity assessed by VAS in symptomatic spectacle wearers. All values are given as group mean ± SD (minimum to maximum values in brackets).

Symptom severity (mm)	Time (months)					
	Baseline	3	6	12	18	24
Eligible sample (n = 9)*	30 ± 22 (0 to 63)	35 ± 25 (0.0 to 69.5)	34 ± 25 (4 to 71)	31 ± 25 (9 to 78)	40 ± 29 (4 to 92)	36 ± 21 (13 to 71)
Main sample	30 ± 21 (0 to 63)	37 ± 25 (0.0 to 69.5)	38 ± 27 (4 to 72)	31 ± 24 (9 to 78)	44 ± 30 (4 to 92)	36 ± 22 (13 to 71)
N	11	10	10	10	10	9

*I.e. those subjects who reported symptoms at all visits. These values were used in time-dependent comparisons (Friedman test).

3.1.3 Tear film characteristics

Twelve of the originally enrolled 15 subjects finished the study, but for a further two subjects there was missing data for tear meniscus height (TMH), and also three cases of missing data for the fluorescein-tear break-up time (f-TBUT) measurements (Table 3.1-5).

The quantity of the tear film, as assessed by the tear meniscus height (TMH), averaged 0.22 ± 0.07 mm at baseline (mean ± SD; range 0.15 to 0.30 mm) (Table 3.1-5). Over time, the range of observed values was reasonably consistent although a few subjects showed abnormally large TMH values (i.e. 0.50 mm). Notwithstanding, the average values showed little change, differing by a maximum of only 0.06 mm between visits. The average and range of TMH values were marginally lower at the 18-month visit. However, this was not significantly lower than the average TMH at other visits; none of the sets of measures was significantly different ($p > 0.664$, Friedman test). A set of box plots to show the time-dependent changes in TMH are shown in Figure 3.1-3. Overall, the tear meniscus height in spectacle wearers averaged 0.23 mm (95% CI = 0.20 to 0.25 mm) during this study.

Table 3.1-5
Mean ± SD (range) of the tear film tests of the spectacle wearers (n = 12). For missing data, see text.

Tear film test	Time (months)						
	Valid n	Baseline	3	6	12	18	24
TMH (mm)	10	0.22 ± 0.07 (0.15 to 0.30)	0.24 ± 0.11 (0.12 to 0.50)	0.22 ± 0.05 (0.15 to 0.30)	0.26 ± 0.10 (0.15 to 0.50)	0.20 ± 0.05 (0.12 to 0.25)	0.21 ± 0.07 (0.10 to 0.35)
PRT (mm)	12	18.6 ± 7.5 (7.0 to 30.0)	24.4 ± 8.1 (13.0 to 42.0)	18.8 ± 5.3 (11.0 to 27.0)	18.8 ± 8.8 (6.0 to 30.0)	16.0 ± 5.1 (8.0 to 22.0)	17.5 ± 6.1 (8.0 to 27.0)
NIBUT (sec)	12	40.1 ± 37.8 (7.7 to 125.9)	55.5 ± 99.0 (6.4 to 359.7)	50.1 ± 105.6 (5.0 to 382.0)	44.7 ± 96.9 (4.8 to 347.0)	41.7 ± 56.3 (9.3 to 201.0)	38.5 ± 51.1 (4.4 to 184.0)
f-TBUT (sec)	9	10.0 ± 6.0 (5.0 to 24.0)	9.2 ± 6.9 (4.0 to 26.0)	7.2 ± 4.2 (3.0 to 15.0)	6.0 ± 2.5 (3.0 to 12.0)	9.6 ± 6.0 (5.0 to 24.0)	15.2 ± 21.0 (3.0 to 70.0)

The tear film volume was also evaluated using the phenol red thread (PRT) test. It averaged 18.6 ± 7.5 mm at baseline and the wetting length values at later visits had average values usually differing by only 1 to 2 mm between visits (Table 3.1-5), although the average PRT value at 3 months was clearly slightly greater than at other visits (Figure 3.1-4). A Friedman test revealed slight and just significant between-visit differences ($p = 0.019$). The average PRT measures at baseline, 18 and 24 months were all found to be significantly lower than the average value of 24 ± 8 mm at 3 months ($p \leq 0.01$, Wilcoxon signed rank test). The higher average value at 3 months was, in part, the result of one subject who showed a wetting length of 42 mm (Figure 3.1-4).

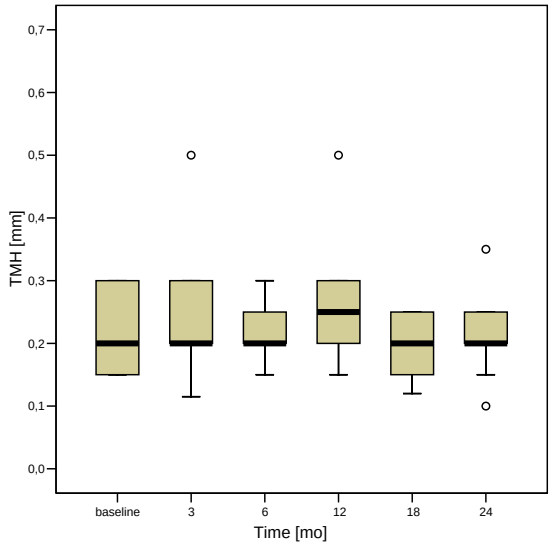


Figure 3.1-3
Box-plots of time-related changes of tear meniscus height (TMH) in spectacle wearers (n = 10).

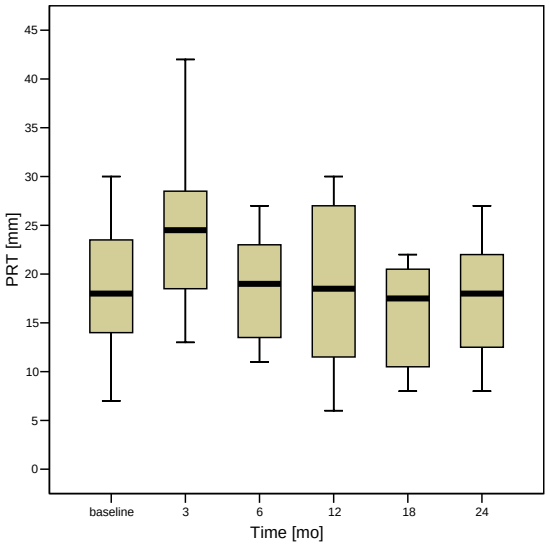


Figure 3.1-4
Box-plots of the time-related changes of phenol red thread test (PRT) in spectacle wearers (n = 12).

A regression analysis for time-related changes in PRT using all data showed an apparent trend of shorter wetting length per year (Figure 3.1-5), that just failed to be statistically significant ($p = 0.070$, $r = -0.215$). However, with the 42 mm value removed, there was no longer any statistically detectable time-dependent change in PRT in these spectacle wearers ($p = 0.140$, $r = -0.183$). The ranges were relative large and they varied considerably between visits, from 14 mm at 18 months to 29 mm at 3 months. Overall, the group average PRT over the 24 months was 19.0 mm (95% CI 15.4 to 22.6 mm).

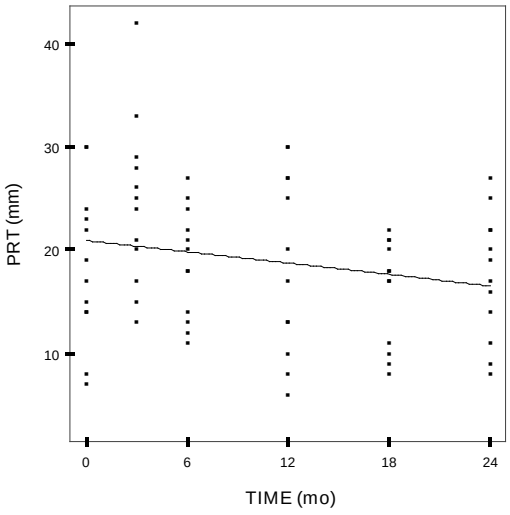


Figure 3.1-5
Scatter plot with linear regression line to illustrate time-related changes of phenol red thread (PRT) values in spectacle wearers. Pearson's $r = -0.215$, $p = 0.070$. With removal of the high 42 mm value at 3 months, this trend was no longer evident ($r = -0.183$, $p = 0.140$).

NIBUT assessments in the spectacle wearers yielded a very wide range of values from 4.4 to 382 sec (Table 3.1-5), although most of the values fell within a much smaller range (Figure 3.1-6). The average NIBUT values from each visit ranged from 40.1 sec at baseline to 38.5 sec at the 24-month visit. On the other hand, the SD values were substantial ranging from 37.8 sec to 105.6 s. Overall, the average NIBUT values fluctuated over the 24 month period, but no statistically-significant changes could be detected, probably because of the large inter-subject differences ($p = 0.422$, Friedman test). The overall group average NIBUT over the 24 months was 45.1 sec (95% CI = -0.26 to 90.5 sec).

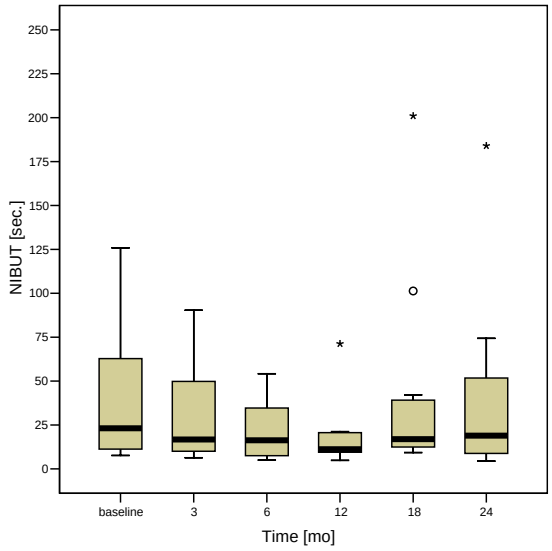


Figure 3.1-6
Box-plots of time-related changes of non-invasive tear break-up time (NIBUT) in spectacle wearers (n = 12). Note that three extreme values (one subject with NIBUTs of 360, 382 and 347 sec at 3, 6 and 12 m) are not visible on this plot.

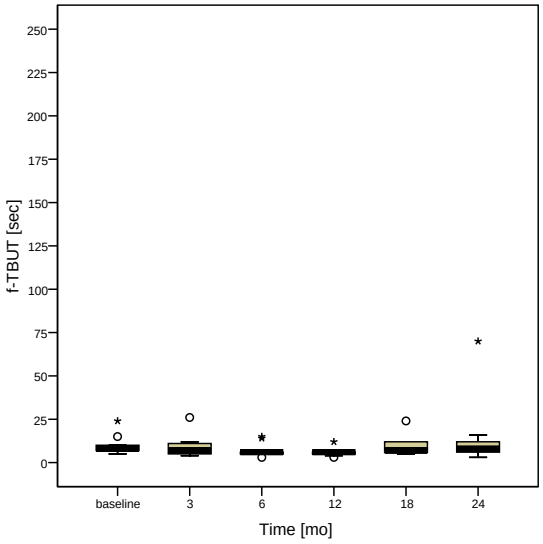


Figure 3.1-7
Box-plots to show time-related changes of fluorescein-tear break-up time (f-TBUT) in spectacle wearers (n = 9).

In contrast to the NIBUT measures, the fluorescein measures of tear stability (f-TBUT) in the spectacle wearers were much lower, ranging from 3 to just 70 sec over the 2 year period (Table 3.1-5). The average f-TBUT value at baseline was 10.0 ± 6.0 sec and these average values fluctuated only slightly (between 6.0 to 9.6 sec) for the assessments over 18 months. The f-TBUT values were slightly higher at the 24-month assessment, averaging 15.2 s. This higher overall measure was the result of an extreme outlier (with an f-TBUT value of 70 sec) and the 24 month data was not statistically significantly different from any other measures over the 24 months ($p = 0.132$, Friedman test). A box plot to show the time-related differences in f-TBUT measures is given in Figure 3.1-7. Non-parametric correlation analysis indicated no time-related changes or trends ($p = 0.725$, Spearman's rho = -0.047). A scatter-plot is illustrated in Figure 3.1-8. Overall, the group average f-TBUT in spectacle wearers who finished the study was 9.4 sec (95% CI = 5.4 to 13.7 sec).

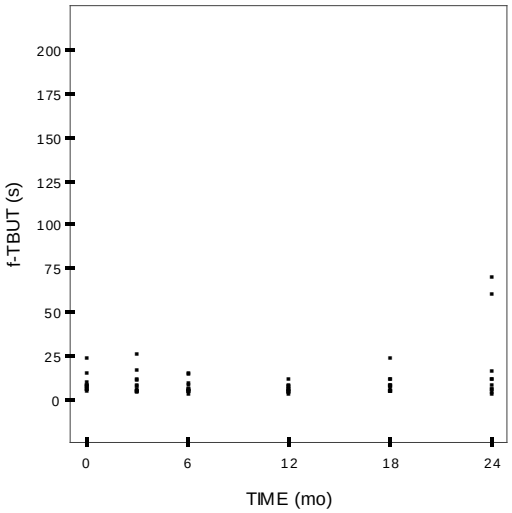


Figure 3.1-8
Scatter plot to illustrate time-related changes of fluorescein-tear break-up time (f-TBUT) in spectacle wearers. Correlation analysis indicated no statistically significant trend (Spearman's rho = -0.047, p = 0.725).

3.1.4 Ocular surface characteristics

At baseline, the eyelid margins of all spectacle wearers showed only minor or no signs of blepharitis, with a mean grade of 0.4 ± 0.3 (range 0.0 to 1.0) (Table 3.1-6). Likewise, the Meibomian glands were assessed to be open and healthy looking for all subjects and graded 0.5 ± 0.4 (range 0.0 to 1.3). Both these characteristics remained stable over the next five assessments, and no significant inter-visit changes could be detected ($p \geq 0.217$, Friedman test).

Redness of the visible part of the bulbar conjunctiva of the spectacle wearers was graded to a mean of 0.9 ± 0.4 (range 0.4 to 1.6) at baseline (Table 3.1-6). Over time, bulbar hyperaemia grades increased slightly, with the mean bulbar hyperaemia at 24 months being 1.3 ± 0.4, which was significantly more than at baseline ($p = 0.003$, Wilcoxon Signed Rank test). A similar pattern was seen for the degree of limbal redness and corneal vascularisation. At the first assessment, these were graded to 0.6 ± 0.2 and 0.5 ± 0.4, respectively. A Friedman test revealed statistically significant inter-visit differences ($p \leq 0.014$), although these were small with the largest mean differences being only 0.5 for bulbar and limbal hyperaemia and 0.3 for corneal vascularisation.

Some corneal fluorescein staining was seen in many spectacle wearers with eight (66.7%) out of the twelve who finished the study having grades ranging from 0.3 to 2.0 at baseline (Table 3.1-6). At later visits, the number of subjects having staining varied between 8 and 10, except at the last visit at 24 months where only five subjects had staining. The mean level of staining was rather stable through the study; the largest mean inter-visit difference being 0.2 ($p = 0.372$, Friedman test). When only including those subjects who actually showed staining (as opposed to the values displayed in Table 3.1-6 where all 12 subjects were included in the analyses) the mean levels of staining ranged from 0.73 to 1.1.

Some conjunctival fluorescein staining was also seen in most of the spectacle-wearing subjects. Over the six visits, seven to eleven subjects had conjunctival staining, but always only mild to moderate (ranging from 0.96 to 1.63). For all 12 subjects (Table 3.1-6), the mean level of conjunctival staining ranged from 0.4 to 0.7, and no inter-visit differences of significance were detected by Friedman test ($p = 0.742$).

Tarsal papillary conjunctivitis was also graded for most spectacle wearers, although three subjects refused to undergo this examination. At baseline, papillary conjunctivitis was graded to a mean of 1.2 ± 0.4 (range 0.4 – 1.7). The maximum mean difference between two visits was 0.3, which was not statistically significant ($p = 0.082$, Friedman test).

Table 3.1-6
Ocular surface characteristics as graded with Efron's grading scale [0 - 4] in spectacle wearers who completed the study (n = 12, except for papillary conjunctivitis where n = 9). The numbers represents group mean ± SD and range (in brackets).

Ocular surface characteristic	Time (months)					
	baseline	3	6	12	18	24
Eyelids	0.4 ± 0.3 (0.0 to 1.0)	0.4 ± 0.3 (0.0 to 1.0)	0.2 ± 0.3 (0.0 to 0.7)	0.4 ± 0.4 (0.0 to 1.5)	0.2 ± 0.2 (0.0 to 0.5)	0.4 ± 0.3 (0.0 to 1.2)
Meibomian glands	0.5 ± 0.4 (0.0 to 1.3)	0.4 ± 0.2 (0.1 to 0.8)	0.3 ± 0.2 (0.0 to 0.7)	0.5 ± 0.5 (0.0 to 1.5)	0.2 ± 0.2 (0.0 to 0.5)	0.4 ± 0.3 (0.2 to 1.0)
Bulbar hyperaemia	0.9 ± 0.4 (0.4 to 1.6)	0.8 ± 0.3 (0.5 to 1.2)	1.0 ± 0.3 (0.4 to 1.5)	1.1 ± 0.2 (0.8 to 1.4)	1.1 ± 0.3 (0.8 to 1.8)	1.3 ± 0.4 (0.8 to 2.2)
Limbal redness	0.6 ± 0.2 (0.2 to 0.9)	0.7 ± 0.3 (0.4 to 1.3)	0.7 ± 0.3 (0.3 to 1.3)	1.1 ± 0.2 (0.8 to 1.4)	0.8 ± 0.3 (0.5 to 1.6)	1.0 ± 0.4 (0.3 to 1.4)
Corneal vascularisation	0.5 ± 0.4 (0.0 to 1.4)	0.4 ± 0.2 (0.2 to 0.8)	0.6 ± 0.4 (0.2 to 1.4)	0.5 ± 0.3 (0.0 to 1.1)	0.3 ± 0.1 (0.1 to 0.5)	0.4 ± 0.3 (0.0 to 1.2)
Corneal staining*	0.5 ± 0.6 (0.0 to 2.0)	0.7 ± 0.8 (0.0 to 2.3)	0.7 ± 0.8 (0.0 to 1.9)	0.8 ± 0.5 (0.0 to 1.5)	0.4 ± 0.5 (0.0 to 1.4)	0.3 ± 0.6 (0.0 to 1.8)
Conjunctival staining	0.6 ± 0.6 (0.0 to 1.4)	0.6 ± 0.4 (0.2 to 1.5)	0.7 ± 0.8 (0.0 to 2.0)	0.6 ± 0.7 (0.0 to 1.8)	0.5 ± 0.3 (0.0 to 1.1)	0.4 ± 0.4 (0.0 to 1.2)
Papillary conjunctivitis	1.2 ± 0.4 (0.4 to 1.7)	1.2 ± 0.3 (0.7 to 1.6)	1.3 ± 0.3 (0.8 to 1.7)	1.5 ± 0.3 (1.0 to 1.9)	1.2 ± 0.5 (0.5 to 2.2)	1.4 ± 0.3 (1.1 to 1.8)

* Corneal staining was graded with CCLRU's grading scales [0-4]. See methods chapter for details.

3.1.5 Corneal thickness and corneal curvature

At baseline, mean central corneal thickness (CCT) was 504 ± 29 µm in the group of spectacle wearers who finished the study (n = 12) (Table 3.1-7). Over the six assessments, the overall mean CCT value was 511 µm (range 504 to 516 µm). By comparison, the mean mid-peripheral corneal thickness (MPCT, 3 mm from centre in superior-nasal quadrant) was 611 ± 32 µm, with the overall mean mid-peripheral CT value being 613 µm over the six visits (range 609 to 617 µm).

Table 3.1-7
Mean ± 1SD central and mid-peripheral corneal thickness (CCT and MPCT) and central corneal radius of curvature (K), in spectacle wearers (n = 12). Minimum and maximum values are given in brackets.

	Time (months)					
	Baseline	3	6	12	18	24
CCT (µm)	504 ± 29 (467 to 563)	514 ± 28 (477 to 563)	508 ± 26 (476 to 561)	511 ± 27 (475 to 555)	516 ± 30 (471 to 564)	512 ± 33 (470 to 579)
MPCT (µm)	611 ± 32 (571 to 665)	612 ± 31 (567 to 653)	609 ± 19 (582 to 638)	617 ± 32 (570 to 663)	614 ± 31 (572 to 658)	617 ± 38 (554 to 675)
Central K (mm)	7.87 ± 0.30 (7.27 to 8.45)	7.82 ± 0.30 (7.25 to 8.43)	7.84 ± 0.29 (7.27 to 8.44)	7.86 ± 0.29 (7.30 to 8.47)	7.84 ± 0.29 (7.20 to 8.42)	7.90 ± 0.29 (7.29 to 8.53)

Box plots of the central and mid-peripheral thickness values are shown in Figure 3.1-9 and Figure 3.1-10. At the different assessments, the mean thickness values obtained fluctuated very slightly with differences from baseline of up to 12 µm for central location sets and 6 µm for mid-peripheral locations (Table 3.1-7). The maximum differences in mean values were thus 2.4% and 1.0%. However, some of these differences, although small, proved to be statistically different, e.g. at the 18 month visit, the mean central corneal thicknesses was 12 µm larger than at baseline (p = 0.015, repeated ANOVA, Bonferroni correction). However, none of the repeated mid-peripheral corneal thickness values were statistically different from baseline (p = 0.704, repeated ANOVA).

Separate linear regression analyses for any time-dependent variations in corneal thickness measures revealed no detectable changes with r values of 0.084 and 0.076 (p = 0.482 and 0.527) for the central and mid-peripheral regions, respectively (Figure 3.1-11 and Figure 3.1-12). If the overall mean thickness values over the 6 visits are taken as a reference value (i.e. 511 µm and 613 µm), then the maximum differences (fluctuations) in group-mean thickness values at each assessment can be calculated to be just ± 1.2% (range -1.4 to +1.0%) and ± 0.6% (range -0.6 to + 0.6%) respectively for central and mid-peripheral sites.

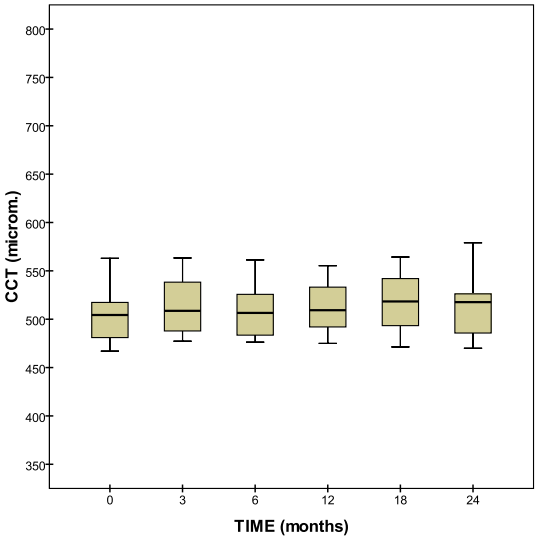


Figure 3.1-9
Box plot to show central corneal thickness over time in spectacle wearers (n = 12).

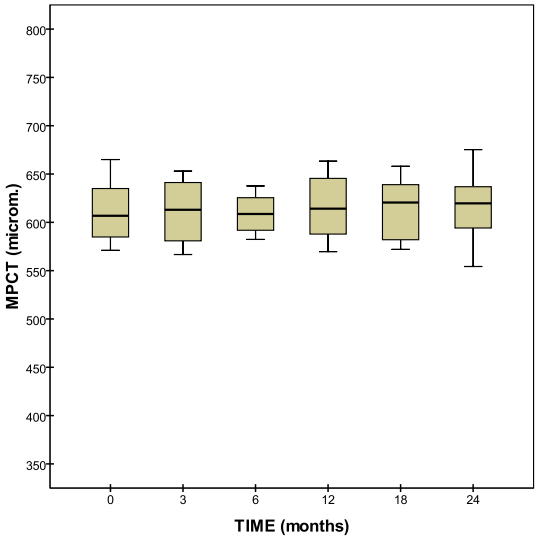


Figure 3.1-10
Box plot to show mid-peripheral corneal thickness over time in spectacle wearers (n = 12).

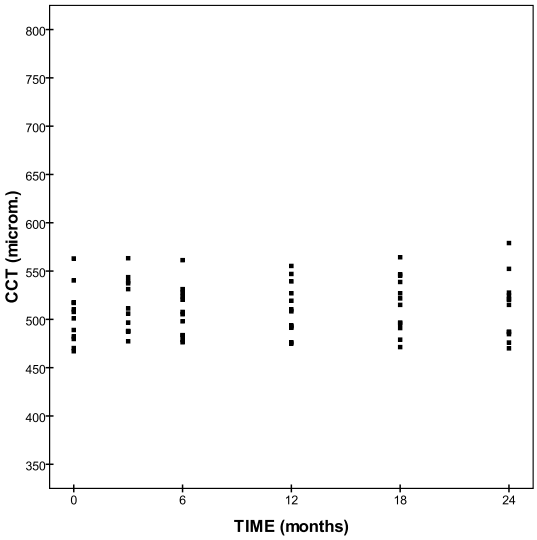


Figure 3.1-11
Regression analysis to show time-related changes of central corneal thickness (µm) in spectacle wearers (n = 12, r = 0.084, p = 0.482).

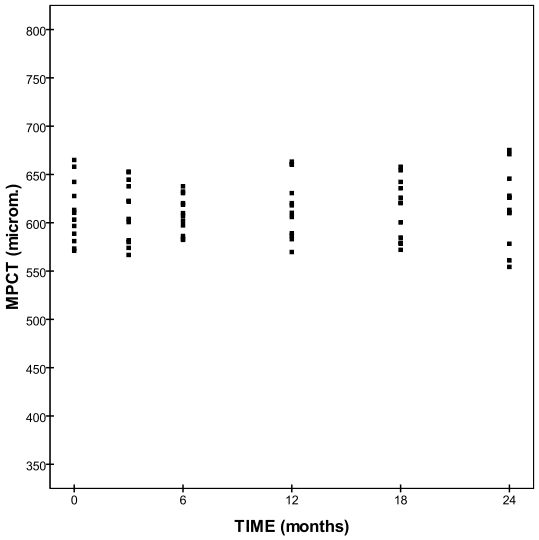


Figure 3.1-12
Regression analysis to show time-related changes of mid-peripheral corneal thickness (µm) in spectacle wearers (n = 12, r = 0.076, p = 0.527).

At baseline, mean central corneal curvature (K) was 7.87 ± 0.30 mm (range 7.27 to 8.45) in the group of spectacle wearers (Table 3.1-7). The largest mean difference between two visits (3 and 24 months) was only 0.08 mm (p=0.006, Wilcoxon Signed Rank test), which is not clinically significant. A box plot presentation shows that the median and inter-quartile range of corneal power stayed largely unchanged (Figure 3.1-13). Although the Friedman test indicated inter-visit differences in the anterior corneal curvature (p=0.002), correlation analysis revealed no detectable time-related trend (p = 0.526, Spearman's rho = 0.076, Figure 3.1-14). Overall mean central corneal curvature was 7.89 mm (95% CI = 7.67 - 8.04 mm) in the spectacle wearers.

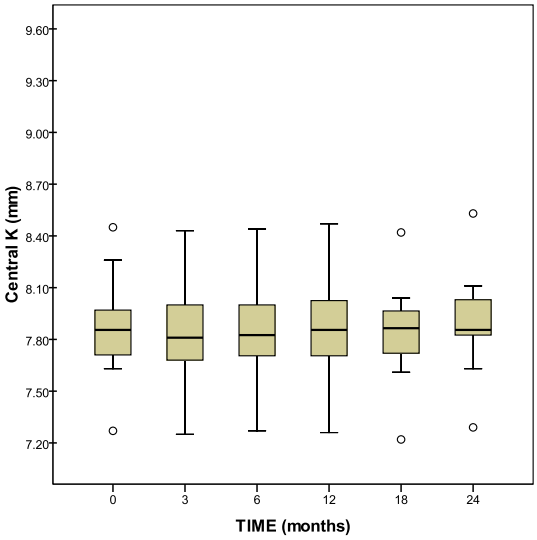


Figure 3.1-13
Box plot to show central corneal radius of curvature values over time (mm) in spectacle wearers (n = 12).

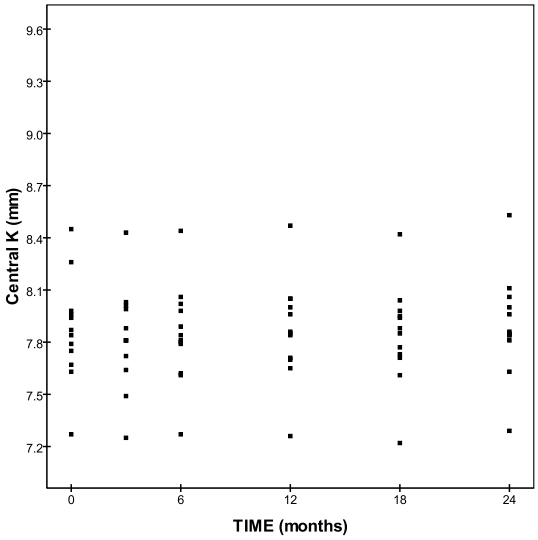


Figure 3.1-14
Scatter plot to illustrate time-related changes in central corneal radius of curvature values (mm) over time in spectacle wearers (n = 12). Non-parametric correlation analysis indicated no statistically significant effect ($p = 0.526$, Spearman's rho = 0.076).

3.1.6 Corneal endothelial cell morphometry

Endothelial cell density – ECD

Central endothelial cell density (CECD) was on average 2676 ± 174 cells / mm^2 with a range of individual CECD values from 2264 to 2862 cells/mm^2 for the 12 spectacle wearers (with a complete set of measures) at baseline (Table 3.1-9). Inspection of a box plot (Figure 3.1-15) indicated that the group median CECD was slightly higher at baseline compared to most other values, but none of the CECD estimates at different visits over the 24 months were statistically different from the baseline values (p = 0.799, repeated ANOVA). The average CECD values at each assessment were generally consistent within ± 0.66% of an overall group mean value of 2658 cells/mm^2. For example, at 12 months, the average CECD was 1.2% lower than at baseline, but at 24 months, the observed reduction was only 0.4%.

The average ECD in the mid-peripheral region (MPECD) was 2705 ± 257 cells / mm^2 at baseline. This difference of 29 cells/mm^2 was not significantly different from CECD values at baseline (p = 0.683, paired t-test). Regional differences were also not evident at later occasions (Table 3.1-8). Comparable to the CECD values, no obvious time related change in MPECD was detected. The box plot (Figure 3.1-16) shows that the median mid-peripheral ECD values fluctuated a little, and the only minor difference was that the ± 25% inter-quartile intervals appeared to decline with time. Inter-visit differences of up to 3.3% were noted for the average values, but no differences were statistically significant (p ≥ 0.322, Bonferroni post-hoc test).

The overall mean CECD for spectacle wearers over the 24 months was 2658 cells / mm^2, with average values from each assessment ranging from 2641 to 2676 cells / mm^2 (Table 3.1-8). Comparison of sets of individual data from each assessment is shown as a scatter-plot in Figure 3.1-17. Linear regression analysis indicated no detectable change in CECD values over time (p = 0.900). The slope of the regression line was close to zero at -3.12 ± 50.4 cells/mm^2 per year (range -82.3 to 96.0), which simply indicates that the apparent CECD values would increase or decrease by about 50 cells/mm^2 / year for an individual (r = -0.015) (see also Table 5.2-1 in the appendix). Similarly, the overall mean MPECD was 2707 cells / mm^2, with average values at each visit ranging from 2679 to 2770 cells / mm^2 (Table 3.1-8).

Time-dependent analysis of the individual MPECD values (Figure 3.1-18) revealed no statistically significant change as assessed with a linear model (p = 0.410), and a very slight (non-statistically significant) negative slope of -29.2 ± 63.7 cells/mm^2 per year (range -111.4 to 94.2) (r = -0.099) was calculated. Again, the large variance in the slope estimate (i.e. ± 64 cells /mm^2 / year) should be noted (Table 5.2-1 in the appendix).

Table 3.1-8
Cell density (mean ± 1SD) of the central (CECD) and mid-peripheral (MPECD) corneal endothelium in *spectacle wearers* (n = 12) at six occasions over a two-year period. Numbers in brackets represent the minimum and maximum observations. A paired t-test was used to explore any regional differences and repeated ANOVA for time related changes (α = 0.05).

ECD	Time (months)						
	Baseline	3	6	12	18	24	p (ANOVA)
CECD (cells/mm²)	2676 ± 174 (2264 - 2862)	2667 ± 164 (2409 - 2900)	2641 ± 136 (2439 - 2858)	2643 ± 160 (2280 - 2846)	2658 ± 134 (2452 - 2825)	2665 ± 138 (2456 - 2917)	0.799
MPECD (cells/mm²)	2705 ± 256 (2220 - 3005)	2770 ± 235 (2395 - 3166)	2717 ± 208 (2289 - 3082)	2684 ± 201 (2307 - 2960)	2689 ± 190 (2346 - 2995)	2679 ± 191 (2218 - 2907)	0.262
p (paired t-test)	0.683	0.112	0.274	0.422	0.605	0.789	
MPECD:CECD ratio	1.012 ± 0.088 (0.840 - 1.127)	1.040 ± 0.077 (0.920 - 1.164)	1.030 ± 0.086 (0.886 - 1.157)	1.017 ± 0.066 (0.902 - 1.111)	1.013 ± 0.077 (0.895 - 1.132)	1.006 ± 0.070 (0.875 - 1.094)	0.607

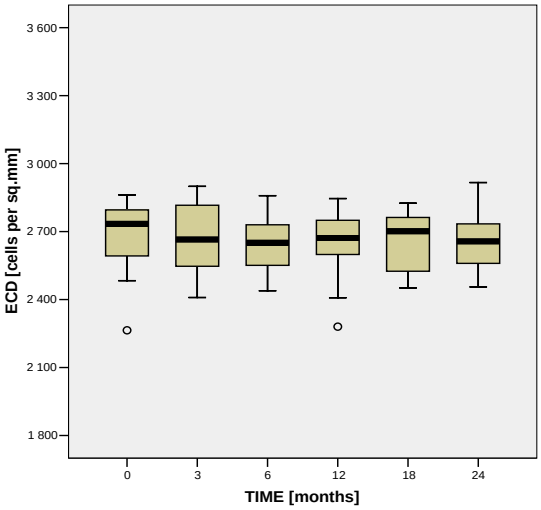


Figure 3.1-15
Box plots of the endothelial cell density in the central region of the cornea (CECD) over a two-year period in *spectacle wearers* (n = 12).

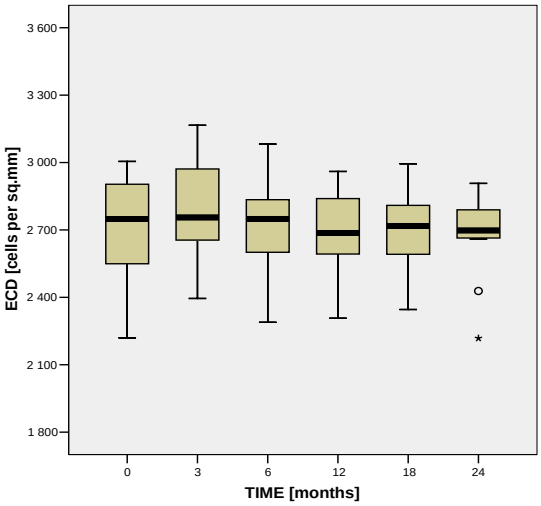


Figure 3.1-16
Box plots of the endothelial cell density in the mid-peripheral region of the cornea (MPECD) over a two-year period in *spectacle wearers* (n = 12)

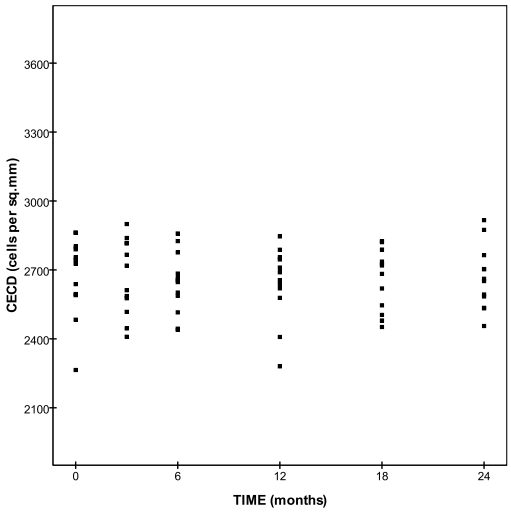


Figure 3.1-17
Linear regression analysis of the central cell density (CECD) and time in *spectacle wearers* (n = 12, p = 0.900, r = -0.015).

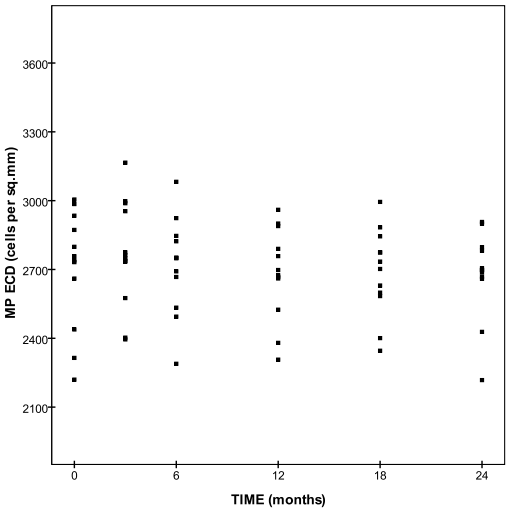


Figure 3.1-18
Linear regression analysis of the mid-peripheral cell density (MPECD) and time in *spectacle wearers* (n = 12, p = 0.410, r = -0.099).

As detailed previously in this section, only 12 spectacle wearers had a complete set of measurements. Three other spectacle-wearing individuals were assessed on five different occasions over the 24-month period, but their data was not included in the time-dependent analyses. Since some interesting differences between spectacle wearers and contact lens wearers were found (see section 3.5.6), it was recognised that at a single assessment information on spectacle lens wearers would be useful. Therefore, in addition to using the baseline data from the 15 (i.e. 12 + 3) myopic spectacle wearers just mentioned, a further six spectacle wearers were also assessed on a single occasion. These were of similar age but had significantly smaller refractive error (see comparisons of demographic data for the initially assigned subjects and the extra subjects in Table 5.2-2 in the appendix).

The addition of six more sets of data had the effect of both slightly increasing both the CECD and MPECD (Table 3.1-9). However, no statistically significant difference between the initial and additional groups of subjects was found. Moreover, a key feature, noted earlier, was that there was only a slight difference between the average CECD value (of 2779 cells / mm^2) and MPECD value (of 2844 cells/mm^2) for the additional set of subjects too. The MPECD was, on average, 2.2 ± 5.6% higher than CECD values with a range of -3.9% to + 8.7%.

Since no statistically significant differences could be demonstrated, endothelial cell density data for the total group of 21 spectacle wearers was used when comparisons with the other three test groups were made (see section 3.5.6).

Table 3.1-9
CECD and MPECD (Mean ± 1SD) for the initial groups of *spectacle wearers* (N = 15) and an extra set of myopic subjects wearing spectacles (n = 6) evaluated on a single occasion ('baseline', total N = 21). Numbers in brackets represent the minimum and maximum observations.

	Initial group	Extra subjects	p (t-test)	Total
CECD (cells/mm^2)	2689 ± 169 (2264 - 2929)	2779 ± 131 (2631 - 3014)	0.260	2715 ± 161 (2264 - 3014)
MPECD (cells/mm^2)	2753 ± 297 (2220 - 3210)	2844 ± 256 (2555 - 3270)	0.518	2779 ± 2583 (2220 - 3270)
p (paired t-test)	0.362	0.356		0.220
MPECD:CECD ratio	1.024 ± 0.096 (0.840 - 1.197)	1.022 ± 0.056 (0.961 - 1.087)	0.957	1.024 ± 0.085 (0.840 - 1.197)

Even though the differences between mean MPECD and CECD were small, individual endothelia could have MPECD:CECD ratios ranging from 0.840 to 1.197 (Table 3.1-9). This type of inter-variability was noted for the images across all visits over the two-year period. In Figure 3.1-19 and Figure 3.1-20 are shown histograms of the MPECD:CECD ratio at baseline and mean MPECD:CECD ratio from all six assessments for the spectacle wearers (N=15). However, the group mean MPECD:CECD ratio varied very little from visit to visit, which is illustrated in Figure 3.1-21. Linear regression analysis of the scatter plot of the time-related changes in the MPECD:CECD ratio revealed no statistically significant trends (p = 0.556, r = -0.064).

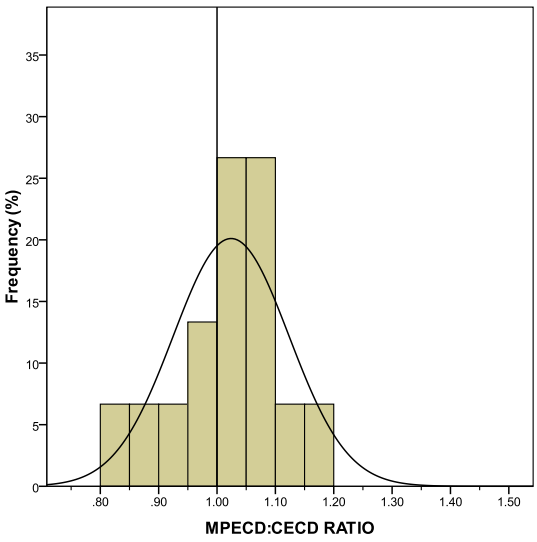


Figure 3.1-19
Histogram showing the frequencies of individual endothelial MPECD:CECD ratios in a group of *spectacle wearers* (N = 15) at a single occasion (baseline). The reference line shows the level of no difference between MPECD and CECD (ratio = 1).

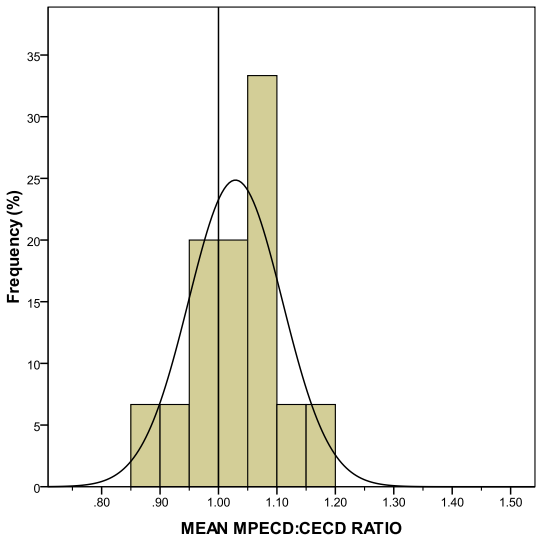


Figure 3.1-20
Histogram showing the frequencies of individual mean endothelial MPECD:CECD ratios in a group of *spectacle wearers* (N = 15) from six occasions over a period of two years. The reference line shows the level of no difference between MPECD and CECD (ratio = 1).

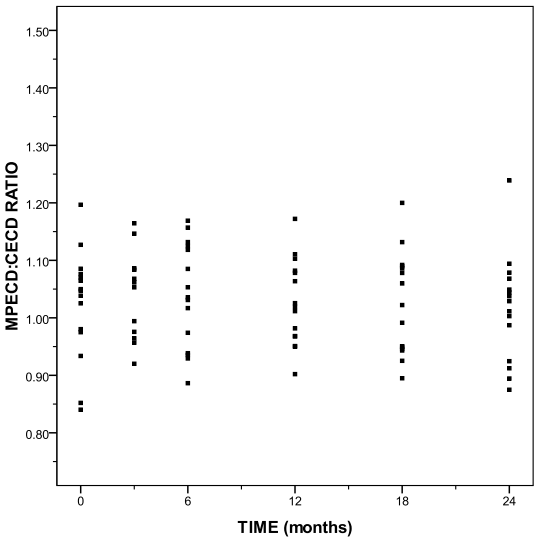


Figure 3.1-21
Regression analysis of the MPECD:CECD ratio in *spectacle wearers* over a period of two years (p = 0.556, r = -0.064).

Endothelial polymegethism -COV

At baseline, the variances in endothelial cell areas (polymegethism) for the central region, as expressed by the coefficient of variation in cell area (CCOV), was 28.7 ± 6.0% and 29.5 ± 5.0% for the mid-peripheral images (MPCOV, Table 3.1-10). Individual endothelial images had CCOV values between 21 and 39.3% and MPCOV values between 24.0 to 39.6. This difference in COV between locations was not significant at baseline (p = 0.490, paired t-test) or at later occasions (Table 3.1-10). As shown in the pair of box plots (Figure 3.1-22 and Figure 3.1-23), there were no obvious changes in COV at either location over the 24 month period. The largest mean difference in CCOV between two visits was 0.7% (95% CI = -3.7 to 5.1%) and no differences were significant (p = 0.952, repeated ANOVA).

Overall, the group mean CCOV across the six visits was 28.4%, with the average values ranging from 28.0 to 28.7%. The overall mean MPCOV was slightly greater at 29.4 ± 5.1%, with the range of average values being from 28.6 to 30.5% (see Table 3.1-10). Assessment of any time-related changes in COV applying a simple linear regression analysis (see Table 5.2-1 in the appendix), showed no detectable changes for neither the central location (p = 0.900, r = -0.015) nor for the mid-peripheral location (p= 0.710, r = 0.05) (graphs not shown).

For the assessment of polymegethism, the CCOV and MPCOV values were essentially unchanged when comparing the data from 15 spectacle wearers with 6 extra spectacle wearers (See Table 3.1-11).

Table 3.1-10
Polymegethism (mean ± 1SD of the coefficient of variation in cell area; COV) of the central (CCOV) and mid-peripheral (MPCOV) corneal endothelium in *spectacle wearers* (n = 12) at six occasions over a two-year period. Numbers in brackets represent the minimum and maximum observations. A paired t-test was used to explore any regional differences and repeated ANOVA for time related changes (α = 0.05).

COV	Time (months)						
	Baseline	3	6	12	18	24	p (ANOVA)
CCOV (%)	28.7 ± 6.0 (21.0 - 39.3)	28.6 ± 5.2 (22.1 - 39.3)	28.0 ± 4.9 (21.6 - 37.8)	28.4 ± 3.5 (22.3 - 33.2)	28.0 ± 4.5 (20.9 - 33.8)	28.6 ± 4.8 (23.9 - 38.1)	0.952
MPCOV (%)	29.5 ± 5.0 (24.0 - 39.6)	29.4 ± 3.4 (24.8 - 34.6)	29.2 ± 5.1 (23.0 - 36.5)	28.6 ± 5.8 (22.6 - 40.5)	29.2 ± 5.9 (22.6 - 39.5)	30.5 ± 6.1 (22.1 - 40.5)	0.493
p (paired t-test)	0.490	0.500	0.310	0.874	0.197	0.121	
MPCOV:CCOV ratio	1.046 ± 0.145 (0.834 - 1.322)	1.043 ± 0.136 (0.845 - 1.295)	1.053 ± 0.148 (0.797 - 1.351)	1.003 ± 0.128 (0.829 - 1.219)	1.040 ± 0.096 (0.863 - 1.215)	1.070 ± 0.143 (0.906 - 1.306)	0.853

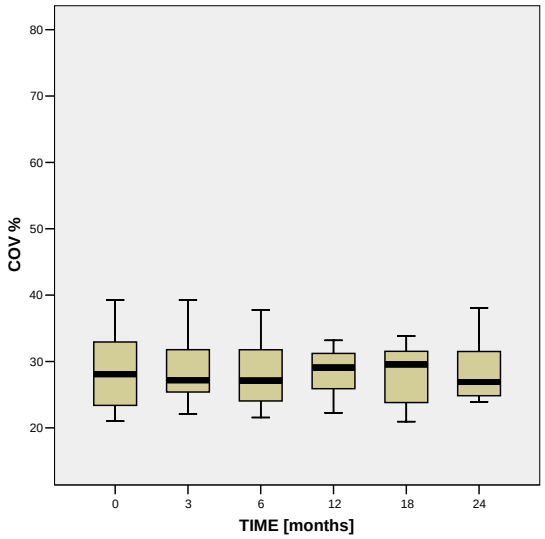


Figure 3.1-22
Box-plots of the degree of endothelial polymegethism in the central cornea (CCOV) over a two-year period in *spectacle wearers* (n = 12).

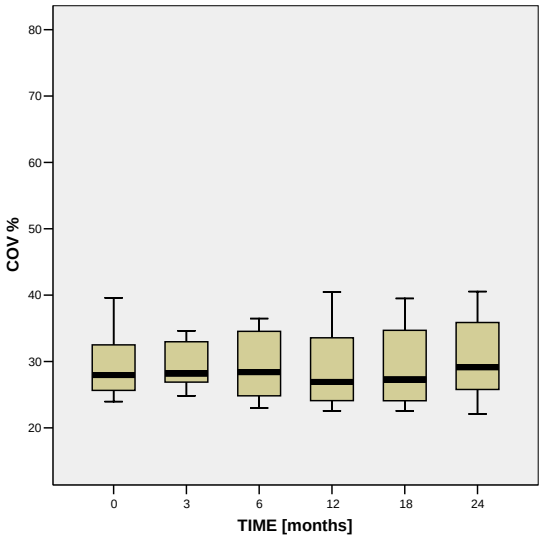


Figure 3.1-23
Endothelial polymegethism in the mid-peripheral cornea (MPCOV) over a two-year period in *spectacle wearers* (n = 12)

Table 3.1-11
CCOV and MPCOV (Mean ± 1SD) for the initial groups of *spectacle wearers* (n = 15) and an extra set of myopic subjects wearing spectacles (n = 6) evaluated on a single occasion (baseline, total N = 21). Numbers in brackets represent the minimum and maximum observations.

COV	Initial group	Extra subjects	p (t-test)	Total
CCOV (%)	27.4 ± 6.3 (16.7 - 39.3)	24.5 ± 4.7 (20.5 - 32.9)	0.315	26.6 ± 5.9 (16.7 - 39.3)
MPCOV (%)	28.0 ± 5.7 (18.0 - 39.6)	28.4 ± 6.0 (22.4 - 37.1)	0.914	28.1 ± 5.7 (18.0 - 39.6)
p (paired t-test)	0.544	0.028		0.083
MPCOV:CCOV ratio	1.035 ± 0.137 (0.834 - 1.322)	1.160 ± 0.133 (0.973 - 1.295)	0.085	1,071 ± 0,144 (0,834 - 1,322)

Endothelial pleomorphism - %SIX

The proportion of six-sided cells in the central region of the endothelium (C%SIX) in spectacle wearers averaged 63.5 ± 12.0% at baseline. As indicated by the SD value, a wide range of values was encountered from 41.8% to 82.4%, although none of the values was obvious outliers (Figure 3.1-24). A similarly wide range of values for the proportion of 6-sided cells was seen at most subsequent assessments (Table 3.1-12) and can also be seen in the broad ± 25% inter-quartile intervals on the box plot (Figure 3.1-24). The C%SIX averaged 60.3 ± 11.1% at the end of the study (Table 3.1-12), but this net reduction of 3.2% was not statistically significant (p = 1.000, Bonferroni correction).

For the mid-peripheral regions of the cornea, the %SIX averaged 64.3 ± 11.0% at baseline, a set of values that was not statistically different from that seen at central locations (p = 0.896). No obvious time-dependent changes occurred in the MP%SIX of the corneas of the spectacle wearers (Figure 3.1-25). The average value at 24 months (of 62.3 ± 9.4%) was neither different from the set of baseline values (p = 1.000, repeated ANOVA, Bonferroni correction) nor from the set of values at the central region (p = 0.600). The box plot analyses (Figure 3.1-24 and Figure 3.1-25) also show no predictable changes in the %SIX values, and none of the slight differences between visits were statistically significant (p = 0.924, repeated ANOVA).

The overall mean value for the C%SIX was 61.9% (with average values ranging from 60.3% to 63.5%). A similar group mean value of 64.3 ± 9.1% was noted for the MP%SIX, with the average values now being from 62.3 to 65.7%. There were no detectable time-related changes in the %SIX at either location as assessed using linear regression analysis (p = 0.600 for central locations and p = 0.510 for mid-periphery) (graphs not shown). In either analyses, the correlation coefficient values were very weak (r = -0.06 for central sites and -0.08 at mid-peripheral sites; see Table 5.2-1).

The values for the %SIX were also essentially unchanged when the initial (n = 15) and extra (n = 6) group were compared, with the range of values being the same and the average values only differing by less than 1-2% at either location (Table 3.1-13).

Table 3.1-12
Percentage of six-sided cells (%SIX) (mean ± 1SD) of the central (C%SIX) and mid-peripheral (MP%SIX) corneal endothelium in *spectacle wearers* (n = 12) at six occasions over a two-year period. Numbers in brackets represent the minimum and maximum observations. A paired t-test was used to explore any regional differences and repeated ANOVA for time related changes (α = 0.05).

%SIX	Time (months)						
	Baseline	3	6	12	18	24	p (ANOVA)
C%SIX (%)	63.5 ± 12.0 (41.8 - 82.4)	61.2 ± 9.7 (43.9 - 81.2)	62.7 ± 6.8 (53.3 - 72.1)	61.0 ± 8.9 (47.5 - 75.0)	62.8 ± 11.5 (43.1 - 75.0)	60.3 ± 11.1 (43.6 - 79.1)	0.858
MP%SIX (%)	64.3 ± 11.0 (50.8 - 80.3)	65.7 ± 8.7 (54.0 - 79.7)	64.2 ± 8.5 (46.5 - 73.8)	64.8 ± 10.9 (45.9 - 78.3)	64.4 ± 7.2 (54.2 - 78.7)	62.3 ± 9.4 (41.9 - 79.7)	0.924
p (paired t-test)	0.856	0.092	0.604	0.226	0.563	0.600	
MP%SIX:C%SIX ratio	1.043 ± 0.245 (0.645 - 1.559)	1.088 ± 0.161 (0.870 - 1.448)	1.031 ± 0.143 (0.649 - 1.184)	1.072 ± 0.188 (0.867 - 1.424)	1.051 ± 0.187 (0.858 - 1.447)	1.059 ± 0.221 (0.720 - 1.427)	0.983

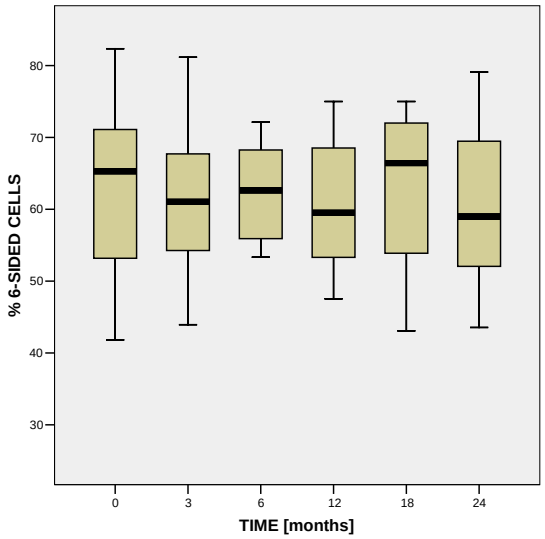


Figure 3.1-24
Box-plots of the degree of endothelial pleomorphism in the central cornea (C%SIX) over a two-year period in *spectacle wearers* (n = 12).

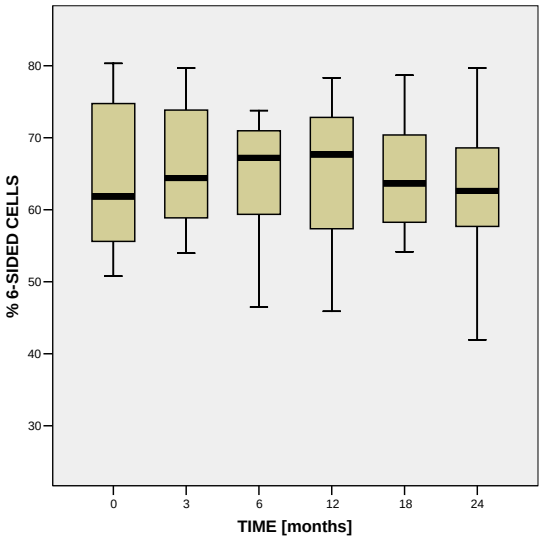


Figure 3.1-25
Box-plots of the degree of endothelial pleomorphism in the mid-peripheral cornea (MP%SIX) over a two-year period in *spectacle wearers* (n = 12).

Table 3.1-13
C&SIX and MP%SIX (Mean ± 1SD) for the initial groups of *spectacle wearers* (n = 15) and an extra set of myopic subjects wearing spectacles (n = 6) evaluated on a single occasion ('baseline', total N = 21). Numbers in brackets represent the minimum and maximum observations.

%SIX	Initial group	Extra subjects	p (t-test)	Total
C%SIX	64.4 ± 11.7 (41.8 - 82.4)	63.1 ± 7.1 (49.3 - 67.7)	0.811	64.0 ± 10.4 (41.8 - 82.4)
MP%SIX	64.6 ± 11.2 (49.2 - 80.3)	66.1 ± 7.4 (52.2 - 73.7)	0.764	65.0 ± 10.1 (49.2 - 80.3)
p (paired t-test)	0.962	0.079		0.721
MP%SIX:C%SIX ratio	1.030 ± 0.235 (0.645 - 1.559)	1.048 ± 0.051 (0.998 - 1.119)	0.780	1.035 ± 0.199 (0.645 - 1.559)

Endothelial morphometric inter-relationships

Twelve of the spectacle wearers completed the study and thus had six measurements of endothelial morphometric parameters. Three subjects had five measurements; hence, the total group size for spectacle wearers was 15 for the correlation analyses.

Endothelial cell density (ECD) showed no obvious relationship to the degree of polymegethism (COV), in neither the central nor the mid-peripheral parts of the endothelium. Similarly, ECD showed no relationship to the %SIX. See Table 5.2-3 in the appendix for correlation coefficients and probability values. On the other hand, the degree of polymegethism (COV) correlated strongly with the degree of pleomorphism (%SIX), both in the central ($r = -0.535$, $p = 0.040$) and mid-peripheral part ($r = -0.807$, $p < 0.001$) of the endothelium. As might be expected, highly polymegethous endotheliae were found to be associated with a lower percentage of 6-sided cells (see Figure 3.1-26 and Figure 3.1-27).

Morphometric parameters centrally correlated strongly with the respective mid-peripheral morphometric parameters. For example, as shown in Figure 3.1-28, high CECD was associated with high MPECD ($r = 0.563$, $p = 0.029$). Similar strong relationships were also found for the COV variable and %SIX (see Figure 3.1-29 and Figure 3.1-30). Moreover, as is clearly reflected in Figure 3.1-28, an individual could be expected to have very similar ECD values centrally and mid-peripherally. Likewise, the COV values were also very similar centrally and mid-peripherally for an individual (Figure 3.1-29). This was not found for the %SIX, where about half of the spectacle wearers were more likely to have a slightly higher MP%SIX than in C%SIX (Figure 3.1-30).

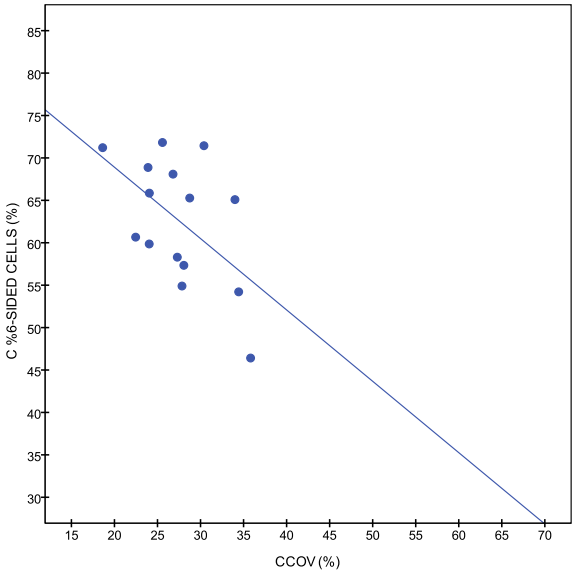


Figure 3.1-26
Scatter plots and linear regression analysis showing the relationship between mean CCOV and C%SIX in *spectacle wearers* (n = 15, p = 0.040, r = -0.535).

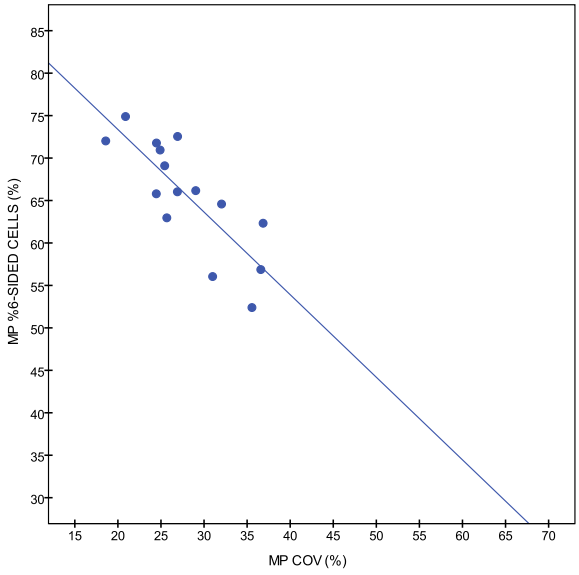


Figure 3.1-27
Scatter plots and linear regression analysis showing the relationship between mean MPCOV and MP%SIX in *spectacle wearers* (n = 15, p < 0.001, r = -0.807).

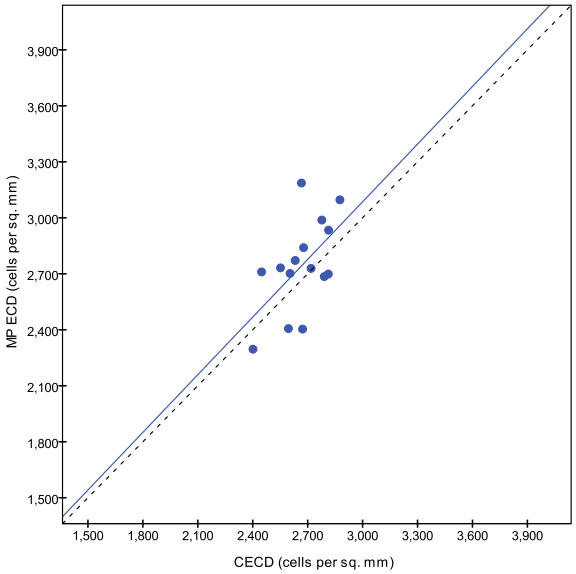


Figure 3.1-28
Scatter plots and linear regression analysis showing the relationship between mean CECD and MPECD in spectacle wearers (n = 15, p = 0.029, r = 0.563). The dotted line shows the one:one correlation.

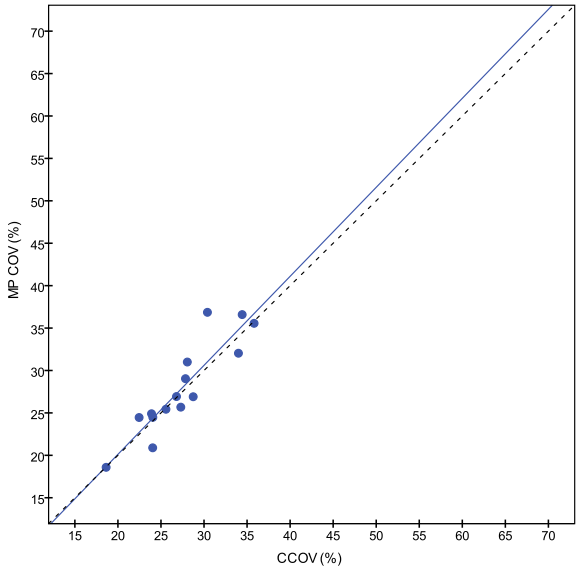


Figure 3.1-29
Scatter plots and linear regression analysis showing the relationship between mean CCOV and MPCOV in spectacle wearers (n = 15, p < 0.001, r = 0.904). The dotted line shows the one:one correlation.

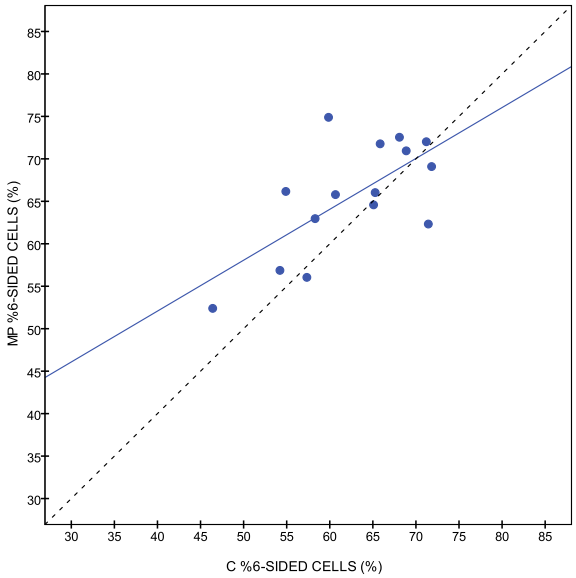


Figure 3.1-30
Scatter plots and linear regression analysis showing the relationship between mean C%SIX and MP%SIX in spectacle wearers (n = 15, p = 0.006, r = 0.674). The dotted line shows the one:one correlation.

3.2 RESULTS FOR SOFT CONTACT LENS WEARERS

3.2.1 Group demographics and vision assessments

Twenty eight soft lens wearers initially wanted to participate, however 3 were excluded for the following reasons: One subject was not willing to travel to the clinic in Oslo; one had an eye injury the same week as the first appointment (unrelated to the study) and a third subject had astigmatism larger than 0.75 DC. Since this subject also wore toric lenses, he was excluded based on the eligibility criteria (i.e. too high astigmatism; see Method's chapter, exclusion criteria). Of the 25 subjects successfully enrolled, 23 attended at all visits. However, for the two other subjects, one was not able to make the appointment at 3 months and another at 6 months. No subjects dropped out of the study for contact lens related reasons. Overall, 23 daily soft contact lens wearers were studied for the entire study period, but the data of the other two subjects is included where appropriate (see Table 3.2-1).

The soft contact lens wearers were aged 29.0 ± 4.4 years (mean ± SD, n = 25) at the first visit. The group was comprised of 14 women (56%) and 11 men (44%). All subjects were experienced contact lens wearers and had successfully worn contact lenses on a daily wear basis for at least 3.5 years. On average, the subjects had worn contact lenses for 10.6 ± 4.0 years at the first visit, and this was essentially unaltered when just considering the subgroup of 23 subjects (average 10.8 ± 4.0 y).

Table 3.2-1
Subject details of the *soft lens wearers* at baseline

	Initial assignment	Completed study
Number of subjects	25	23
Age at first visit (years)	29.0 ± 4.4	29.3 ± 4.4
Age range (years)	21 to 39	21 to 39
Gender (F:M)	14:11	13:10
Duration of lens wear at first visit (years)	10.6 ± 4.0	10.8 ± 4.0
Refractive error (MSE)[a]	-3.65 ± 1.40	-3.54 ± 1.38

[a] Mean refractive error (MSE) in spherical equivalent power (DS). All other data are mean ± S.D.

Most subjects reported that they wore their lenses daily (80%). Furthermore, over 90% wore their lenses for 10 hours or more per day (see also Table 3.2-2). The subjects reported a high level of satisfaction of their *quality of vision* with lens wear: On a 100 mm long horizontal line (Visual Analogue Scale) where the minimum end point was marked "svært misfornøyd" (i.e. "very dissatisfied") and the maximum end point was marked "svært fornøyd" (i.e. "very satisfied") the average score was 91 ± 9 (mean ± 1SD). The range was from 72 to 100. When asking about eye *comfort* with lenses in situ the level of satisfaction was somewhat lower. The average VAS score at baseline was 76 ± 18 mm (range 32 to 100) with the endpoints representing "svært ukomfortable" (i.e. "very uncomfortable") and "svært comfortable" (i.e. "very comfortable").

All subjects replaced their lenses on a regular basis. Almost half the group wore daily disposable lenses, whereas 44% replaced their lenses on a monthly basis (Table 3.2-3). Of these 11 subjects, four had worn SiH lenses on a daily wear basis.

The various types of disinfection system used by the soft lens wearers group are shown in Table 3.2-4. As expected, almost half the group did not use any system for cleaning or disinfection since they disposed of their lenses daily. The majority of those who stored their lenses for re-use used a chemical system. Only one subject used a special cleaner in addition to the disinfection system.

Table 3.2-2
Contact lens-wear modality of the *soft lens wearers* at baseline

Hours per day	Initial assignment N (%)	Completed study n (%)
5-10	2 (8)	2 (9)
10-15	15 (60)	15 (65)
> 15	8 (32)	6 (26)
Total	25 (100)	23 (100)

Table 3.2-3
Replacement schedule of soft contact lenses for the *soft lens wearers* at baseline

	Initial assignment N (%)	Completed study n (%)
Planned replacement (6, 9 or 12 m)	2 (8)	2 (9)
Monthly replacement	11 (44)	10 (43)
Daily disposables	12 (48)	11 (48)
Total	25 (100)	23 (100)

Table 3.2-4
Disinfection systems used by the *soft lens wearers*

	Initial assignment N (%)	Completed study n (%)
Chemical based	11 (44)	10 (43)
H_2O_2 based	2 (8)	2 (9)
None	12 (48)	11 (48)
Total	25 (100)	23 (100)

At baseline, the mean spherical refractive error for all the soft contact lens wearers was -3.55 ± 1.39 DS (N=25). Half the group had some astigmatism. However, the measured cylinder did not exceed 0.75 DC in any cases, and mean cylinder error was -0.38 ± 0.19 DC. All subjects wore spherical corrections, which imply slight under-correction of the astigmatism for some of them. Figure 3.2-1 shows the distribution of refractive error (MSE) as spherical equivalent power (DS) at each assessment.

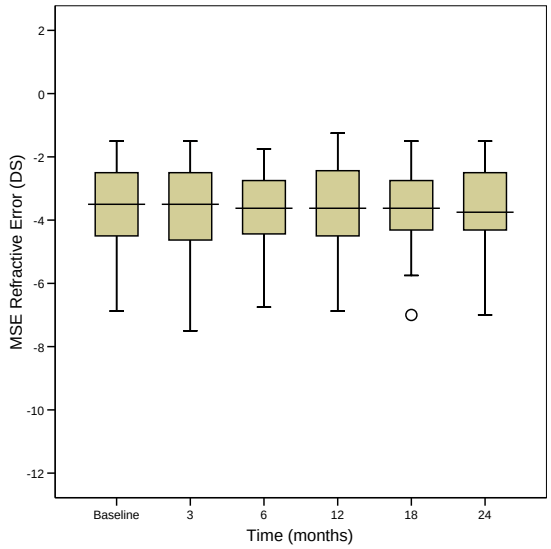


Figure 3.2-1
Box plot to show refractive error (MSE) in *soft contact lens wearers* (n = 23) over 2 years

The MSE refractive error remained essentially at the same levels at all follow-up visits (Table 3.2-5). Some slight differences in MSE were noted ($p = 0.026$, Friedman test) with the average refractive errors being statistically significantly different from baseline at 6 and 18 months ($p \leq 0.01$, Wilcoxon signed rank test), but such fluctuations did not exceed 0.25 DS and did not reflect an obvious time-related progressive change.

On average, the best-corrected high contrast visual acuity (BCHCVA) in the soft contact lens wearers was -0.09 ± 0.06 logMAR units at baseline. As summarized in Table 3.2-5, the mean and the range of BCHCVA for the soft lens wearers were very similar at each visit. No statistically significant trend in the BCHCVA was seen ($p = 0.476$, Friedman test). At all visits, the habitual (i.e. with contact lenses) HCVA was marginally poorer than the vision assessed with the best spectacle correction (Table 3.2-5). For example, at baseline, the habitual HCVA averaged -0.07 ± 0.06, which was significantly poorer than the BCHCVA of -0.09 ± 0.06 ($p < 0.029$, Wilcoxon signed rank test). However, such small changes represent only 1-2 letters differences on the charts. Moreover, as with the BCHCVA, the habitual HCVA values showed no obvious trend or change from visit to visit ($p = 0.077$, Friedman test).

Table 3.2-5
Refractive error (MSE) and visual acuity measurements: LogMAR Best Corrected High- and Low Contrast Visual Acuity (BCHCVA and BCLCVA, respectively) and Habitual HCVA and LCVA of *soft lens wearers* (n = 23) over a period of two years. All values are mean ± 1SD with ranges in brackets.

	Time (months)					
	baseline	3	6	12	18	24
Refractive error[a]	-3.54 ± 1.38 (-6.88 to -1.50)	-3.72 ± 1.49 (-7.50 to -1.50)	-3.71 ± 1.29 (-6.75 to -1.75)	-3.53 ± 1.37 (-6.88 to -1.25)	-3.71 ± 1.33 (-7.00 to -1.50)	-3.57 ± 1.33 (-7.00 to -1.50)
BCHCVA	-0.09 ± 0.06 (-0.20 to 0.04)	-0.10 ± 0.06 (-0.20 to 0.04)	-0.10 ± 0.05 (-0.20 to 0.00)	-0,09 ± 0,06 (-0,20 to 0,04)	-0.09 ± 0.05 (-0.18 to 0.00)	-0.10 ± 0.06 (-0.20 to 0.00)
Habitual HCVA	-0.07 ± 0.06[b] (-0.18 to 0.04)	-0.08 ± 0.08 (-0.20 to 0.14)	-0.09 ± 0.07 (-0.20 to 0.14)	-0.06 ± 0.07 [b] (-0.20 to 0.08)	-0.07 ± 0.07 [b] (-0.18 to 0.08)	-0.05 ± 0.07 [b] (-0.20 to 0.10)
BCLCVA	0.09 ± 0.07 (-0.08 to 0.22)	0.09 ± 0.05 (-0.04 to 0.20)	0.09 ± 0.05 (-0.04 to 0.18)	0.10 ± 0.05 (0.00 to 0.20)	0.06 ± 0.06 (-0.08 to 0.16)	0.08 ± 0.07 (-0.02 to 0.20)
Habitual LCVA	0.13 ± 0.07 (0.00 to 0.30)	0.10 ± 0.07 (-0.04 to 0.26)	0.10 ± 0.07 (0.02 to 0.24)	0.14 ± 0.07 (0.04 to 0.26)	0.09 ± 0.07 (0.00 to 0.20)	0.13 ± 0.09 (-0.02 to 0.38)

[a] Mean refractive error (MSE) in spherical equivalent power (DS)
[b] Significantly different from the respective HCVA with best correction ($p < 0.042$, Wilcoxon Signed Rank test)

At baseline, average best-corrected low contrast visual acuity (BCLCVA) in the soft contact lens wearers was 0.09 ± 0.06 logMAR. BCLCVA did change significantly during the study, according to the Friedman test ($p = 0.008$). Wilcoxon signed rank test revealed a significantly better BCLCVA at 18 compared to 3, 6 and 12 months ($p < 0.009$). However, the change of 0.03 logMAR units equals only 1.5 letters on the VA chart (Table 3.2-5) and this improvement could be a learning effect.
The habitual low contrast visual acuity (LCVA) averaged 0.13 ± 0.07 logMAR units at baseline, which was slightly worse (2 letters) than the BCLCVA ($p = 0.052$, Wilcoxon signed rank test). This trend was also apparent at some of the later visits (12, 18 and 24 months), where the average habitual LCVA was significantly poorer than the average BCLCVA ($p < 0.025$, Wilcoxon signed rank test) (Table 3.2-5). Friedman tests also revealed significant inter-visit differences in habitual LCVA ($p = 0.007$). The average habitual LCVA at 18 months was significantly (2-3 letters) better than at baseline and 12 months ($p < 0.01$ WSR test) and the poorest group mean habitual LCVA at 12 months was significantly different from the result at 3 months ($p = 0.005$, WSR test). However, these small and at some points statistically significantly different changes did not demonstrate any consistent time-dependent trend (Table 3.2-5).

3.2.2 Ocular comfort

Most of the subjects wearing soft contact lenses reported, at each visit, at least one of the listed symptoms, with 24 of 25 reporting ocular symptoms at baseline. Of the 23 subjects who completed the study, only one did not report any symptoms at baseline, 3, 6, 12 and 18 months. At the last visit (24 months) the number of subjects reporting symptoms of ocular discomfort had dropped to 19; i.e. another three did not report any symptoms (Figure 3.2-2).

The majority of symptomatic subjects responded that they experienced the symptoms “sometimes” (Table 3.2-6); the percentage of such subjects ranged from 82 to 96% (group mean 89%) over the study period. A few subjects, comprising 5% to 18% of the group, replied that they had symptoms ‘often’, while none of the subjects reported having symptoms ‘always’.

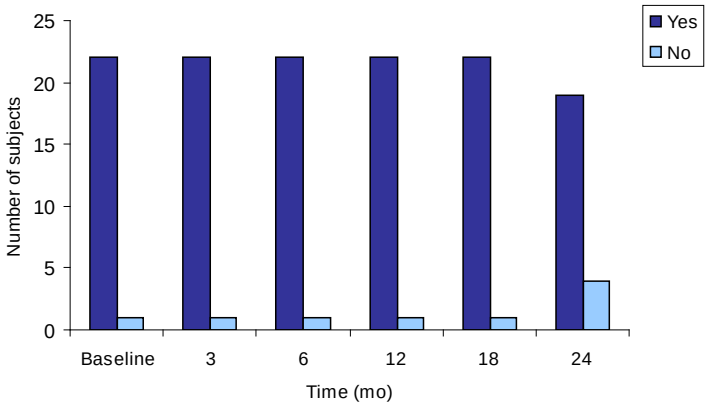


Figure 3.2-2
Frequency of reporting one or more ocular symptoms in *soft contact lens* wearing subjects (n = 23)

Table 3.2-6
Symptom frequency (count) in *soft lens wearers* who completed the study (N=23).

Symptom frequency (n)	Time (months)					
	Baseline	3	6	12	18	24
Never	1	1	1	1	1	4
Sometimes	19	19	18	21	21	17
Often	3	3	4	1	1	2
Always	0	0	0	0	0	0

The severity of ocular symptoms (on a 100 point VAS) in the symptomatic soft contact lens wearers averaged 31 ± 19 at baseline (N = 22). The range of symptom intensities was again substantial being from 56 to 70. Nevertheless, the average values for the soft contact lens wearers remained essentially unchanged over the 24 months with differences of less than 2 between visits (Table 3.2-7). There were no obvious time-dependent changes in symptom severity, and no statistically significant differences or trends were identified ($p > 0.900$, Friedman test). Over the six visits, the overall mean level of symptoms in symptomatic soft lens wearers was 32 (95% CI = 29 – 35).

Table 3.2-7
Symptom severity assessed by VAS in symptomatic *soft contact lens wearers*. All values are given as group mean ± SD (minimum to maximum values in brackets).

Symptom severity (mm)	Time (months)					
	Baseline	3	6	12	18	24
Eligible sample (n = 19)*	31 ± 18 (0 to 60)	29 ± 18 (8 to 69)	32 ± 20 (5 to 76)	32 ± 16 (5 to 60)	33 ± 16 (7 to 63)	32 ± 20 (7 to 74)
Main sample	31 ± 19 (0 to 60)	30 ± 17 (8 to 69)	33 ± 20 (5 to 76)	32 ± 16 (5 to 60)	32 ± 15 (7 to 63)	32 ± 20 (7 to 74)
N	22	22	22	22	22	19

*I.e. those subjects who reported symptoms at all visits. These values were used in time-dependent comparisons (Friedman test).

3.2.3 Tear film characteristics

The average tear meniscus height (TMH) for the 23 (of 25 originally enrolled) soft contact lens wearers who finished the study was 0.21 ± 0.08 mm at baseline. Some subjects had a rather high value (i.e. > 0.30 mm) and this was especially evident at the 12-month assessment (Figure 3.2-3). As seen in Table 3.2-8, a few rather large TMH values were always evident, even as high as 0.7 mm, but none of the sets of values were statistically different from each other (p = 0.059, Friedman test). Over time, the median values remained largely stable and were within less than one grading unit (0.05 mm, Figure 3.2-3). For the soft contact lens wearers, the overall group-average TMH was 0.22 mm (95% CI = 0.20 to 0.25 mm).

The group-averaged tear volume, as estimated by the PRT test, was 15.9 ± 8.1 mm for the soft contact lens wearing group at baseline (Table 3.2-8). As indicated by the slightly larger SD and range (7 to 35), the PRT data in the soft contact lens wearers was a little more variable than in the spectacle wearers. As shown in Figure 3.2-4, the inter-quartile intervals for the PRT data were slightly wider and fluctuated a little more. Both mean values (19.5 ± 9.5, Table 3.2-8) and the median value (20.0 mm, Figure 3.2-4) appeared higher at the 3 month assessment, but this was not significantly higher than the baseline value (p = 0.069).

Table 3.2-8
Mean ± SD (range) of the tear film tests of the subjects that completed the study, *soft contact lens* group (n = 23).

Tear film test	Time (months)					
	Baseline	3	6	12	18	24
TMH (mm)	0.21 ± 0.08 (0.10 to 0.45)	0.18 ± 0.08 (0.10 to 0.40)	0.23 ± 0.10 (0.10 to 0.45)	0.29 ± 0.17 (0.10 to 0.70)	0.22 ± 0.08 (0.10 to 0.40)	0.23 ± 0.09 (0.10 to 0.40)
PRT (mm)	15.9 ± 8.1 (7.0 to 35.0)	19.5 ± 9.5 (7.0 to 35.0)	16.9 ± 6.9 (6.0 to 29.0)	15.4 ± 6.6 (6.0 to 30.0)	17.4 ± 7.8 (6.0 to 30.0)	15.0 ± 8.2 (5.0 to 33.0)
NIBUT (sec)	24.9 ± 25.3 (5.7 to 125.8)	28.1 ± 35.8 (7.5 to 177.0)	27.6 ± 19.0 (6.4 to 74.1)	28.3 ± 33.7 (7.3 to 167.5)	26.0 ± 27.0 (7.3 to 134.0)	28.0 ± 27.7 (6.4 to 108.0)
f-TBUT (sec)	19.0 ± 17.7 (5.5 to 83.0)	23.7 ± 21.7 (4.6 to 103.5)	24.9 ± 17.7 (5.0 to 60.4)	19.2 ± 13.0 (5.5 to 51.9)	34.9 ± 60.1 (3.7 to 301.0)	30.7 ± 42.2 (5.2 to 180.0)

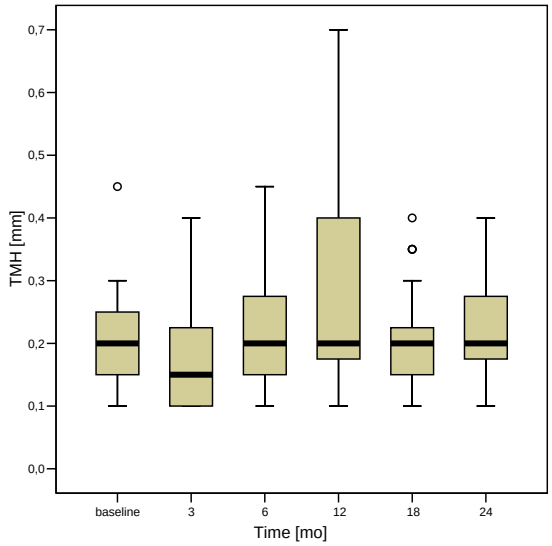


Figure 3.2-3
Box-plots to show time-related changes of tear meniscus height (TMH) in *soft contact lens wearers*.

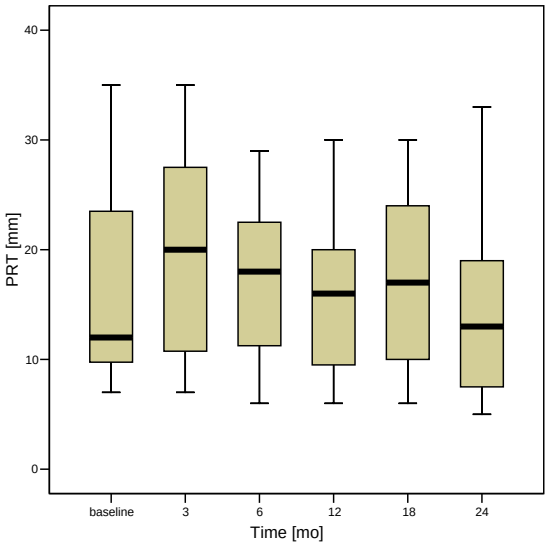


Figure 3.2-4
Box-plots to show time-related changes of phenol red thread test (PRT) in *soft contact lens wearers*.

Furthermore, Figure 3.2-4 gives the impression that the median PRT values got smaller over time after the 3 month's assessment, but no statistically significant changes were detectable by visit-to-visit comparisons ($p > 0.232$, Friedman test) or by a linear regression analysis ($p = 0.335$, $r = -0.083$, Figure 3.2-5). If just the data from follow-up visits were considered (i.e. 3 to 24 months) then there was still no detectable time-related change in PRT ($p=0.129$, $r = -0.142$; not shown).

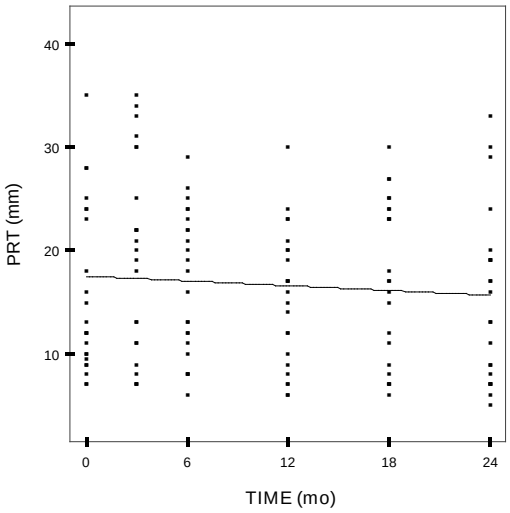


Figure 3.2-5
Scatter-plot to illustrate time-related changes of phenol red thread (PRT) values in *soft lens wearers*. The linear regression line shown indicates no statistically significant effect (slope = -0.96 mm/year, $p = 0.335$, Pearson's $r = -0.083$).

The tear stability values for the soft contact lens wearers were mostly stable over the 24 month study period, and no significant between-visits differences were detected ($p = 0.931$, Friedman test). At baseline, the average NIBUT was 24.9 ± 25.3 sec and the averages changed by no more

than 3.2 sec between visits (Table 3.2-8 and Figure 3.2-6). The range of NIBUT values were somewhat less than those observed in the spectacle wearers (with the largest range of values being 169.5 s, compared to 377.0 sec in the spectacle wearers).

The average f-TBUT for all 25 soft contact lens wearers at baseline was 18.4 ± 17.2 seconds (mean ± 1 S.D.) with a range of 77.5 s. From Table 3.2-8, it seems that at later occasions, the average values were somewhat larger. The high 18-month's group average was partly due to one subject who had an average f-TBUT of 301 seconds. Furthermore, examination of the median values, as indicated in Figure 3.2-7, shows no obvious time-dependent progressive changes. The group average values were increasingly higher because of increasing ranges and increasing positive skewness of the distributions. Overall, the average f-TBUTs at each visit were not significantly different from one another (p = 0.343, Friedman test), and no real trend could be identified by correlation analysis (Figure 3.2-8; p = 0.443, Spearman's rho = 0.066). Overall, the mean f-TBUT in contact lens wearers who finished the study was 25.4 sec (95% CI = 14.9 to 36.0 sec).

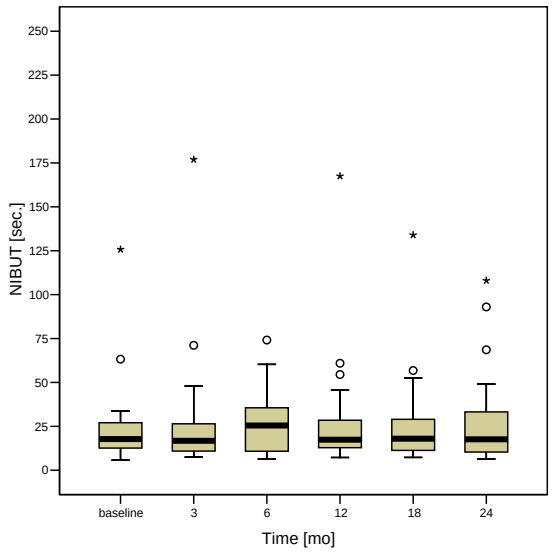


Figure 3.2-6
Box-plots to show time-related changes of non-invasive tear break-up time (NIBUT) in *soft contact lens wearers* (n = 23).

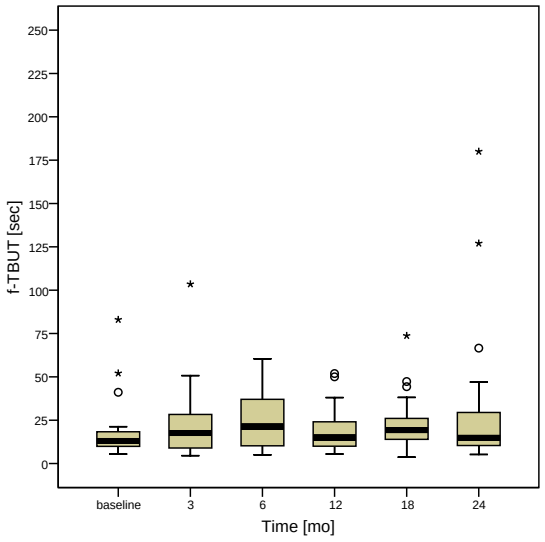


Figure 3.2-7
Box-plots to illustrate time related changes of fluorescein-tear break-up time (f-TBUT) in *soft contact lens wearers* (n = 23). Note that one subject with an extreme f-TBUT at 18 months (301 sec) is not visible on this plot.

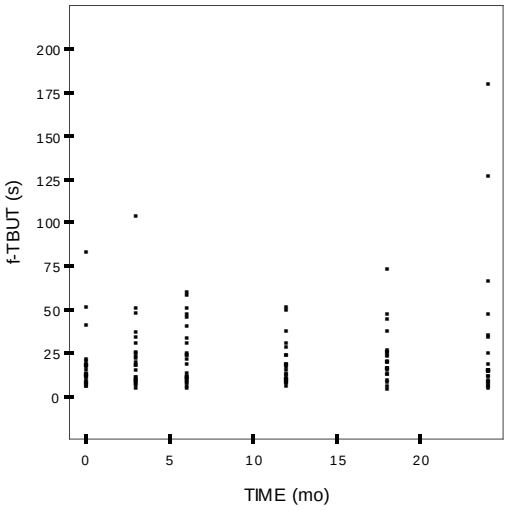


Figure 3.2-8
Scatter plot to illustrate time-related changes of fluorescein-tear break-up time (f-TBUT) in *soft contact lens wearers*. Note that one subject with an extreme f-TBUT at 18 months (301 sec) is not visible on the graph. Correlation analysis indicated no statistically significant trend (Spearman's rho = 0.066, p = 0.443).

3.2.4 Ocular surface characteristics

The results are summarized in Table 3.2-9. Only subtle signs of blepharitis were seen in soft-lens wearers. At baseline, average grading of eyelids was 0.4 ± 0.6 (range 0.0 to 1.9). At later visits, mean grading of eyelids were only 0.1 to 0.2 units different from baseline. However, significant inter-visit differences were seen (p = 0.040, Friedman test), although these only had a maximum magnitude of 0.3. Meibomian glands were graded to a mean of 0.6 ± 0.4 at baseline. At later visits, mean reductions of up to 0.3 were seen (at 18 months) and such differences were just statistically significant (p = 0.015, Friedman test).

The mean grade for bulbar hyperaemia was 1.9 ± 0.4 (range 1.1 to 2.5) at baseline. With ongoing lens wear, the levels of bulbar hyperaemia were up to 0.4 units lower, and these were statistically significant (p = 0.003, Friedman test). Limbal redness was graded to 1.2 ± 0.6 in the soft lens wearers at baseline, ranging from 0.0 to 2.1. No substantial changes were seen at later visits, and the mean inter-visit differences were only up to 0.2 units. Similarly, corneal vascularisation averaged 0.9 ± 0.4 at baseline and changed by only up to 0.2 units the next 4 assessments. However, at 24 months the average corneal vascularisation declined slightly, but significantly, to a mean of 0.6 ± 0.4 (p = 0.001, Wilcoxon Signed Rank test).

Table 3.2-9
Ocular surface characteristics as graded with Efron's grading scale [0 - 4] in *soft contact lens wearers* who completed the study (n = 23, except for papillary conjunctivitis where n = 15). The numbers represents group mean ± SD and range (in brackets).

Ocular surface characteristic	Time (months)					
	baseline	3	6	12	18	24
Eyelids	0.4 ± 0.6 (0.0 to 1.9)	0.2 ± 0.4 (0.0 to 1.2)	0.3 ± 0.4 (0.0 to 1.0)	0.5 ± 0.5 (0.0 to1.8)	0.3 ± 0.4 (0.0 to 1.0)	0.4 ± 0.4 (0.0 to1.0)
Meibomian glands	0.6 ± 0.4 (0.0 to 1.2)	0.4 ± 0.4 (0.0 to 1.0)	0.6 ± 0.4 (0.0 to 1.5)	0.4 ± 0.3 (0.0 to 0.9)	0.3 ± 0.3 (0.0 to 1.0)	0.5 ± 0.3 (0.0 to 0.9)
Bulbar hyperaemia	1.9 ± 0.4 (1.1 to 2.5)	1.7 ± 0.4 (1.0 to 2.3)	1.6 ± 0.3 (1.0 to 2.3)	1.5 ± 0.4 (0.7 to 2.0)	1.7 ± 0.3 (0.8 to 2.2)	1.6 ± 0.3 (0.9 to 2.0)
Limbal redness	1.2 ± 0.6 (0.0 to 2.1)	1.3 ± 0.6 (0.2 to 2.3)	1.3 ± 0.6 (0.5 to 2.2)	1.2 ± 0.5 (0.0 to 2.0)	1.3 ± 0.5 (0.5 to 2.4)	1.1 ± 0.4 (0.3 to 1.8)
Corneal vascularisation	0.9 ± 0.4 (0.0 to 1.8)	0.8 ± 0.4 (0.0 to 1.8)	0.8 ± 0.5 (0.0 to 1.9)	0.8 ± 0.4 (0.0 to 1.7)	0.7 ± 0.3 (0.3 to 1.5)	0.6 ± 0.4 (0.0 to 1.4)
Corneal staining*	0.5 ± 0.6 (0.0 to 1.5)	0.7 ± 0.7 (0.0 to 2.0)	0.8 ± 0.8 (0.0 to 1.9)	0.6 ± 0.9 (0.0 to 3.3)	0.4 ± 0.7 (0.0 to 1.8)	0.4 ± 0.6 (0.0 to 1.8)
Conjunctival staining	1.8 ± 1.1 (0.0 to 3.0)	1.7 ± 0.8 (0.0 to 3.0)	1.3 ±´0.8 (0.0 to 3.0)	0.8 ± 0.9 (0.0 to 3.0)	0.8 ± 0.8 (0.0 to 2.0)	1.0 ± 0.8 (0.0 to 3.0)
Papillary conjunctivitis	1.7 ± 0.4 (0.7 to 2.5)	1.7 ± 0.5 (1.0 to 2.5)	1.5 ± 0.5 (0.5 to 2.3)	1.6 ± 0.6 (0.0 to 2.5)	1.6 ± 0.3 (1.0 to 2.0)	1.5 ± 0.5 (0.0 to 2.4)

* Corneal staining was graded with CCLRU's grading scales [0-4]. See methods chapter for details.

Approximately half (48%) the soft lens wearers had corneal fluorescein staining at baseline. At later visits, the percentage having staining ranged from 35% at 18 months to 52% at 3 and 6 months. The mean grading of staining in those subjects who actually had staining was 1.1 ± 0.2 (range 0.8 to 1.5) at baseline, and the mean grade of staining was up to 0.5 units higher at later visits. For all subjects, mean corneal fluorescein staining grade at baseline was 0.5 ± 0.6 (Table 3.2-9). At later visits, mean differences of up to 0.2 units were seen ($p = 0.429$, Friedman test).
Over 80% of the subjects wearing soft lenses had conjunctival staining at baseline but the grades, at 2.2 ± 0.8 (range 0.1 to 3.0), were substantially greater than those seen in spectacle wearers. At the following visits, the percentage of subjects having conjunctival staining ranged from 52 to 87%, while the average grading for the subjects who had conjunctival staining declined from visit to visit by 0.1 to 0.4 units. Thus, at 24 months the mean grading of conjunctival staining for 18 subjects was just 1.3 ± 0.6 (range 0.5 to 3.0). If all subjects were included in the analyses (i.e. those with grading = 0.0, see Table 3.2-9), significant inter-visit differences were revealed by Friedman test ($p < 0.001$). The level of conjunctival staining at 12, 18 and 24 months, ranging from mean 0.8 to 1.0, were significantly lower than the baseline value of 1.8 and the 3 month's value of 1.7 ($p \leq 0.011$, Wilcoxon Signed Rank test).

Papillary conjunctivitis was graded at every visit for 15 of the 23 subjects. The remaining eight subjects refused to undergo this examination at one or more occasion The average grading at baseline was mild at 1.7 ± 0.4 (range 0.7 to 2.5). The average CLPC did not significantly change over time.

3.2.5 Corneal thickness and corneal curvature

The mean central corneal thickness (CCT) was 526 ± 32 µm at the first assessment of the 19 soft contact lens wearers (Table 3.2-10). Their overall CCT mean value over the six assessments was 524 µm (range 520 to 527 µm). Their mean mid-peripheral corneal thickness values were 620 ± 49 µm at baseline, with the overall mid-peripheral CT values being 623 µm over the six visits (range 616 to 630 µm).

As detailed in Table 3.2-10, at the different assessments, the mean thickness values obtained again fluctuated very slightly with differences from baseline of only 6 µm for central location sets and 10 µm for mid-peripheral locations (Figure 3.2-9 and Figure 3.2-10). Therefore, the maximum differences in mean values (compared to baseline) were thus 1.1% and 1.6%. None of these small differences was statistically significant ($p \geq 0.201$, pairwise comparisons with Bonferroni correction). The mean central thickness value at the 24-month assessment was identical to the mean central thickness values at baseline. Likewise, the mean mid-peripheral thickness value was only marginally higher than baseline (626 µm vs. 620 µm).

Table 3.2-10
Mean ± 1SD central and mid-peripheral corneal thickness (CCT and MPCT) and central corneal radius of curvature (K), in *soft lens wearers* (n = 19). Minimum and maximum values are given in brackets.

	Time (months)					
	Baseline	3	6	12	18	24
CCT (µm)	526 ± 32 (472 to 569)	520 ± 30 (459 to 558)	520 ± 32 (467 to 565)	525 ± 30 (469 to 574)	527 ± 35 (452 to 568)	526 ± 29 (465 to 567)
MPCT (µm)	620 ± 49 (551 to 720)	616 ± 48 (553 to 702)	617 ± 44 (541 to 691)	630 ± 45 (573 to 709)	(628 ± 50) 534 to 709	626 ± 46 (535 to 721)
Central K (mm)	7.90 ± 0.20 (7.611 to 8.365)	7.89 ± 0.19 (7.59 to 8.30)	7.92 ± 0.23 (7.577 to 8.480)	7.89 ± 0.18 (7.63 to 8.39)	7.87 ± 0.21 (7.59 to 8.33)	7.84 ± 0.19 (7.58 to 8.22)

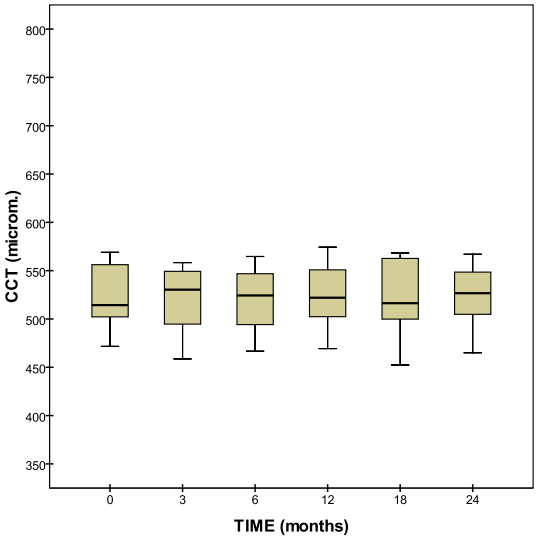


Figure 3.2-9
Box plot to show central corneal thickness over time in *soft lens* wearers (n = 19).

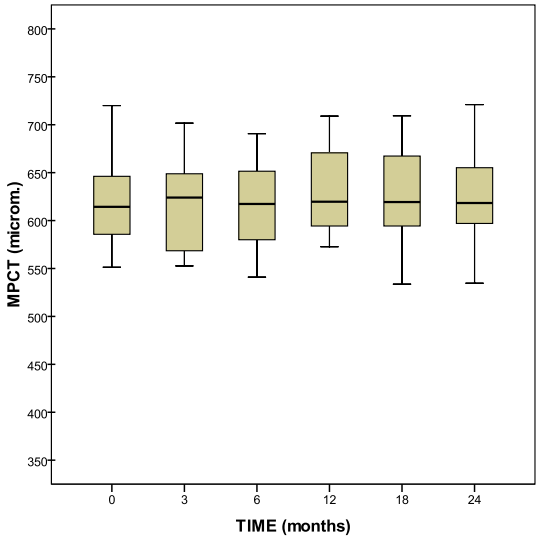


Figure 3.2-10
Box plot to show mid-peripheral corneal thickness over time in *soft lens wearers* (n = 19).

Linear regression analyses for any time-dependent changes in corneal thickness in these soft contact lens wearers are shown in Figure 3.2-11 and Figure 3.2-12. No statistically detectable changes for either central ($p = 0.609$, $r = 0.048$) or mid-peripheral thicknesses ($p = 0.375$, $r = 0.084$) were identified over the different assessments. If the overall mean thickness values over the 6 visits are taken as a reference value (i.e. 524 µm and 623 µm), then the maximum differences (fluctuations) in thickness values can be calculated to be just $\pm$ 0.7% (range -0.8 to 0.5%) and $\pm$ 1.0% (range -1.0 to 1.0%) respectively for central and mid-peripheral sites.

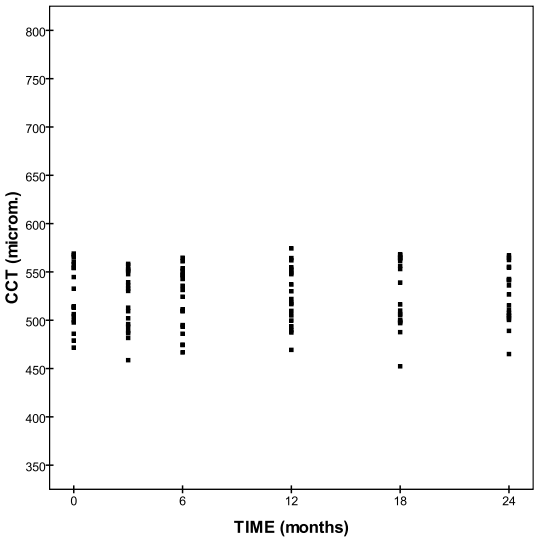


Figure 3.2-11
Regression analysis to show time-related changes of central corneal thickness (µm) in *soft lens wearers* (n = 19). The linear regression line indicates no statistically significant effect (p = 0.609, r = 0.048).

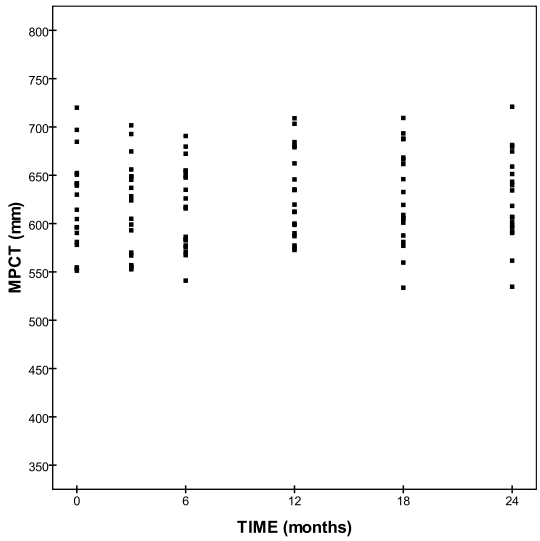


Figure 3.2-12
Regression analysis to show time-related changes of mid-peripheral corneal thickness (µm) in *soft lens wearers* (n = 19). The linear regression line indicates no statistically significant effect (p = 0.375, r = 0.084).

Measurements of corneal curvature from 19 soft lens wearers were obtained at all visits. At baseline, the mean central corneal curvature was 7.90 ± 0.20 mm (range 7.61 to 8.34 mm). As with the spectacle wearers, only very small differences in mean K values were observed between visits (Table 3.2-10 and Figure 3.2-13), although the Friedman test revealed significant differences between visits (p = 0.001). Using Wilcoxon signed rank test as a post-hoc test, it was shown that the mean 24-month's K value was lower than the value obtained at baseline, 3 and 6 months by -0.060, -0.051 and -0.080 mm, respectively (p ≤ 0.004).

Overall, as shown by the general consistency of the values (Figure 3.2-13) and a correlation analysis (Figure 3.2-14) there were no predictable changes in anterior surface curvature during the period of soft contact lens wear (p = 0.343, r = -0.091). Overall mean central radius of curvature was 7.89 mm (95% CI = 7.79 – 7.98) for soft lens wearers.

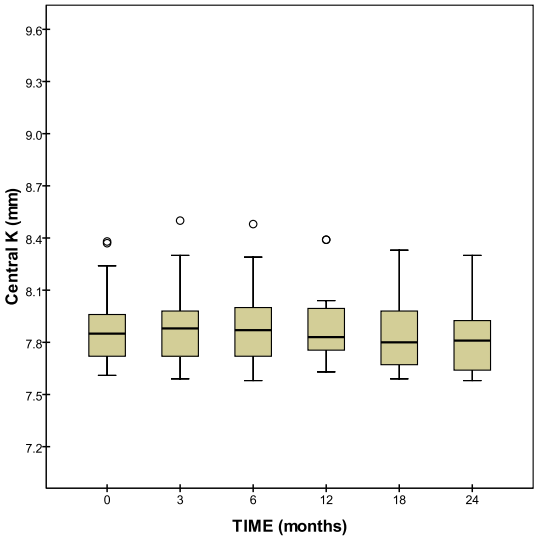


Figure 3.2-13
Box plot to show central corneal radius of curvature values over time (mm) in *soft lens wearers* (n = 19).

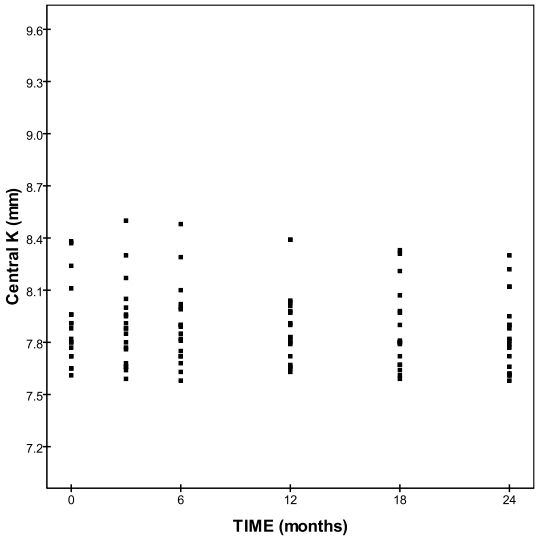


Figure 3.2-14
Scatter plot to illustrate time-related changes in central corneal radius of curvature values (mm) over time in *soft lens wearers* (n = 19). Non-parametric correlation analysis indicated no statistically significant effect (p = 0.305, r = -0.097).

3.2.6 Corneal endothelial cell morphometry

Endothelial cell density – ECD

For the 19 soft contact lens wearers assessed at all visits, the average central endothelial cell density (CECD) was 2711 ± 347 cells / mm^2 at baseline. Individual CECD values ranged from 1890 to 3223 cells/mm^2 (Table 3.2-11). As can be seen in the box plots in Figure 3.2-15, two of the subjects consistently had rather low CECD values, with their data appearing as outliers. However, for these two individuals, as for the rest of the daily wear soft contact lens wearers, the box plot indicates no obvious change in the CECD values over time. The average value was slightly lower at the 6 months assessment (at 2699 ± 329 cells/mm^2) and then appeared to increase again by 24 months to have the same values as at baseline (i.e. 2712 ± 353 cells/mm^2). However, no statistically significant changes in the CECD values were found between visits (p = 0.991, repeated ANOVA).

The MPECD values averaged 2939 ± 306 cells / mm^2 at baseline, as compared to 2717 ± 347 cells/mm^2 for the central region of the same corneas. Individual MPECD values ranged from 2453 to 3380 cells/mm^2. The net difference was 228 cells / mm^2 (95%CI = 107 to 349 cells / mm^2) and this was statistically significant (p = 0.001, paired t-test). As with CECD, the average MPECD did not change substantially over the 2 years (Table 3.2-11). The median MPECD values clearly fluctuated from visit to visit and this is evident in the ± 25% inter-quartile intervals (Figure 3.2-16). However, it can be noted that those subjects with CECD values that were obviously lower than the others, clearly did not have lower MPECD values (Figure 3.2-15 and Figure 3.2-16). The average MPECD at 24 months was lower (at 2851 cells/mm^2) than at baseline, but this 2.9% change was not statistically significant (p = 0.098, repeated ANOVA).

Table 3.2-11
Cell density (mean ± 1SD) of the central (CECD) and mid-peripheral (MPECD) corneal endothelium in *soft lens wearers* (n = 19) at six occasions over a two-year period. Numbers in brackets represent the minimum and maximum observations. A paired t-test was used to explore any regional differences and repeated ANOVA for time related changes (α = 0.05).

ECD	Time (months)						
	Baseline	3	6	12	18	24	p (ANOVA)
CECD (cells/mm^2)	2711 ± 347 (1890 - 3223)	2704 ± 342 (1904 - 3246)	2699 ± 329 (1924 - 3370)	2707 ± 346 (1949 - 3454)	2715 ± 364 (1898 - 3409)	2712 ± 353 (1925 - 3278)	0.991
MPECD (cells/mm^2)	2939 ± 306 (2453 - 3380)	2933 ± 329 (2453 - 3503)	2946 ± 328 (2359 - 3522)	2869 ± 315 (2332 - 3362)	2885 ± 257 (2500 - 3403)	2851 ± 314 (2301 - 3411)	0.098
p (paired t-test)	0.001	0.003	0.001	0.028	0.027	0.053	
MPECD:CECD ratio	1.093 ± 0.111 (0.957 - 1.406)	1.094 ± 0.128 (0.876 - 1.347)	1.099 ± 0.115 (0.842 - 1.291)	1.069 ± 0.124 (0.876 - 1.408)	1.076 ± 0.137 (0.871 - 1.396)	1.061 ± 0.119 (0.790 - 1.268)	0.177

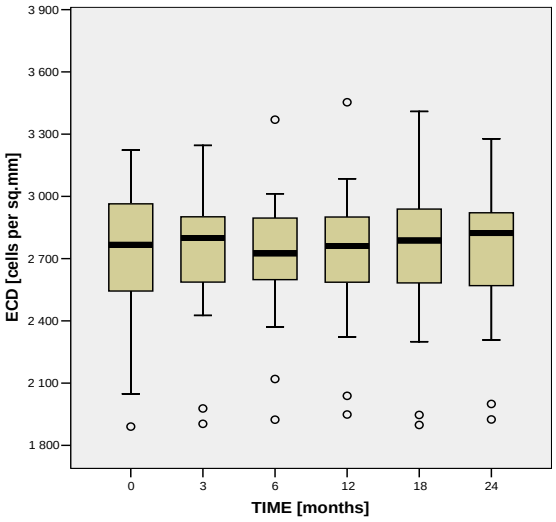


Figure 3.2-15
Box-plots of endothelial cell density in the central region of the cornea (CECD) over a two-year period in subjects wearing *soft lenses* (n = 19).

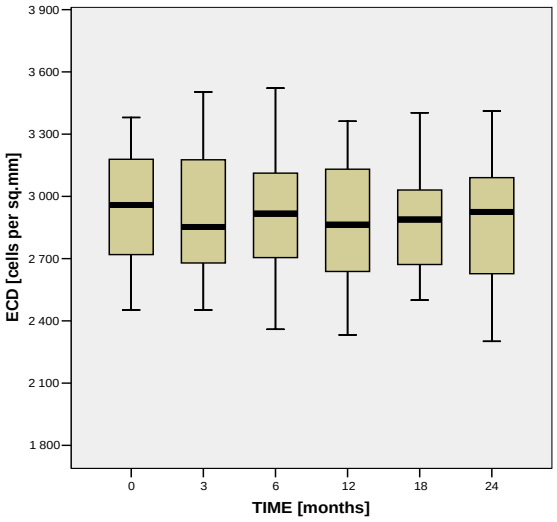


Figure 3.2-16
Box-plots of endothelial cell density in the mid-peripheral region of the cornea (MPECD) over a two-year period in subjects wearing *soft lenses* (n = 19).

More detailed analyses to assess any time-related changes in cell density are given in Figure 3.2-17, and Figure 3.2-18 (see also Table 5.3-1 in the appendix). The overall mean CECD was 2708 cells / mm^2, with average values ranging from 2699 to 2712 cells/mm^2. Comparisons of sets of individual values at central sites by a linear regression analysis (Figure 3.2-17 and Figure 3.2-18) revealed no measurable time-related change in CECD values ($p = 0.930$, $r = 0.008$). For each set of corneal images from each subject, the same regression analyses revealed time-related changes varying from -85 cells/mm^2 per year to + 104 cells/mm^2 per year, with an overall time-related change of just 4 ± 51 cells/mm^2 per year (see Table 5.3-1 in the appendix). Since the average CECD values declined over the first 6 months, an assessment was made also of just this period, using the same linear regression. This indicated a cell loss per year of -24 cells / mm^2. However, the slope was not statistically significant: $p = 0.912$ ($r = -0.15$).

The overall mean MPECD was 2904 cells / mm^2, with average values from 2851 to 2946 cells/mm^2. A scatter-plot of the individual MPECD values at each visit is shown in Figure 3.2-18. The linear regression analysis indicated a negative slope but this was not statistically significant ($p = 0.244$). The net slope was -47 ± 107 cells/mm^2 per year (range from -375 to 160 cells/mm^2 per year) with a poor correlation coefficient ($r = -0.110$, see also Table 5.3-1 in the appendix).

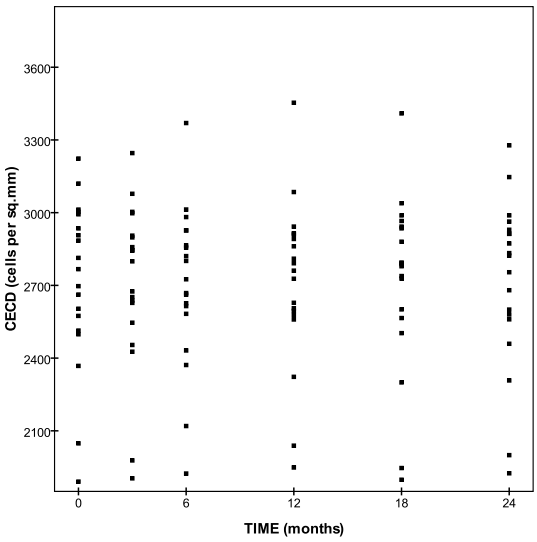


Figure 3.2-17
Linear regression analysis of the central cell density (CECD) and time in *Soft lens wearers* (n = 19, p = 0.930, r = 0.008.

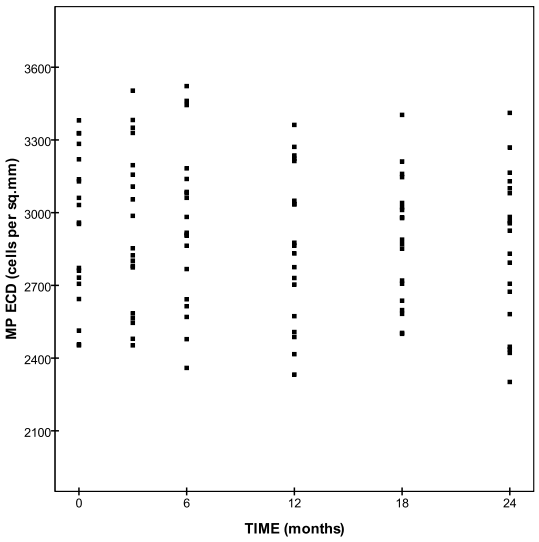


Figure 3.2-18
Linear regression analysis of the mid-peripheral cell density (MPECD) and time in *Soft lens wearers* (n = 19, p = 0.244, r = -0.110).

As mentioned above, most soft lens wearers had a higher MPECD than CECD. However, the inter-subject variance in the MPECD: CECD ratio was large. While, at baseline, some of the soft contact lens wearers had a MPECD:CECD ratio that was 0.9 others had a value as high as 1.4 (N=21, Figure 3.2-19). A similar effect was seen at the subsequent visits (Figure 3.2-20), where the overall mean MPECD:CECD ratio was 1.071 ± 0.012. As assessed over time (Figure 3.2-21), again with the result of linear regression analysis applied, there was no measurable change in the MPECD: CECD ratio (p = 0.130, r = 0.137).

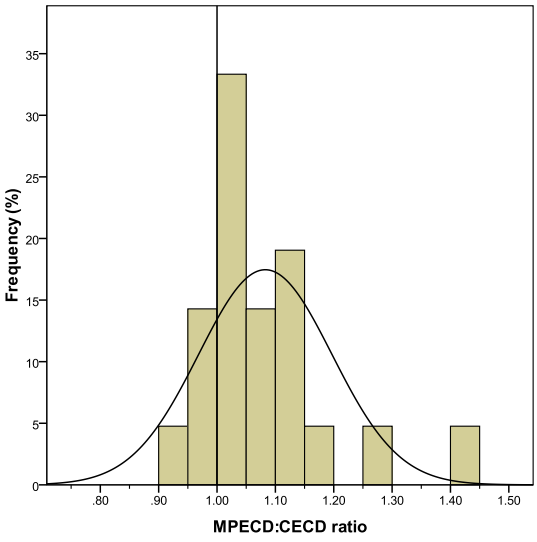


Figure 3.2-19
Histogram showing the frequencies of individual endothelial MPECD:CECD ratios in a group of *soft lens wearers* (N = 21) at a single occasion (baseline). The reference line shows the level of no difference between MPECD and CECD (ratio = 1).

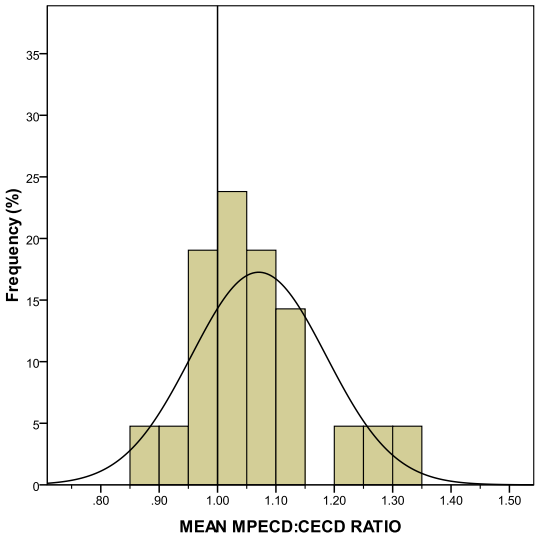


Figure 3.2-20
Histogram showing the frequencies of individual mean endothelial MPECD:CECD ratios in a group of *soft lens wearers* (N = 21) from six occasions over a period of two years .The reference line shows the level of no difference between MPECD and CECD (ratio = 1).

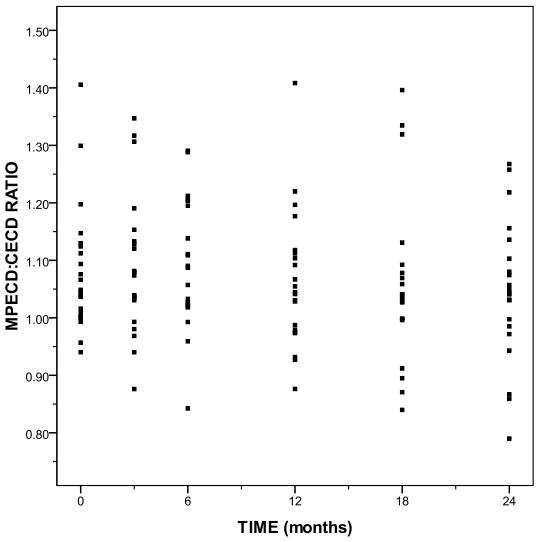


Figure 3.2-21
Regression analysis of the endothelial MPECD:CECD ratio in *soft lens wearers* over a period of two years (p = 0.130, r = -0.137).

Endothelial polymegethism -COV

The CCOV was 32.0 ± 5.7% for soft lens wearers at baseline. Individual endothelia had CCOV values between 23.2 and 42.7% at the baseline assessments (Table 3.2-11). The CCOV did not change during the 2-year period; the largest mean difference between two visits was 1.2% (95% CI = -1.2 to 3.5%), which was not significant (p =0.709, repeated ANOVA, Table 3.2-11). In the mid-peripheral part of the corneal endothelium, the mean degree of polymegethism (MPCOV) was 34.8 ± 10.4% at baseline (Table 3.2-11), and it should be noted that the range of MPCOV values was quite a lot greater than at central sites, being between 20.8 and 63.9%. The net difference of 2.8% (95% CI = -1.3 to 6.9%) between the CCOV and MPCOV at baseline was not statistically significant (p = 0.070, paired t-test). As with the central part of the endothelium, the mean MPCOV did not change significantly with time; the largest mean difference between two visits was 1.8% with a 95% CI from -6.4% to 2.9% (p = 0.528, repeated ANOVA). The box-plots in Figure 3.2-22 and Figure 3.2-23 illustrate the small changes that occurred in endothelial COV in soft lens wearers during the two years of follow-up. The individual with a high MPCOV at baseline (63.9%) showed further development of the polymegethism, as indicated by the outlier data points in Figure 3.2-23.

The overall mean CCOV value was 32.1%, with the range of average values being small at 31.7 to 32.9%. For the mid-peripheral sites, the overall mean MPCOV value was 35.1%, again with a fairly narrow range of average values of between 34.4 to 36.2%. A linear regression analysis did not show any relationship between time and CCOV or MPCOV. The net change at central sites was calculated to be just -0.2% / year with a large variance of ± 2.3% (r = -0.030, p NS at 0.750), and was just 0.8 + 2.3% / year at mid-peripheral sites (r = 0.049, p = 0.603, see also Table 5.3-1 in the appendix).

Table 3.2-12
Polymegethism (mean ± 1SD of the coefficient of variation in cell area; COV) of the central (CCOV) and mid-peripheral (MPCOV) corneal endothelium in *soft lens wearers* (n = 12) at six occasions over a two-year period. Numbers in brackets represent the minimum and maximum observations. A paired t-test was used to explore any regional differences and repeated ANOVA for time related changes (α = 0.05).

COV	Time (months)						p (ANOVA)
	Baseline	3	6	12	18	24	
CCOV (%)	32.0 ± 5.7 (23.2 - 42.7)	32.9 ± 6.1 (24.0 - 49.7)	31.8 ± 5.7 (23.3 - 45.6)	31.7 ± 5.1 (22.9 - 43.0)	32.2 ± 6.1 (22.7 - 43.7)	31.8 ± 5.7 (23.1 - 39.7)	0.709
MPCOV (%)	34.8 ± 10.4 (20.8 - 63.9)	34.9 ± 10.5 (23.6 - 66.4)	34.6 ± 12.6 (20.6 - 76.1)	34.4 ± 9.6 (21.6 - 58.9)	35.9 ± 11.7 (24.3 - 75.1)	36.2 ± 10.7 (22.8 - 70.6)	0.508
p (paired t-test)	0.168	0.325	0.293	0.120	0.083	0.025	
MPCOV:CCOV ratio	1.090 ± 0.264 (0.812 - 1.745)	1.062 ± 0.243 (0.736 - 1.689)	1.087 ± 0.326 (0.692 - 2.004)	1.080 ± 0.217 (0.818 - 1.527)	1.112 ± 0.235 (0.845 - 1.799)	1.135 ±0.221 (0.862 - 1.778)	0.542

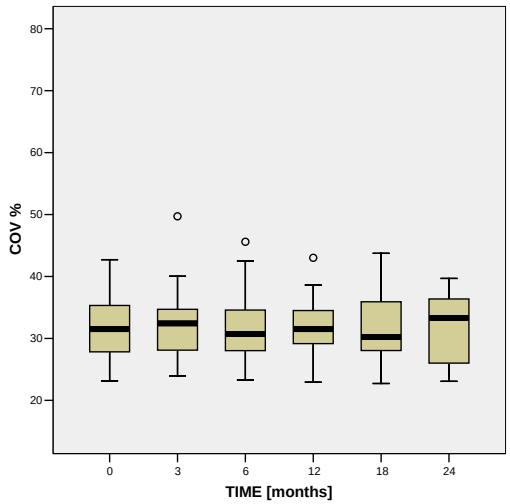


Figure 3.2-22
Box-plots of the degree of endothelial polymegethism in the central cornea (CCOV) over a two-year period in *soft lens wearers* (n = 19).

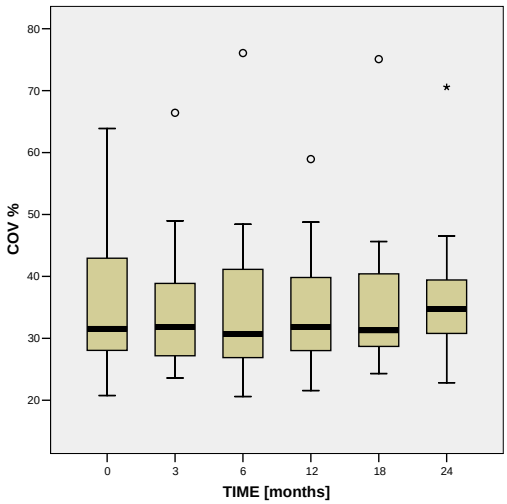


Figure 3.2-23
Box-plots of the degree of endothelial polymegethism in the mid-peripheral cornea (MPCOV) over a two-year period in *soft lens wearers* (n = 19).

Endothelial pleomorphism - %SIX

At baseline, nearly 60% of the endothelial cells, in the central region of the cornea of the soft lens wearers, were 6-sided (C%SIX, Table 3.2-11), although a wide range of values were encountered from 31.2 to 78.9%. While the average C%SIX appeared to vary between visits with up to 4.5% differences between assessments (95% CI = -10.4 to 1.5), no significant differences could be detected (p = 0.370, repeated ANOVA). Figure 3.2-24 illustrates the fluctuations in median values for the C%SIX over the period of two years. In contrast, the MP%SIX averaged 62.5% ± 10.8% at baseline (Table 3.2-11), and there was a slightly narrower range of values observed (from 45.3 to 80.0%). At baseline, the average MP%SIX was however not significantly different from the average C%SIX of 58.2 ± 11.5% (p = 0.200, paired t-test). As illustrated in Figure 3.2-25, the apparent fluctuations in the estimates for the MP%SIX were greater than for the C%SIX of the soft contact lens wearers. Overall, a small decrement in the MP%SIX was noted with time and, at 24 months, the average MP%SIX value was reduced to 54.5 ± 12.0%. This mean difference of -8.0% (95%

CI = -14.9 to -1.2) was statistically significant (p = 0.014, Bonferroni correction). Moreover, MP%SIX was significantly lower than C%SIX at 24 months (p= 0.011, paired t-test).

The overall mean C%SIX was 58.0%, with average values ranging from 56.1 to 60.5% (Table 3.2-13). The overall mean value for the MP%SIX of the same corneas was 58.4%, with average values ranging from 54.5 to 62.5%. No significant time-related trends could be noted, neither for the C%SIX (r = 0.07, p = 0.480) nor for the MP%SIX (r = -0.18, p = 0.057; Table 5.3-1 in the appendix).

Table 3.2-13
Percentage of six-sided cells (%SIX) (mean ± 1SD) of the central (C%SIX) and mid-peripheral (MP%SIX) corneal endothelium in *soft lens wearers* (n = 19) at six occasions over a two-year period. Numbers in brackets represent the minimum and maximum observations. A paired t-test was used to explore any regional differences and repeated ANOVA for time related changes (α = 0.05).

%SIX	Time (months)						p (ANOVA)
	Baseline	3	6	12	18	24	
C%SIX (%)	58.2 ± 11.5 (31.2 - 78.9)	58.6 ± 10.8 (42.9 - 81.4)	56.1 ± 9.3 (40.9 - 72.4)	56.8 ± 9.5 (35.2 - 71.6)	57.8 ± 8.6 (35.8 - 73.0)	60.5 ± 9.0 (43.3 - 80.3)	0.370
MP%SIX (%)	62.5 ± 10.8 (45.3 - 80.0)	58.2 ± 8.9 (40.6 - 71.4)	58.5 ± 11.6 (29.0 - 80.3)	58.8 ± 9.9 (39.6 - 73.0)	58.0 ± 11.2 (33.8 - 75.8)	54.5 ± 11.5 (30.0 - 70.3)	0.015
p (paired t-test)	0.199	0.881	0.465	0.447	0.943	0.011	
MP%SIX:C%SIX ratio	1.118 ± 0.353 (0.762 - 2.371)	1.020 ± 0.233 (0.705 - 1.532)	1.066 ± 0.272 (0.616 - 1.604)	1.056 ± 0.218 (0.778 - 1.533)	1.019 ± 0.229 (0.666 - 1.527)	0.901 ± 0.157 (0.565 - 1.196)	0.025

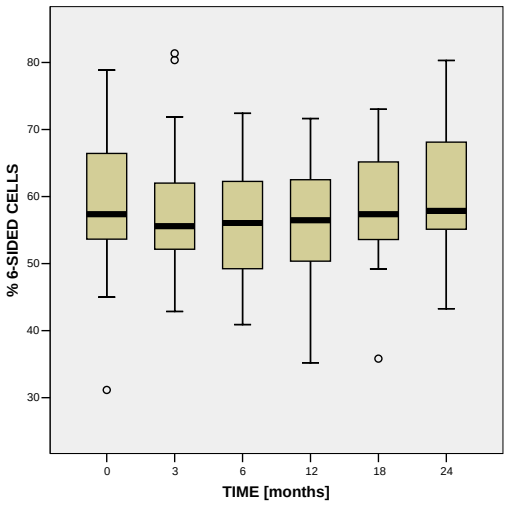


Figure 3.2-24
Box-plots of the degree of endothelial pleomorphism in the central cornea (C%SIX) of *soft lens wearers* (n = 19).

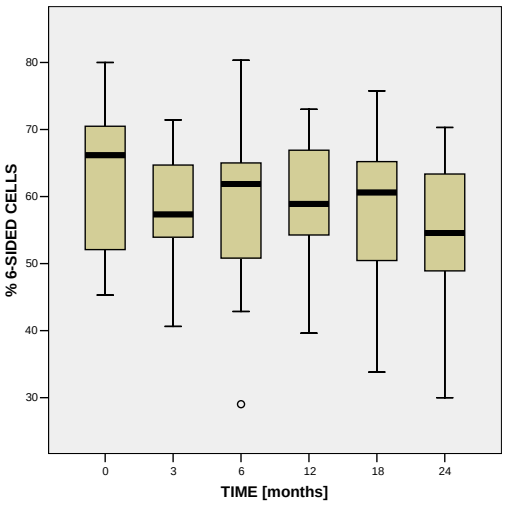


Figure 3.2-25
Box-plots of the degree of endothelial pleomorphism in the mid-peripheral cornea (MP%SIX) of *soft lens wearers* (n = 19).

Endothelial morphometric inter-relationships

In soft lens wearers, 19 subjects completed the study and thus had six measurements of endothelial morphometric parameters. Two subjects had four measurements; hence, the total group size for soft lens wearers was 21 for the correlation analyses.

Cell density (ECD) showed no obvious relationship with the degree of polymegethism (COV), in neither the central nor the mid-peripheral parts of the endothelium. Similarly, ECD showed no relationship to the %SIX. See Table 5.3-2 in the appendix for correlation coefficients and probability values.

On the other hand, the degree of polymegethism (COV) correlated strongly with the degree of pleomorphism (%SIX), both in the central ($r = -0.826$, $p < 0.001$) and mid-peripheral part ($r = -0.759$, $p < 0.001$) of the endothelium. As might be expected, higher COV values were found to be associated with lower %SIX values (see Figure 3.2-26 and Figure 3.2-27). The slope for the regression line for mid-peripheral data in soft lens wearers was apparently less than for the central data (see Figure 3.2-26 and Figure 3.2-27). However, if the outlier with substantial mid-peripheral polymegethism (MPCOV = 68.5%) was left out from the analysis, the slope of the regression line became very similar to the slope of the regression line of the CCOV to C%SIX relationship (graph not shown, however, Figure 3.2-29 and Figure 3.2-30 illustrates the effect).

Morphometric parameters centrally correlated strongly with the respective mid-peripheral morphometric parameters. For example, as shown in Figure 3.2-28, high CECD was associated with high MPECD ($r = 0.639$, $p = 0.002$). Similar strong relationships were also found for the COV and %SIX variables (see Figure 3.2-29 and Figure 3.2-31). Moreover, as is clearly reflected in (Figure 3.2-28), an individual could be expected to have higher MPECD than CECD values. In contrast, the level of polymegethism was similar centrally and mid-peripherally for most individuals (Figure 3.2-29 and Figure 3.2-30). The data for %6-sided cells were more spread and half the group had higher MP%SIX than C%SIX whereas the other half had similar, or slightly higher *central* %SIX (Figure 3.2-31).

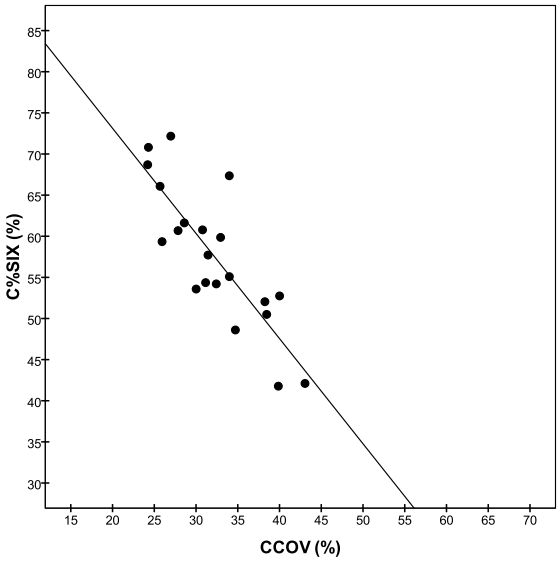


Figure 3.2-26
Scatter plots and linear regression analysis showing the relationship between mean CCOV and C%SIX in *soft lens wearers* (n = 21, p < 0.001, r = -0.826).

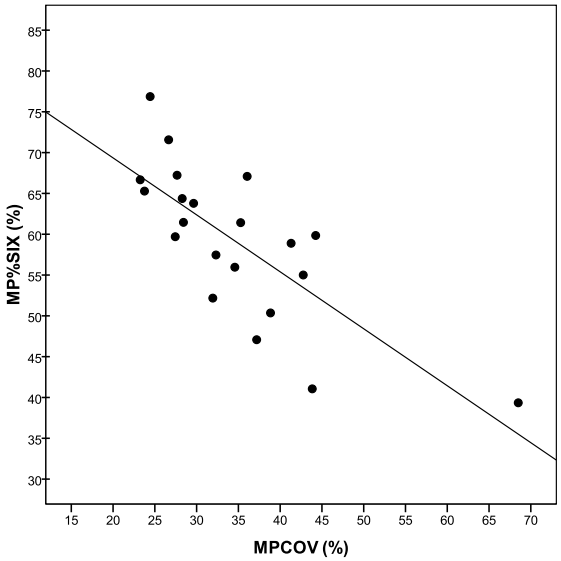


Figure 3.2-27
Scatter plots and linear regression analysis showing the relationship between mean MPCOV and MP%SIX in *soft lens wearers* (n = 21, p < 0.001, r = -0.759).

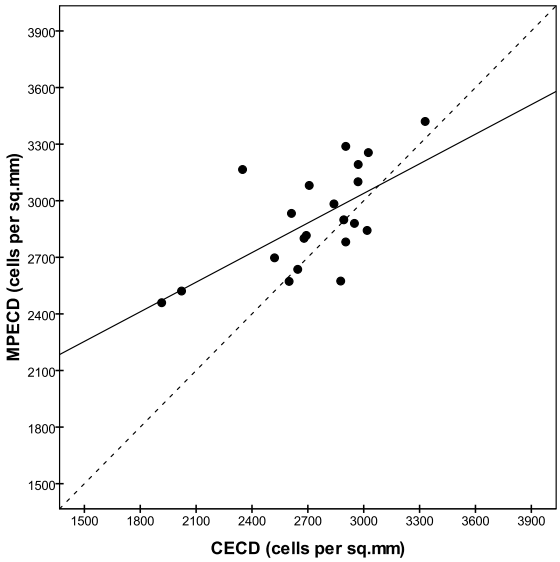


Figure 3.2-28
Scatter plots and linear regression analysis showing the relationship between mean CECD and MPECD in *soft lens wearers* (n = 21, p = 0.002, r = 0.639). The dotted line shows the one:one correlation.

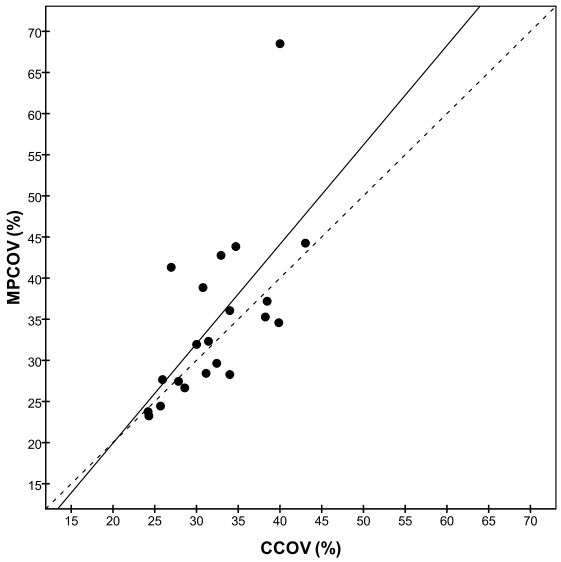


Figure 3.2-29
Scatter plots and linear regression analysis showing the relationship between mean CCOV and MPCOV in *soft lens wearers* (n = 21, p = 0.001, r = 0.648). The dotted line shows the one:one correlation.

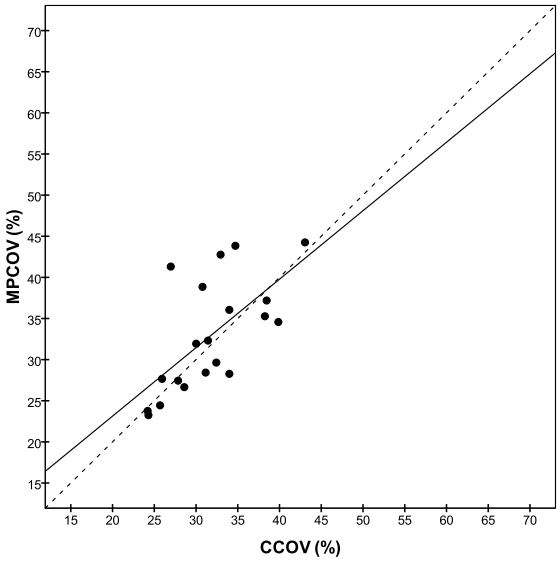


Figure 3.2-30
Scatter plots and linear regression analysis showing the relationship between mean CCOV and MPCOV in *soft lens wearers* (n = 20, p = 0.002, r = 0.648). One subject with MPCOV=69% is left out of the analysis.

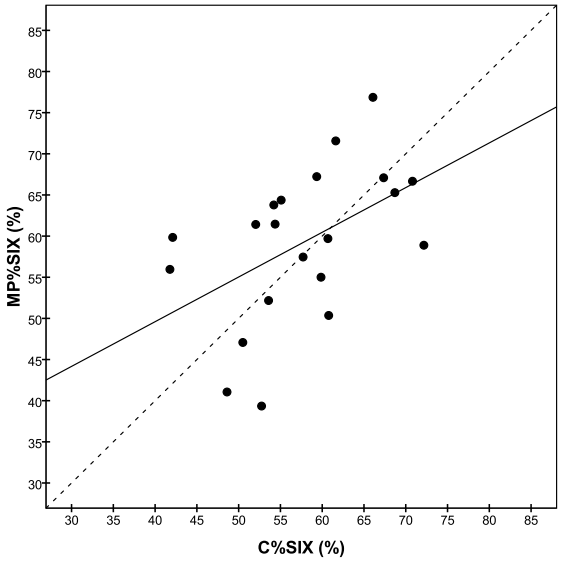


Figure 3.2-31
Scatter plots and linear regression analysis showing the relationship between mean C%SIX and MP%SIX in *soft lens wearers* (n = 21, p = 0.024, r = 0.490). The dotted line shows the one:one correlation.

3.3 RESULTS FOR SiH CONTACT LENS WEARERS

3.3.1 Group demographics and vision assessments

Initially, 28 subjects were interested in participating in this group. They were all well established soft contact lens wearers who wanted to try a new contact lens material allowing for 30 days of continuous wear. However, after receiving further information about the study, two subjects withdrew and instead they joined the soft contact lens wearing group. In total, 26 soft lens wearers were re-fitted with SiH lenses and 19 subjects completed the study. The 26 subjects in the SiH lens group consisted of 10 men and 16 women. During the study, six subjects stopped wearing their SiH lenses for various reasons (Table 3.3-1), and one subject did not attend the 6 months' visit. Thus, the number of subjects completing the study was 19 (Table 3.3-2).

No subject in either group experienced MK (microbial keratitis). However, two adverse events took place. One subject experienced severe blepharitis, and scars from focal sub-epithelial infiltrates in the superior peripheral cornea (CLPUs) were present in one eye at the 18-month visit. She was given the advice of an extensive lid care regime and to return to daily lens wear. Another subject had severe CLPC in one eye at the 18-month visit (Figure 3.3-1). She was advised to temporarily ceasing lens wear and to use Livostin Eyedrops (antihistamines, 0.5mg/ml levocabastine) for one month. She recommenced continuous SiH lens wear after some time of daily disposable lens wear when the signs and symptoms had subsided (Dumbleton, 2002). Both subjects were excluded from further participation.

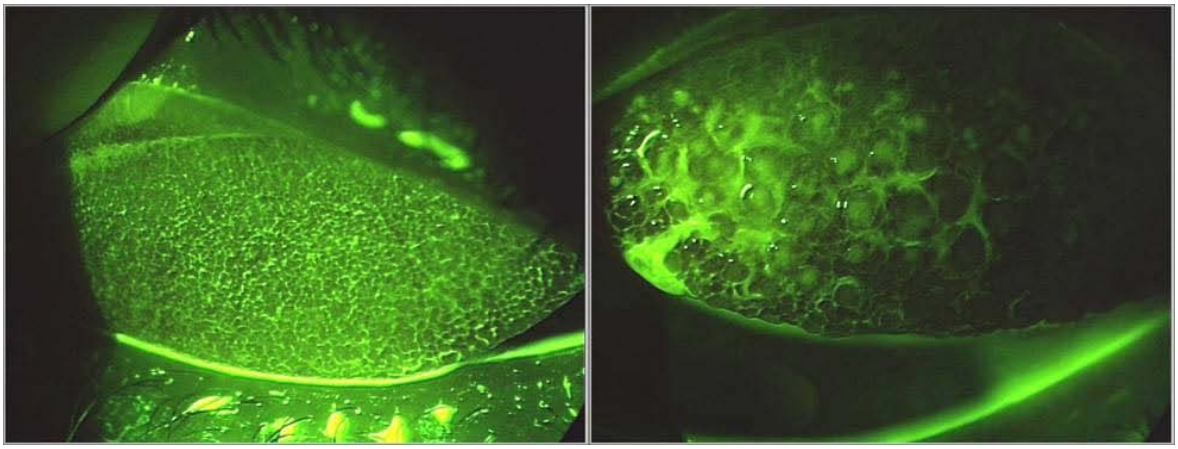


Figure 3.3-1
Severe CLPC observed in one subject's left eye (right picture) after 18 months of SiH lens wear (Photo: Ann E. Ystenæs)

Table 3.3-1
Details of the subjects in the *SiH group* who dropped out of the study

ID#	Time	Reason
404	3 months	Discomfort, lens deposits
415	3 months	Visual acuity unstable
402	6 months	Discomfort
413	6 months	Discomfort, lens deposits (lipids)
403	18 months	Blepharitis, corneal staining and episodes of infiltrates (CLPUs)
418	18 months	Contact lens induced papillary conjunctivitis

Table 3.3-2
Subject details of the *silicone hydrogel* (SiH) lens wearing group at baseline (prior to refitting)

	Initial assignment	Completed study
Number of subjects	26	19
Age at the first visit (years)	26.9 ± 6.0	27.8 ± 6.3
Age range (years)	20 to 41	20 to 41
Gender (F:M)	16:10	10:9
Duration of lens wear at the first visit (years)	7.8 ± 4.0	8.5 ± 4.0
Refractive error (MSE)[a]	-3.69 ± 2.37	-3.89 ± 2.64

[a] Mean refractive error (MSE) in spherical equivalent power (DS). All other data are mean ± S.D.

Over 60% of these subjects initially wore their soft lenses every day, and around 70% wore them more than 10 hours per day (Table 3.3-3). Moreover, these subjects graded their *quality of vision* when wearing lenses as high. On a 100 mm long visual analogue scale the mean score was 86 ± 17 (range 49 to 100). Average eye comfort when wearing lenses was also graded high at 77 ± 16 (32 to 98).

The predominant type of contact lens wear prior to refitting with the SiH lenses was a daily disposable soft lens. Some subjects had worn soft lenses on a monthly replacement schedule and had used a chemical "all-in-one" disinfection solution (Table 3.3-4). One of these nine subjects reported using a special daily cleaner.

Table 3.3-3
Contact lens-wear modality of the subjects in the *SiH lens wear* group at baseline (prior to refitting)

Hours per day	Initial assignment N (%)	Completed study n (%)
5-10	8 (31)	5 (26)
10-15	15 (58)	11 (58)
> 15	3 (12)	3 (16)
Total	26 (100)	19 (100)

Table 3.3-4
Replacement schedule of soft contact lenses for the subjects in the *SiH lens wear* group at baseline.

	Initial assignment N (%)	Completed study n (%)
Planned replacement (6, 9 or 12 m)	0 (0)	0 (0)
Monthly replacement	9 (35)	8 (42)
Daily disposables	17 (65)	11 (58)
Total	26 (100)	19 (100)

Of the 26 subjects initially assigned for the study, 14 had some astigmatism. Mean cylindrical error was only -0.38 ± 0.13 DC, and none wore lenses that corrected for the astigmatism since it did not exceed 0.75D. At baseline, the average refractive error (mean spherical equivalent; MSE) was -3.69 ± 2.37 DS (N=26). This was not significantly different from the MSE of a subgroup of 19 who completed the study whose MSE at baseline was -3.89 ± 2.64 DS (Table 3.3-5). Even though some individuals showed greater time-dependent variations of refraction than other, the MSE remained largely stable throughout the study (p = 0.298, Friedman test) (Figure 3.3-2).

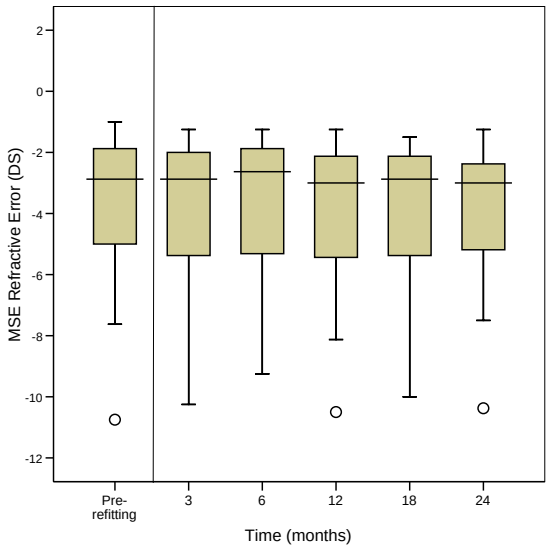


Figure 3.3-2
Box plot to show refractive error (MSE) before and during two years after refitting with *SiH contact lenses* (n = 19).

Table 3.3-5
Refractive error (MSE) and visual acuity measurements: LogMAR Best Corrected High- and Low Contrast Visual Acuity (BCHCVA and BCLCVA, respectively) and Habitual HCVA and LCVA of *SiH lens wearers* (n = 19) over a period of two years. All values are mean ± 1SD with ranges in brackets.

	Time (months)					
	baseline	3	6	12	18	24
Refractive error[a]	-3.89 ± 2.64 (-10.75 to -1.00)	-3.91 ± 2.48 (-10.25 to -1.25)	-3.83 ± 2.36 (-9.25 to -1.25)	-4.03 ± 2.53 (-10.50 to -1.25)	-3.86 ± 2.35 (-10.00 to -1.50)	-3.95 ± 2.38 (-10.38 to -1.25)
BCHCVA	-0.12± 0.07 (-0.20 to 0.00)	-0.11 ± 0.08 (-0.20 to 0.10)	-0.13 ± 0.05 (-0.20 to -0.06)	-0.10 ± 0.21 (-0.20 to 0.74)	-0.15 ± 0.04 (-0.20 to -0.06)	-0.15 ± 0.07 (-0.20 to 0.02)
Habitual HCVA		-0.06 ± 0.08 (-0.20 to 0.14)	0.00 ± 0.18 (-0.18 to 0.68)	-0.08 ± 0.07 (-0.18 to 0.08)	-0.07 ± 0.04 (-0.14 to 0.02)	-0.07 ± 0.08 (-0.18 to 0.08)
BCLCVA	0.06 ± 0.07 (-0.08 to 0.18)	0.06 ± 0.11 (-0.08 to 0.34)	0.09 ± 0.15 (-0.12 to 0.54)	0.03 ± 0.09 (-0.10 to 0.24)	0.05 ± 0.08 (-0.08 to 0.16)	0.02 ± 0.10 (-0.10 to 0.28)
Habitual LCVA		0.16 ± 0.10 (0.04 to 0.36)	0.18 ± 0.14 (-0.02 to 0.50)	0.11 ± 0.08 (-0.10 to 0.26)	0.13 ± 0.06 (0.00 to 0.26)	0.14 ± 0.12 (-0.08 to 0.38)

[a] Mean refractive error (MSE) in spherical equivalent power (DS)

At baseline, the average best-corrected visual acuity (high contrast) was -0.12 ± 0.07 logMAR for the 19 subjects who finished the study (Table 3.3-5). The presence of significant inter-visit difference was revealed by Friedman test (p = 0.030). Wilcoxon signed rank test demonstrated that

the mean difference of 2 letters between average BCHCVA at 24 and 3 months was significant ($p = 0.004$).

Since most subjects did not wear their lenses at the first visit, habitual visual acuity was not measured at baseline for this group. However, habitual HCVA and LCVA was measured at all later occasions (Table 3.3-5). At the 3-month's assessment, average habitual HCVA was -0.06 ± 0.08 logMAR. No inter-visit differences proved to be significant by the Friedman test ($p = 0.059$). However, habitual HCVA measures were consistently poorer than BCHCVA ($p \leq 0.007$, Wilcoxon signed rank test).

Before being refitted with SiH lenses, the 19 soft lens wearers had an average BCLCVA of 0.06 ± 0.07 logMAR. Despite visit-to-visit differences in the order of three letters (between 6 and 12 months), no statistically significant differences between the mean measures of BCLCVA over the 6 occasions were demonstrated ($p = 0.104$, Friedman test, Table 3.3-5).

As with the high-contrast VA, no measures of low-contrast VA were done at baseline for subjects wearing their lenses. At all later visits, habitual LCVA was measured. The group-mean values of LCVA ranged from 0.11 to 0.18 logMAR (Table 3.3-5). However, no significant inter-visit differences were demonstrated by the Friedman test ($p = 0.301$). As expected, VA with lenses in was significantly poorer than with best-corrected spectacles. The average difference between the two VA measures ranged from to 0.07 to 0.11 logMAR units, or 2.5 to 5.5 letters. Low contrast VA with lenses was consistently significantly poorer than BCLCVA ($p \leq 0.004$, Wilcoxon signed rank test) over the 6 occasions.

3.3.2 Ocular comfort

At baseline, all subjects in this group had worn soft contact lenses on a daily wear basis. Prior to the refitting to SiH lenses, 23 of these 26 subjects reported one or more ocular symptoms (Table 3.3-6).

Of the 19 subjects completing all visits, 18 of them reported having at least one symptom at 3, 6 and 12 months, and 17 of them still reported at least one symptom at the 18 and 24-month visits (Figure 3.3-3). At both baseline and at the follow-up assessments, most of the symptomatic SiH contact lens wearers (81 to 100%) reported that they experienced symptoms only 'sometimes' (Table 3.3-6), with only very few subjects (0 to 19%) reporting symptoms 'often'.

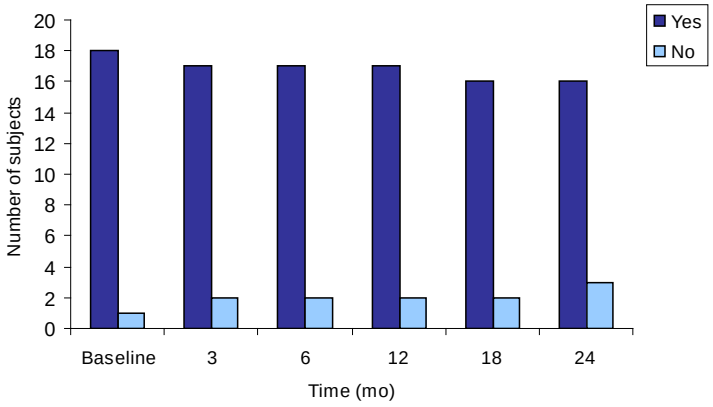


Figure 3.3-3
Frequency reporting one or more ocular symptoms in *SiH contact lens* wearing subjects (n = 19).

Table 3.3-6
Symptom frequency (count) in *SiH lens wearers* (n = 19) over a 2-year period

Symptom frequency (n)	Time (months)					
	Baseline	3	6	12	18	24
Never	1	2	2	2	3	3
Sometimes	16	17	16	17	15	13
Often	2	0	1	0	1	3
Always	0	0	0	0	0	0

The ocular symptom severity in the SiH contact lens wearers as assessed by VAS averaged 24 ± 19 mm (range 2 to 76 mm) for the 18 symptomatic subjects (Table 3.3-7). At later visits, the mean symptom severity grading fluctuated somewhat, ranging from 21 to 31. Again, the ranges were large being from 50 to 74 mm.

Fourteen subjects reported one or more symptom at every visit (eligible sample) and there was little change in the ocular symptom severity reported by these over time, with there usually being less than a difference of four (on a 100-point scale) between visits. However, at the last

assessment (at 24 months), the severity of reported symptoms increased slightly (by 7 points, to an average of 31), but neither this change nor any differences in this group's visit-to-visit mean symptom severity were statistically significant (p = 0.117, Friedman test). Over the 24 months, the mean level of ocular symptoms was 25 (95% CI = 21 – 28).

Table 3.3-7
Symptom severity assessed by VAS in *symptomatic* SiH lens wearers. All values are given as group mean ± SD (minimum to maximum values in brackets).

Symptom severity (mm)	Time (months)					
	Baseline	3	6	12	18	24
Eligible sample (n = 14)*	28 ± 20 (7 to 76)	22 ± 20 (3 to 68)	23 ± 19 (1 to 64)	28 ± 19 (2 to 68)	26 ± 18 (3 to 50)	31 ± 20 (3 to 72)
Main sample	24 ± 19 (2 to 76)	21 ± 19 (2 to 68)	23 ± 18 (1 to 64)	24 ± 19 (2 to 68)	25 ± 18 (0 to 50)	31 ± 20 (3 to 72)
N	18	17	17	17	16	16

*I.e. those subjects who reported symptoms at all visits. These values were used in time-dependent comparisons (Friedman test).

3.3.3 Tear film characteristics

Of those 26 subjects who initially entered the study, 19 were assessed at all occasions. However, for measurements of tear meniscus height (TMH) and fluorescein-tear break-up time (f-TBUT) a few additional data points were missing due to oversight.

The average TMH (mean ± 1 S.D.) at baseline was 0.21 ± 0.04 mm for the soft contact lens wearers who were later refitted into SiH contact lenses for continuous wear and who were assessed at every visit (Table 3.3-8). The group-average TMH remained largely unchanged after the intervention. The visit-to-visit group-averages differed by a maximum of 0.02 mm and no significant differences could be found (p = 0.453, Friedman test). Furthermore, the variability of TMH measurements was low for this group with ranges from 0.10 to 0.25 mm (Figure 3.3-4). Over the duration of the study, the overall TMH for the SiH lens wearers was 0.21 mm (95% CI = 0.21 to 0.24 mm).

Table 3.3-8
Mean ± SD (range) of the tear film tests of the *SiH wearers* that completed the study (n = 19). For missing data, see text.

Tear film test	Time (months)						
	Valid n	Baseline	3	6	12	18	24
TMH (mm)	14	0.21 ± 0.04 (0.15 to 0.30)	0.21 ± 0.03 (0.15 to 0.25)	0.21 ± 0.05 (0.10 to 0.30)	0.23 ± 0.06 (0.15 to 0.40)	0.24 ± 0.05 (0.15 to 0.30)	0.24 ± 0.05 (0.15 to 0.30)
PRT (mm)	19	20.6 ± 7.7 (7.0 to 30.0)	25.3 ± 6.2 (13.0 to 37.0)	24.5 ± 5.3 (10.0 to 31.0)	17.9 ± 4.7 (8.0 to 25.0)	20.1 ± 3.8 (13.0 to 30.0)	16.3 ± 4.9 (7.0 to 22.0)
NIBUT (sec)	19	30.2 ± 22.3 (5.3 to 93.7)	19.7 ± 11.5 (6.0 o 45.0)	28.9 ± 27.3 (7.2 to 117.1)	21.4 ± 33.9 (6.0 to 149.0)	23.5 ± 24.8 (5.6 to 107.7)	18.8 ± 26.9 (5.3 to 127.5)
f-TBUT (sec)	16	11.0 ± 8.2 (4.0 to 37.0)	11.4 ± 8.5 (4.0 to 33.0)	8.4 ± 4.8 (4.0 to 20.0)	6.2 ± 1.8 (4.0 to 9.0)	9.6 ± 7.0 (4.0 to 26.0)	8.0 ± 7.9 (3.0 to 36.0)

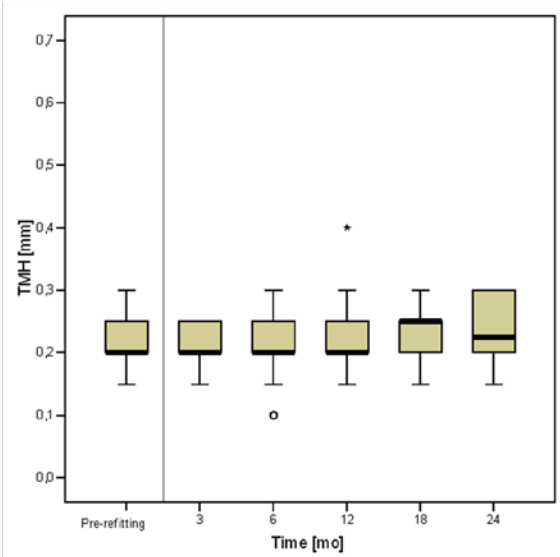


Figure 3.3-4
Box-plots to show time-related changes of tear meniscus height (TMH) in *SiH lens wearers* (n = 14).

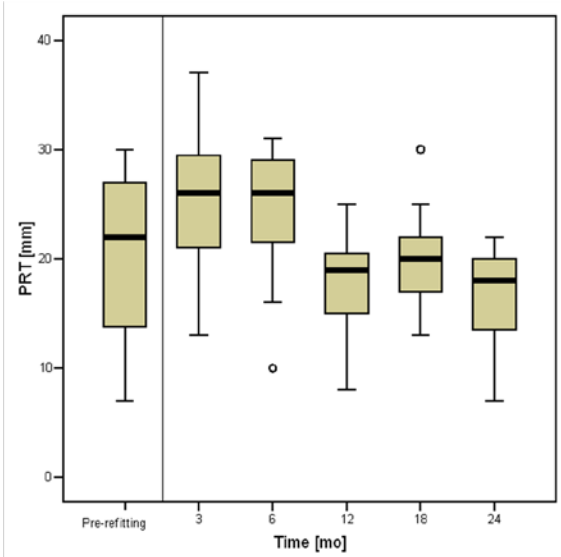


Figure 3.3-5
Box-plots to show time-related changes of phenol red thread (PRT) test in *SiH contact lens wearers* (n = 19)

The tear volume, as estimated by phenol red thread (PRT) test, measured 20.6 ± 7.7 mm, (7 to 30 mm), at baseline for those SiH wearers who finished the study (Table 3.3-8). Statistically significant differences between each assessment were evident by Friedman analysis ($p < 0.001$). After three and six months of SiH-lens wear, the average PRT values had increased with 4.7 and 3.9 mm, respectively ($p=0.019$ and 0.032, WSR-test. Figure 3.3-5) Thereafter, the PRT values seemed to change in a discontinuous way sometime between 6 and 12 months. The group mean PRT values at 12, 18 and 24 months were all significantly lower than at 3 and 6 months ($p \leq 0.002$, WSR-test). However, the net reduction from baseline seen at 12 months was not sustained; the values at 18 months were again as high as 30 mm, with an average of 20.1 mm, which was not significantly different from baseline. At 24 months, the PRT values were also similar to those measured at 12 months.

A linear regression analysis was applied to further examining any time-dependent changes in PRT (Figure 3.3-6). The scatter plot clearly shows evidence of a time related change in PRT since the refitting with SiH lenses. The slope shown, using a linear regression analysis, indicates very significant reduction with time ($p < 0.001$, $r = -0.52$), albeit not a very continuous one. The slope on the regression indicated a progressive reduction in the PRT measures, since refitting into SiH lenses, of -4.9 mm / year. Overall, mean PRT measured 20.8 mm in SiH lens wearers (95%CI = 19.1 to 22.5 mm).

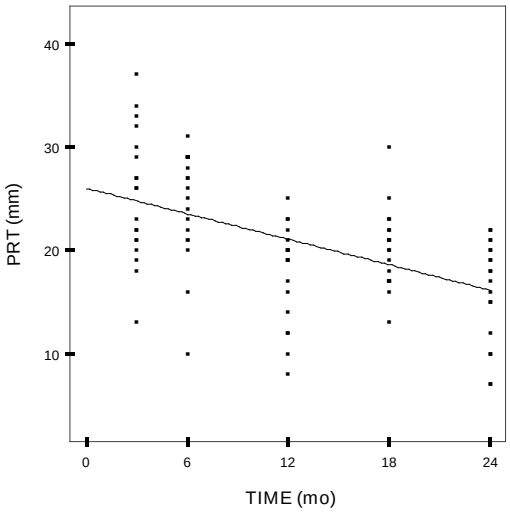


Figure 3.3-6
Scatter-plot to illustrate time-related changes of phenol red thread (PRT) test in *SiH contact lens wearers*. The linear regression line shown indicates a statistically significant effect (slope = -4.92 mm/year, $p < 0.001$, Pearson's $r = -0.52$).

The quality of the pre-ocular tear film as evaluated by non-invasive tear break-up time (NIBUT) was 30.2 ± 22.3 sec at baseline for the 19 SiH lens-wearing subjects who completed this study. As can be seen from Table 3.3-8, the NIBUT assessments yielded a wide range of values from 5.3 to 149 sec. The average values at later visits ranged from 28.9 sec at 6 months to 18.8 sec. at 24 months. The differences between NIBUTs from visit-to-visit were in the order of 5-10 sec (Table 3.3-8). Although baseline group-average NIBUT was significantly higher than the NIBUT

measurements observed at 24 months (Friedman test p = 0.001, Wilcoxon Signed Rank test p = 0.007), no apparent time-dependent changes could be observed (not shown). In summary, similar to NIBUTs in spectacle wearers and soft contact lens wearers, NIBUT measurements showed a high level of variability, both between subjects and between visits (Figure 3.3-7). Overall, NIBUT in SiH wearers was 23.7 sec. (95% CI = 14.6 – 32.9 sec).

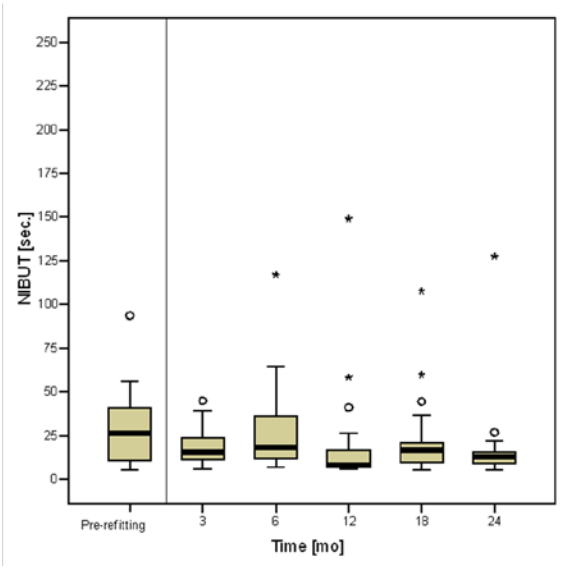


Figure 3.3-7
Box-plots to show time-related changes of non-invasive tear break-up time (NIBUT) in *SiH lens wearers*

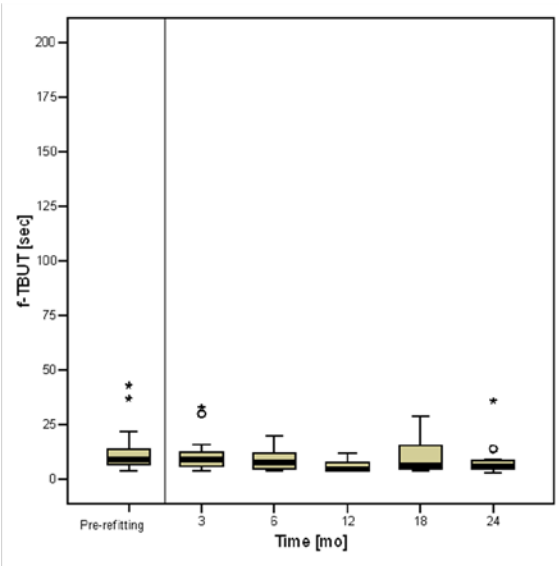


Figure 3.3-8
Box-plots to illustrate time related changes of fluorescein-tear break-up time (f-TBUT) in *SiH lens wearers*.

In contrast to NIBUT, fluorescein-TBUT values were again much lower, averaging 11.1 ± 7.9 sec. at baseline. The f-TBUT also varied less than NIBUT ranging from 3.0 to 37.0 sec (Table 3.3-8). The group-average f-TBUT value at three months had a similar duration as baseline, but thereafter it dropped somewhat (Table 3.3-8). Compared to baseline, f-TBUT tended to be lower at 24 months with group-averages of 8.0 ± 7.9 seconds (p = 0.010, Wilcoxon signed rank test, Friedman test p = 0.013). This apparent tendency of a time-dependent reduction in f-TBUT was also reflected in the median values, which were 9.0 and 10.0 sec. at baseline and 3 months respectively, whereas at the 6 month's visit and later it dropped to 6.0 and finally 5.5 sec. (Figure 3.3-8). However, no real trend could be identified by inspection of a scatter plot and no relationship between time and f-TBUT was discovered by non-parametric correlation analysis (p = 0.084, Spearman's rho = -0.181, Figure 3.3-9). Overall, mean f-TBUT in SiH wearers was 9.1 sec. (95% CI = 7.0 to 11.0 sec).

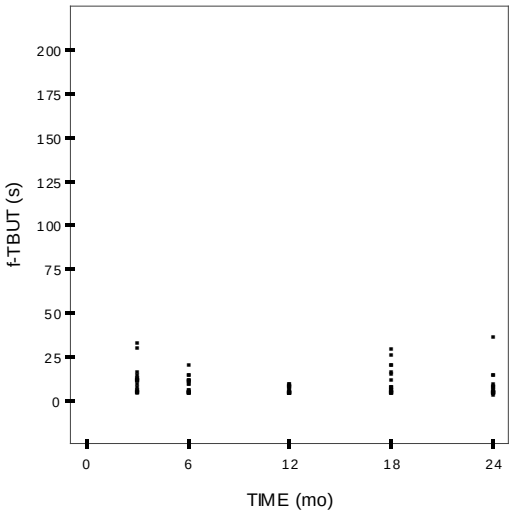


Figure 3.3-9
Scatter plot to illustrate time related changes of fluorescein-tear break-up time (f-TBUT) in *SiH lens wearers*. Non-parametric correlation analysis indicated no statistically detectable relationship (p = 0.084, Spearman's rho = -0.181).

3.3.4 Ocular surface characteristics

Before being refitted with SiH lenses, the eyelids of these soft lens wearers showed only mild or no signs of blepharitis and abnormal Meibomian glands. Mean grades of 0.5 ± 0.5 and 0.4 ± 0.3, respectively, were assigned. After the lens change, the mean grading of the eyelids and Meibomian glands stayed unchanged ($p \geq 0.213$, Friedman test) (Table 3.3-9).

Bulbar hyperaemia in the SiH lens group was slight at baseline, with a mean of 1.0 ± 0.3 (range 0.4 to 1.7). The level of redness declined very slightly over the next two visits to an average of 0.8 ± 0.3 at 6 months; this was significantly lower than at baseline ($p = 0.007$, Wilcoxon Signed Rank test). Thereafter, mean bulbar redness increased again and at 24 months mean bulbar hyperaemia was slightly greater than at baseline ($p = 0.001$, WSR test) although still mild at 1.3 ± 0.3 (range 0.9 to 1.9). Limbal redness followed a similar pattern. At baseline, redness in the limbal region averaged 1.0 ± 0.3 (range 0.5 to 1.5). At later visits, this area became paler, reaching a lower level at 0.7 ± 0.2 after 6 months ($p = 0.0003$, WSR test). Thereafter, limbal redness increased back to baseline levels, reaching a mean of 1.1 ± 0.3 at 24 months (Table 3.3-9).

Corneal vascularisation was graded to a mean of 0.8 ± 0.4 before the lens change. Over the next 4 visits, mean corneal vascularisation declined gradually and the mean grading of 0.5 ± 0.3 at 18 months differed significantly from baseline ($p = 0.002$, WSR test). A small increment was seen at the last visit, and the mean level of 0.6 ± 0.4 at 24 months was not significantly different from baseline ($p = 0.070$).

Ten (53%) of the 19 original soft-lens wearers showed corneal fluorescein staining at baseline (Table 3.3-9) and up to 63% showed staining at later visits (at 6 months). The lowest proportion of subjects showing corneal staining was 47% at 18 and 24 months. The average level of staining (in the subjects who actually had staining) ranged from 0.9 ± 0.5 at baseline to 0.6 ± 0.5 at 3 and 24 months. The mean level of staining for all subjects was only 0.4 ± 0.4 at baseline and even lower at later visits at 0.2 ± 0.3 (3 months) and 0.3 ± 0.5 (18 and 24 months,). These differences were not statistically significant, however ($p = 0.296$, Friedman test).

At baseline, all but 4 subjects (84%) had conjunctival fluorescein staining, but this was less intense than that seen in the soft contact lens groups. After lens change, the frequency of conjunctival staining remained largely unchanged. All but one subject (95%) had some conjunctival staining at 6, 18 and 24 months, but the severity of the staining remained mild. It averaged 0.9 ± 0.7 at baseline and ranged from 0.9 ± 0.6 to 1.1 ± 0.5 at later visits (Table 3.3-9). None of these inter-visit differences were statistically significant ($p = 0.649$, Friedman test).

Papillary conjunctivitis was graded at every visit for 17 of the 19 subjects. The average grading at baseline was mild at 1.1 ± 0.5 (range 0.4 to 1.7, see Table 3.3-9). After lens change, the average

CLPC increased to 1.5 ± 0.3 (p = 0.019, WSR test) and average CLPC at 24 months was significantly different from baseline at 1.6 ± 0.3 (range 1.2 to 2.1) (p = 0.002, WSR test).

Table 3.3-9
Ocular surface characteristics as graded with Efron's grading scale [0 - 4] in *SiH lens wearers* who completed the study (n = 19, except for papillary conjunctivitis where n = 17). The numbers represents group mean ± SD and range (in brackets).

Ocular surface characteristic	Time (months)					
	baseline	3	6	12	18	24
Eyelids	0.5 ± 0.5 (0.0 to 1.2)	0.4 ± 0.3 (0.0 to 1.0)	0.4 ± 0.5 (0.0 to 1.8)	0.4 ± 0.3 (0.0 to 1.4)	0.4 ± 0.4 (0.0 to 1.4)	0.4 ± 0.3 (0.0 to 1.3)
Meibomian glands	0.4 ± 0.3 (0.0 to 1.1)	0.4 ± 0.2 (0.0 to 0.8)	0.6 ± 0.4 (0.0 to 1.3)	0.4 ± 0.3 (0.0 to 1.0)	0.4 ± 0.2 (0.2 to 1.1)	0.4 ± 0.3 (0.0 to 0.9)
Bulbar hyperaemia	1.0 ± 0.4 (0.4 to 1.7)	0.9 ± 0.4 (0.4 to 2.0)	0.8 ± 0.3 (0.4 to 1.5)	0.9 ± 0.3 (0.4 to 1.3)	1.1 ± 0.2 (0.7 to 1.4)	1.3 ± 0.3 (0.9 to 1.9)
Limbal redness	1.0 ± 0.3 (0.5 to 1.5)	0.9 ± 0.3 (0.3 to 1.6)	0.7 ± 0.2 (0.3 to 1.2)	1.0 ± 0.3 (0.5 to 1.5)	1.1 ± 0.3 (0.6 to 1.6)	1.1 ± 0.3 (0.7 to 1.8)
Corneal vascularisation	0.8 ± 0.4 (0.2 to 1.5)	0.7 ± 0.3 (0.3 to 1.5)	0.7 ± 0.4 (0.2 to 1.5)	0.6 ± 0.3 (0.2 to 1.4)	0.5 ± 0.3 (0.2 to 1.3)	0.6 ± 0.4 (0.3 to 1.6)
Corneal staining*	0.4 ± 0.4 (0.0 to 1.0)	0.2 ± 0.3 (0.0 to 0.9)	0.4 ± 0.5 (0.0 to 1.9)	0.4 ± 0.4 (0.0 to 1.4)	0.3 ± 0.5 (0.0 to 1.7)	0.3 ± 0.5 (0.0 to 2.0)
Conjunctival staining	0.9 ± 0.7 (0.0 to 2.5)	0.9 ± 0.6 (0.0 to 2.0)	1.1 ± 0.5 (0.2 to 1.8)	1.0 ± 0.5 (0.0 to 1.6)	0.9 ± 0.6 (0.0 to 1.8)	0.9 ± 0.5 (0.2 to 1.8)
Papillary conjunctivitis	1.1 ± 0.5 (0.4 to 1.7)	1.5 ± 0.3 (0.8 to 2.0)	1.4 ± 0.3 (0.9 to 2.2)	1.3 ± 0.2 (0.8 to 1.8)	1.4 ± 0.3 (0.7 to 2.2)	1.6 ± 0.3 (1.2 to 2.1)

* Corneal staining was graded using CCLRU's grading scales [0-4]. See methods chapter for details.

3.3.5 Corneal thickness and corneal curvature

A similar overall result was obtained for the soft lens wearers refitted with SiH lenses. Their mean central corneal thickness (CT) was 550 ± 38 µm at baseline (n = 18) prior to being refitted with SiH lenses. Similarly, their overall mean value for central CT mean over the six assessments was the same at 548 µm (range 548 to 550 µm,Table 3.3-10).

In the SiH lens wearers, the mean mid-peripheral corneal thickness values were consistently greater than the central CT values. At baseline the mid-peripheral thickness values in these soft lens wearers was somewhat greater than in the other group of SCL wearers either at their initial assessment or after a further 2 years of SCL wear (Table 3.3-10). The average mid-peripheral thickness was 650 ± 54 µm at baseline and with the overall mid-peripheral CT values being 653 µm over the six visits (range 649 to 661 µm).

Table 3.3-10
Mean ± 1SD central and mid-peripheral corneal thickness (CCT and MPCT) and central corneal radius of curvature (K), in *SiH lens wearers* (n = 18). Minimum and maximum values are given in brackets.

	Time (months)					
	Baseline	3	6	12	18	24
CCT (µm)	550 ± 38 (471 to 634)	549 ± 47 (468 to 632)	545 ± 44 (461 to 635)	548 ± 37 (469 to 622)	548 ± 41 (469 to 627)	549 ± 37 (472 to 626)
MPCT (µm)	650 ± 54 (538 to 759)	661 ± 63 (551 to 769)	649 ± 57 (544 to 757)	651 ± 48 (540 to 748)	651 ± 55 (555 to 755)	654 ± 56 (545 to 761)
Central K (mm)	7.83 ± 0.23 (7.39 to 8.31)	7.83 ± 0.20 (7.44 to 8.34)	7.84 ± 0.21 (7.37 to 8.38)	7.82 ± 0.19 (7.43 to 8.33)	7.82 ± 0.20 (7.40 to 8.32)	7.82 ± 0.21 (7.45 to 8.37)

Box plots of the central and mid-peripheral thickness values for the silicone hydrogel contact lens wearers are shown in Figure 3.3-10 and Figure 3.3-11. At the different assessments, the mean thickness values again showed almost no changes with differences from baseline of only 5 µm for central location sets and 11 µm for mid-peripheral locations (Table 3.3-10). The maximum differences in mean values were thus just 0.9% and 1.7%. Neither differences, from baseline nor when comparing two different visits, could be shown to be statistically significant ($p \geq 0.503$, repeated ANOVA). The mean central thickness values were just 1 µm different from baseline at the 24-month assessment, while the mean mid-peripheral thickness values were only 4 µm higher than baseline (654 µm vs. 650 µm).

The box-plots, especially for the mid-periphery, show a larger variance in the data over time as compared to either the spectacle wearers (Figure 3.1-10) or the subjects staying in soft contact lenses (Figure 3.2-10), but with no obvious time-related changes. Linear regression analyses of time-dependent changes in corneal thickness after refitting to SiH lenses (i.e. from 3 to 24 months) revealed no detectable change in CCT values over time (Figure 3.3-12, $p = 0.867$, $r = 0.018$) or the MPCT values (Figure 3.3-13, $p = 0.987$, $r = 0.004$). If the overall mean thickness values over the 6 visits are taken as a reference value (i.e. 547 µm and 653 µm), then the maximum differences

(fluctuations) in thickness values can be calculated to be just ± 0.6% (range – 0.7 to 0.4%) and ± 0.9% (-0.6 to□1.3%) respectively for central and mid-peripheral sites.

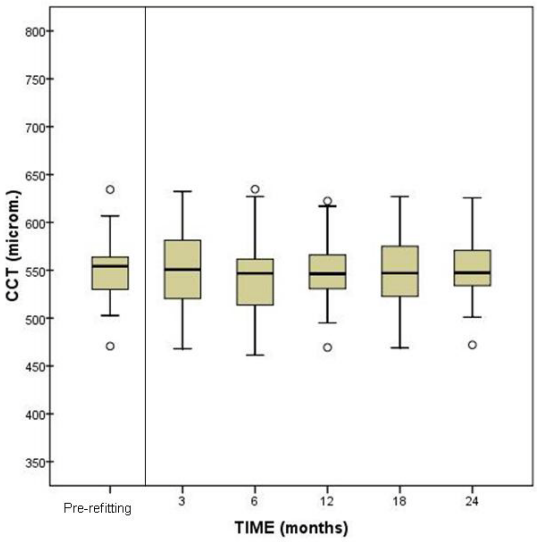


Figure 3.3-10
Box plot to show central corneal thickness over time in *SiH lens wearers* (n= 18).

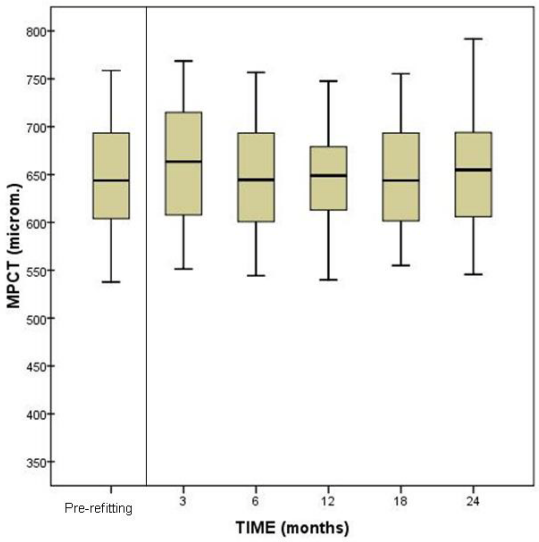


Figure 3.3-11
Box plot to show mid-peripheral corneal thickness over time in *SiH lens wearers* (n = 18).

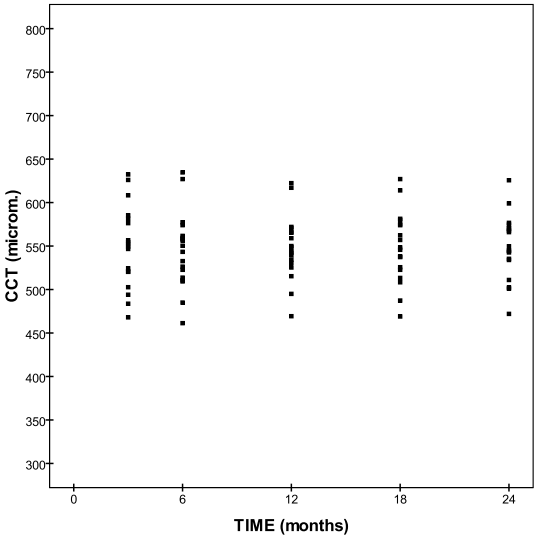


Figure 3.3-12
Regression analysis to show time-related changes of central corneal thickness (µm) in *SiH lens wearers* (n = 18). The linear regression line indicates no statistically significant effect (p = 0.867, r = 0.018).

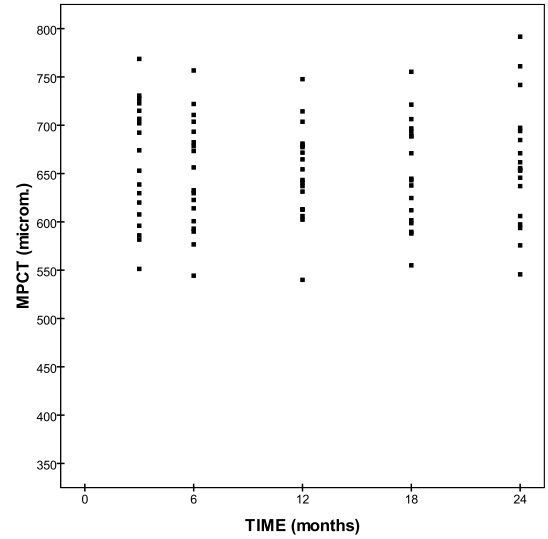


Figure 3.3-13
Regression analysis to show time-related changes of mid-peripheral corneal thickness (µm) in *SiH lens wearers* (n = 18). The linear regression line indicates no statistically significant effect (p = 0.987, r = 0.004).

Before being refitted with SiH lenses, the mean central K-value in this group of soft lens wearers was 7.83 ± 0.23 mm (Table 3.3-10). Essentially, the mean central K-values remained stable and the box plot in Figure 3.3-14 shows remarkable consistency to most of the K readings. The largest difference between two visits was very small indeed at 0.03 mm, which was just statistically significant (p = 0.036, Friedman test, p = 0.010, Wilcoxon signed rank test). This change cannot be regarded as clinically significant.

A correlation analysis (Figure 3.3-15) shows that there were no relationship between the anterior corneal curvature and time (p = 0.807, Spearman's rho = -0.023) when soft lens wearers were refitted with SiH lenses. Overall mean central radius of curvature was 7.83 mm (95% CI = 7.73 – 7.92) in SiH lens wearers.

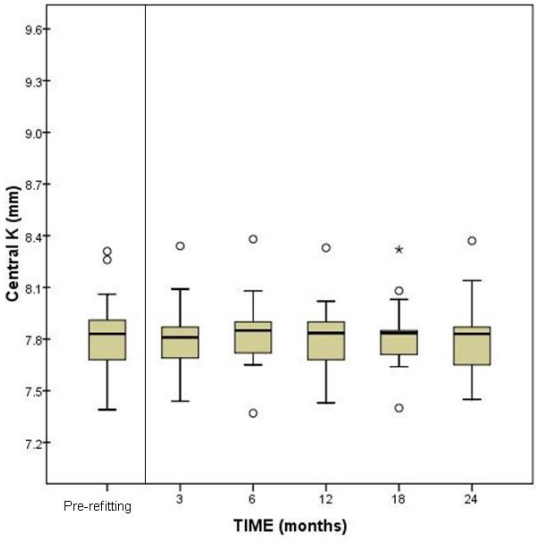


Figure 3.3-14
Box plot to show central corneal radius of curvature values over time (mm) in *SiH lens wearers* (n = 19).

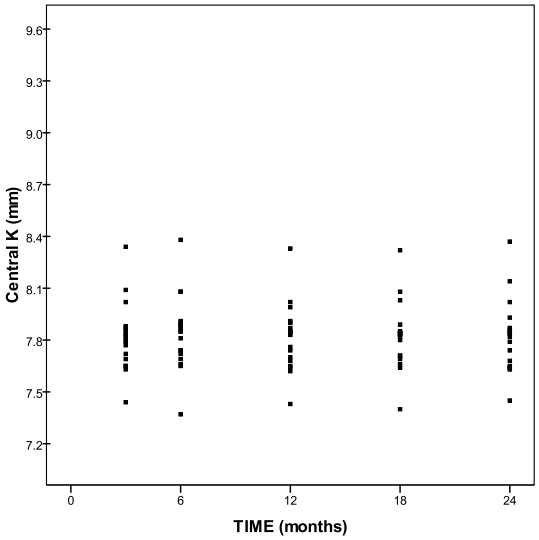


Figure 3.3-15
Scatter plot to illustrate time-related changes in central corneal radius of curvature values (mm) over time in *SiH lens wearers* (n = 19). Non-parametric correlation analysis indicated no statistically significant effect (p = 0.807, Spearman's rho = -0.023).

3.3.6 Corneal endothelial cell morphometry

Endothelial cell density – ECD

Endothelial data from all visits was only available for 18 of the 19 subjects due to poor quality of the endothelial photographs for one subject. The CECD before refitting averaged 2781 ± 252 cells / mm^2 (Table 3.3-11). After refitting, the CECD decreased slightly over the two-year period (Table 3.3-11). At 24 months, the CECD was 1.8% lower than at baseline (averaging 2738 ± 266 cells / mm^2), however, neither this or any other differences between visits were significant ($p = 0.197$, repeated ANOVA). The box plot (Figure 3.3-16) indicates a rather remarkable consistency to the CECD values over time, with neither the median values nor the ± 25% inter-quartile intervals showing much obvious change.

The MPECD averaged 3059 ± 311 cells / mm^2 before refitting (Table 3.3-11). This average MPECD value was nearly 10% higher than the CECD (95% CI = 5.0 to 10.0%), and the difference was highly significant ($p = 0.001$, paired t-test). Overall, the MPECD values appeared to decrease, with a change of -2.9% within the first 3 months, and after 2 years of SiH lens wear, the MPECD was 4.8% lower than at baseline (95% CI = 0.3 to 9.5%, $p = 0.032$, Bonferroni correction) (Figure 3.3-17). With the reduction in the MPECD values with time, the difference between MPECD and CECD became smaller (being just 6.6%, instead of 10.3%) at 24 months. However, the difference was still statistically significant ($p = 0.001$, paired t-test).

Table 3.3-11
Cell density (mean ± 1SD) of the central (CECD) and mid-peripheral (MPECD) corneal endothelium before and at five occasions (over a two-year period) after *refitting into SiH lenses* (n = 18). Numbers in brackets represent the minimum and maximum observations. A paired t-test was used to explore any regional differences and repeated ANOVA for time related changes ($\alpha = 0.05$).

ECD	Time (months)						p (ANOVA)
	Pre-refitting	3	6	12	18	24	
CECD (cells/mm^2)	2781 ± 252 (2262 - 3193)	2794 ± 248 (2259 - 3174)	2761 ± 247 (2308 - 3125)	2769 ± 296 (2193 - 3177)	2747 ± 286 (2200 - 3142)	2738 ± 266 (2143 - 3091)	0.197
MPECD (cells/mm^2)	3059 ± 311 (2631 - 3683)	2970 ± 333 (2536 - 3742)	2989 ± 345 (2400 - 3655)	2993 ± 282 (2528 - 3479)	2936 ± 276 (2544 - 3398)	2911 ± 257 (2403 - 3430)	0.002
p (paired t-test)	0.001	0.005	0.003	0.001	0.004	0.001	
MPECD:CECD ratio	1.103 ± 0.100 (0.899 - 1.339)	1.064 ± 0.080 (0.884 - 1.179)	1.084 ± 0.099 (0.904 - 1.250)	1.086 ± 0.087 (0.917 - 1.209)	1.074 ± 0.089 (0.883 - 1.199)	1.066 ± 0.070 (0.910 - 1.175)	0.103

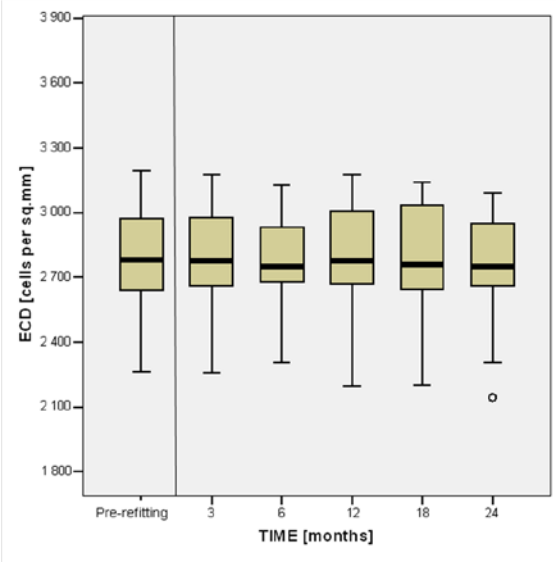


Figure 3.3-16
Box plots of the endothelial cell density in the central region of the corneas (CECD) before and at five occasions over 24 months after refitting with *SiH lenses* (n = 18).

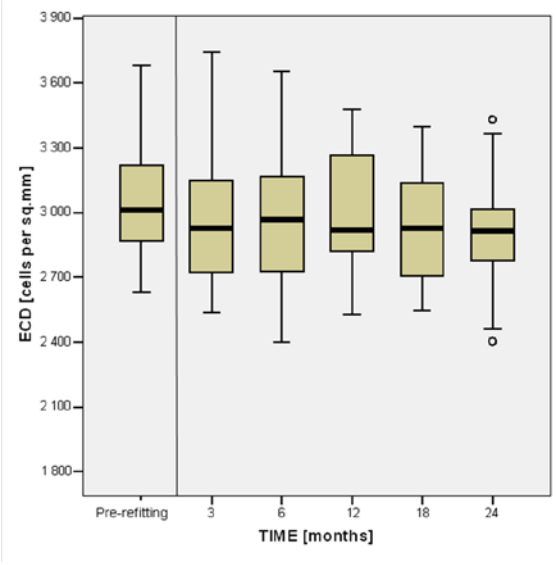


Figure 3.3-17
Box plots of the endothelial cell density in the mid-peripheral region of the corneas (MPECD) before and at five occasions over 24 months after refitting with *SiH lenses* (n = 18).

The overall mean CECD value of the SiH lens wearers, over the 6 visits, was 2765 cells / mm^2, with average values at each visit ranging from 2738 to 2794 cells / mm^2 (Table 3.3-11). The overall mean MPECD value was 2976 cells / mm^2, (with average values at each visit from 2911 to 3059 cells / mm^2). As shown in Figure 3.3-18 and Figure 3.3-19 and summarized in Table 5.4-1 (in the appendix), a statistically significant relationship between time and ECD could not be demonstrated for either location, at least not by linear regression analysis. This lack of statistical significance is despite the fact that the best-calculated slope for the central region was -24 cells/ mm^2/ year and was even higher at -57 cells/mm^2 / year for the mid-peripheral region of these endothelia. The slopes were however not significant ($p = 0.500$ for central sites, $r = -0.07$; $p = 0.161$ for mid-peripheral sites, $r = -0.136$).

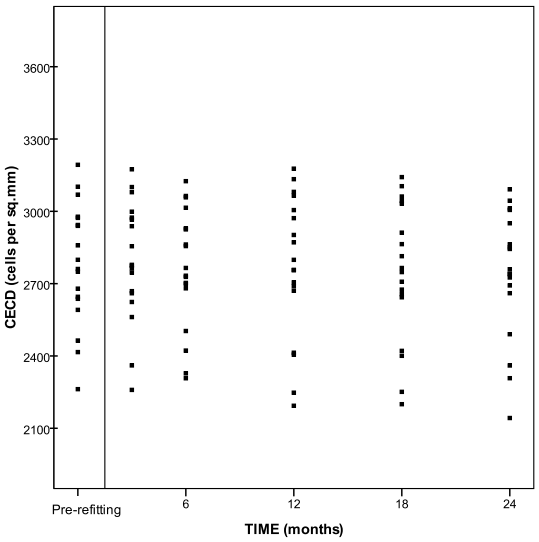


Figure 3.3-18
Linear regression analysis of the central cell density (CECD) and time before and at five occasions over 24 months after refitting with *SiH lenses* (n = 18, p= 0.500, r = -0.066).

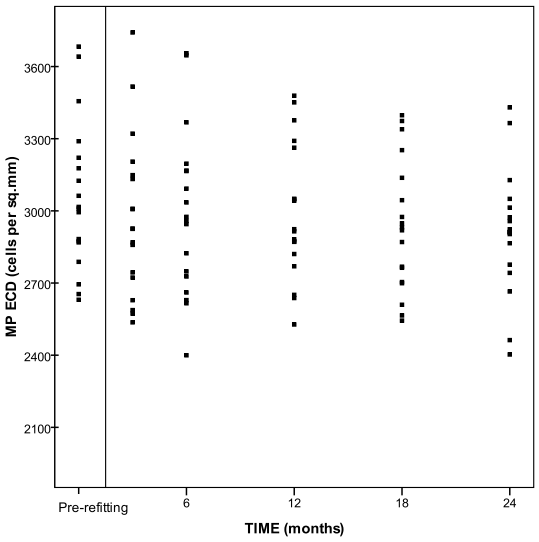


Figure 3.3-19
Linear regression analysis of the mid-peripheral cell density (MPECD) and time before and at five occasions over 24 months after refitting with *SiH lenses* (n = 18, p = 0.161, r = -0.136).

As already described, before refitting the net difference between the MPECD and CECD was quite substantial. Before refitting, the average MPECD:CECD ratio was 1.096 ± 0.095 with the data being positively skewed to values to as high as 1.339 (n = 22, Figure 3.3-20). A slight negative shift was noted over five subsequent occasions during the SiH lens wear (Figure 3.3-21). However, analysis for any predictable time-related change in the MPECD:CECD ratio revealed no detectable change (p = 0.490, r = -0.062, Figure 3.3-22).

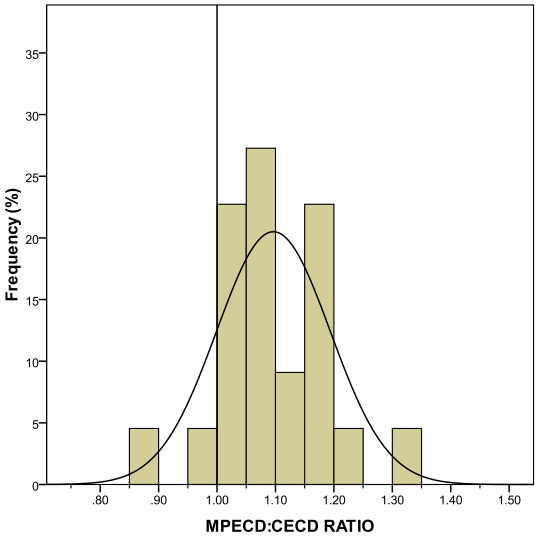


Figure 3.3-20
Histogram showing the frequencies of individual endothelial MPECD:CECD ratios at a single occasion (baseline) before refitting into *SiH lenses*. The reference line shows the level of no difference between MPECD and CECD (ratio = 1). N = 22.

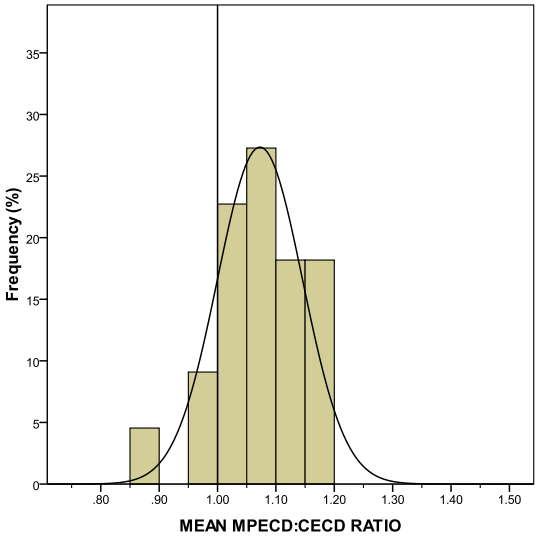


Figure 3.3-21
Histogram showing the overall frequencies of individual mean endothelial MPECD:CECD ratios in a group of *SiH lens wearers* (N = 22) from five occasions over a period of two years. The reference line shows the level of no difference between MPECD and CECD (ratio = 1).

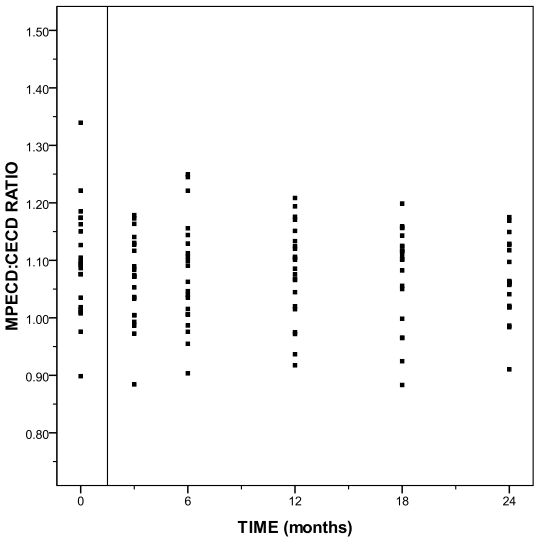


Figure 3.3-22
Regression analysis of the endothelial MPECD:CECD ratio in *SiH lens wearers* over a period of two years (p = 0.490, r = 0.062). The reference line shows the intervention of refitting from soft lenses into SiH lenses.

Endothelial polymegethism -COV

At baseline, the average degree of polymegethism of the central corneal endothelium (CCOV) in those subjects later being refitted with SiH lenses, averaged 35.0 ± 6.8%, ranging from 22.9% to 50.1% (Table 3.3-11). Within 3 months, the average CCOV value was reduced to 29.6%, and this net change of 5.5% (95% CI = 1.3 to 9.5%) was highly statistically significant (p = 0.005, Bonferroni correction.) The range of CCOV values also decreased substantially and halved within 3 months, i.e. from 27.2% to 13.8% (Figure 3.3-23), although the ± 25% inter-quartile intervals changed little. Thereafter, only minor fluctuations in the mean CCOV could be observed. After 2 years of SiH lens wear, the mean CCOV was 28.9%, which was not significantly lower than what was observed at 3 months (p = 1.000, Bonferroni correction), but was still very different from that observed at baseline (p = 0.002, Bonferroni correction).

Table 3.3-12
Polymegethism (mean ± 1SD of the coefficient of variation in cell area; COV) of the central (CCOV) and mid-peripheral (MPCOV) corneal endothelium before and at five occasions (over a two-year period) after *refitting into SiH lenses* (n = 18). Numbers in brackets represent the minimum and maximum observations. A paired t-test was used to explore any regional differences and repeated ANOVA for time related changes (α = 0.05).

COV	Time (months)						p (ANOVA)
	Pre-refitting	3	6	12	18	24	
CCOV (%)	35.0 ± 6.8 (22.9 - 50.1)	29.6 ± 3.9 (21.8 - 35.6)	31.1 ± 4.4 (21.6 - 37.4)	29.0 ± 4.4 (21.2 - 36.8)	29.0 ± 4.6 (18.6 - 34.8)	28.9 ± 4.7 (20.0 - 37.3)	<0.001
MPCOV (%)	33.5 ± 7.5 (18.3 - 45.3)	31.8 ± 6.5 (19.4 - 45.3)	32.2 ± 6.6 (23.3 - 51.5)	29.7 ± 5.1 (21.6 - 44.4)	28.9 ± 4.7 (20.6 - 41.9)	30.1 ± 5.4 (21.9 - 41.8)	0.005
p (paired t-test)	0.364	0.084	0.481	0.516	0.917	0.314	
MPCOV:CCOV ratio	0.969 ± 0.207 (0.752 - 1.365)	1.076 ± 0.176 (0.762 - 1.539)	1.043 ± 0.192 (0.704 - 1.431)	1.036 ± 0.163 (0.798 - 1.366)	1.009 ± 0.163 (0.792 - 1.323)	1.049 ± 0.155 (0.754 - 1.356)	0.314

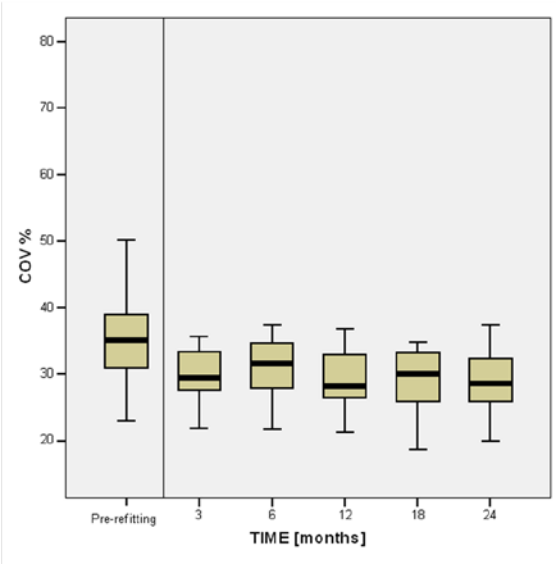


Figure 3.3-23
Box-plots of the degree of endothelial polymegethism in the central cornea (CCOV) before and at five occasions over 24 months after refitting with *SiH lenses* (n = 18).

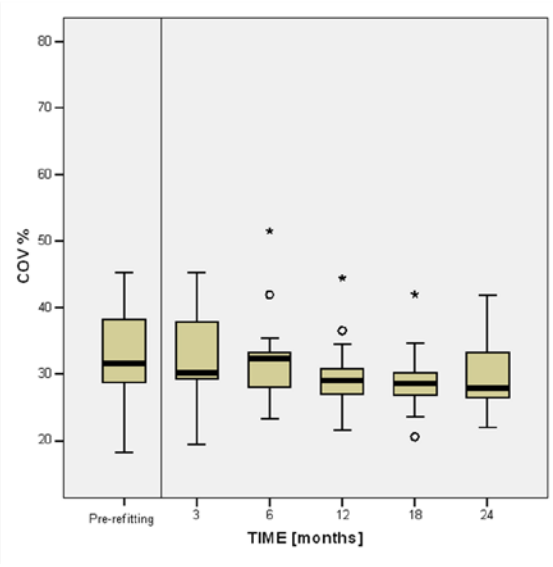


Figure 3.3-24
Box-plots of the degree of endothelial polymegethism in the mid-peripheral cornea (MPCOV) before and at five occasions over 24 months after refitting with *SiH lenses* (n = 18).

The degree of polymegethism in the mid-peripheral parts of the corneal endothelium (MPCOV) averaged 33.5% at baseline (Table 3.3-11), as compared to 35.0% in the central region. The difference in COV between central and mid-peripheral locations was not significant (p = 0.360, paired t-test). Similar to CCOV, the mean MPCOV was lower after 3 months of SiH lens wear, but the reduction was less (by just 1.7% to a new average of 31.8%). Some further reduction occurred and the MPCOV averaged 30.1% after 2 years. Repeated ANOVA analyses indicated statistically significant differences between visits (p = 0.005). However, Bonferroni post hoc test revealed no statistically significant changes or differences between assessments, although a nearly significant difference was seen between the MPCOV at baseline and the MPCOV at 18 months (p = 0.057). The box plot (Figure 3.3-24) shows that this trend for a reduction in MPCOV with time in those wearing SiH lenses was not seen in all subjects. In contrast to the CCOV values, the ranges of MPCOV of did not differ much from visit to visit (Table 3.3-11). However, as seen in Figure 3.3-24, the inter-quartile ranges became narrower (at least at the 6, 12 and 18 months' examinations), so the similarities of the ranges, as given in Table 3.3-11, were a result of outlying and extreme values.

While the ECD values could not be shown to change significantly with time in the SiH lens wearers, changes in the polymegethism (COV) were large enough for some possible time-related trends to be demonstrated. Overall, the mean CCOV over the six visits was 30.4%, the average values at each visit ranging from 29.0 to 35.0%. The overall mean MPCOV was calculated to be 31.0% (with average values ranging from 29.7 to 33.5%), which was *not* different from overall mean CCOV values (p = 0.27). A linear regression analysis indicated a net change with time in CCOV values of -2.2% / year (p = 0.002). The variance in the slope estimate, of ± 2.1%, was still rather substantial, so the correlation coefficient was not strong (r = -0.296, Table 5.4-1 in the appendix). For the mid-peripheral corneal endothelial regions, the net change calculated was slightly less

(at -1.9% / year), but the variance was much greater (at ± 3.1% / year). However, it should be noted that if the baseline values were left out from the analysis, no time dependent changes could be demonstrated, either for the central (r = -0,122, p = 0.254) or for the mid-peripheral (r = -0.167, p = 0.116) corneal endothelium. This again emphasises that the most substantial changes occur during a period shortly after refitting with SiH lenses.

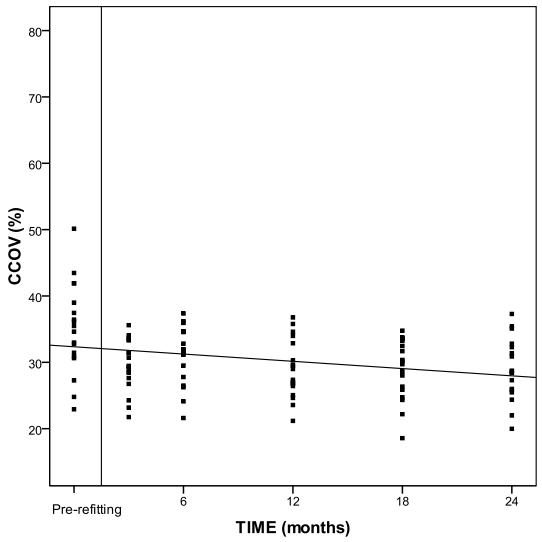


Figure 3.3-25
Scatter plots to show changes in the degree of endothelial polymegethism (COV) in the central cornea of *SiH lens wearers* (n = 18). The result of a linear regression is shown (p = 0.002, r = 0.296).

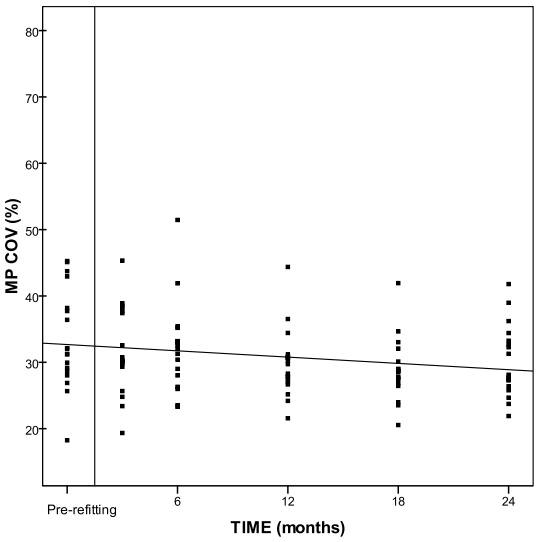


Figure 3.3-26
Scatter plots to show changes in the degree of endothelial polymegethism (COV) in the mid-peripheral cornea of *SiH lens wearers* (n = 18). The result of a linear regression is shown (p = 0.023, r = 0.219)..

Endothelial pleomorphism - %SIX

The average C%SIX in these soft lens wearers before refitting was 56.8 ± 9.5% (Figure 3.3-13), i.e. marginally lower than in the other group of soft contact lens wearers (where the average baseline value was 58.2%; Table 3.2-11). As can be seen from Figure 3.3-27, the C%SIX cells values showed no obvious changes with time after the refitting with SiH lenses. The largest difference between two visits was 2.7% (95% CI = -4.7 to 10.1%) but this magnitude of change was not statistically significant (p = 862, repeated ANOVA).

Table 3.3-13
Percentage of six-sided cells (%SIX) (mean ± 1SD) of the central (C%SIX) and mid-peripheral (MP%SIX) corneal endothelium in soft lens wearers before and at five occasions (over a two-year period) after *refitting into SiH lenses* (n = 18). Numbers in brackets represent the minimum and maximum observations. A paired t-test was used to explore any regional differences and repeated ANOVA for time related changes (α = 0.05).

%SIX	Time (months)						p (ANOVA)
	Pre-refitting	3	6	12	18	24	
C%SIX (%)	56.8 ± 9.5 (41.5 - 74.1)	58.8 ± 8.9 (46.8 - 83.1)	56.1 ± 7.5 (40.3 - 68.8)	56.8 ± 8.8 (45.2 - 78.5)	57.6 ± 8.4 (46.8 - 75.4)	57.9 ± 9.1 (43.3 - 72.1)	0.862
MP%SIX (%)	60.8 ± 12.2 (36.8 - 76.2)	60.7 ± 9.0 (46.8 - 79.0)	57.9 ± 10.5 (33.8 - 73.8)	59.4 ± 9.0 (41.9 - 74.6)	59.7 ± 8.9 (44.3 - 79.0)	59.8 ± 6.9 (49.2 - 72.1)	0.852
p (paired t-test)	0.275	0.382	0.460	0.340	0.368	0.399	
MP%SIX:C%SIX ratio	1.100 ± 0.288 (0.600 - 1.796)	1.043 ± 0.152 (0.800 - 1.291)	1.041 ± 0.185 (0.585 - 1.300)	1.062 ±0.188 (0.723 - 1.328)	1.046 ± 0.160 (0.761 - 1.367)	1.049 ± 0.162 (0.744 - 1.291)	0.895

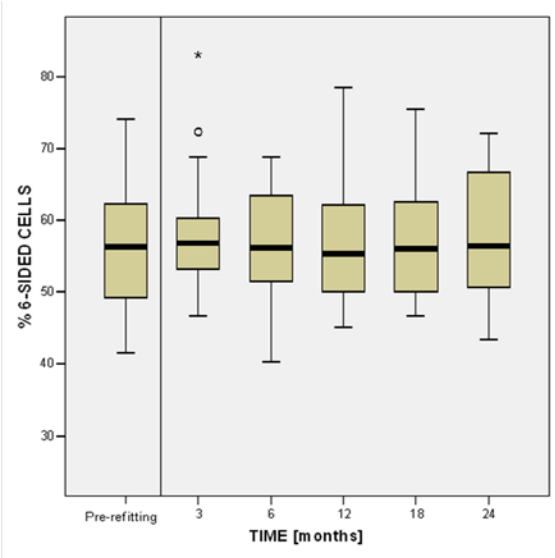


Figure 3.3-27
Box-plots of the degree of endothelial pleomorphism in the central cornea (C%SIX) before and at five occasions over 24 months after refitting with *SiH lenses* (n = 18).

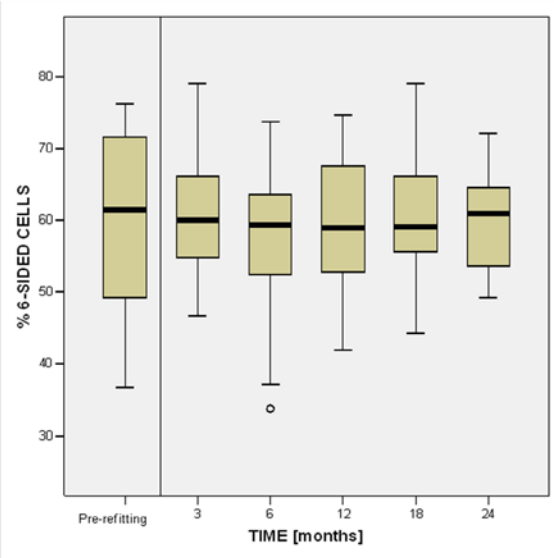


Figure 3.3-28
Box-plots of the degree of endothelial pleomorphism in the mid-peripheral cornea (MP%SIX) before and at five occasions over 24 months after refitting with *SiH lenses* (n = 18).

The MP%SIX averaged 60.8 ± 12.2 at baseline (Table 3.3-11). This was not significantly different from the C%SIX value of 56.8% (p = 0.280, paired t-test). As also observed for the central endothelium, the MP%SIX did not change much over the period of the study (Figure 3.3-28).

Compared to baseline values, the average values for the MP%SIX decreased by only 1% (95% CI = -8.1 to 10.3%) after 2 years (p = 0.852, repeated ANOVA)).

The overall mean value for C%SIX over the 6 visits, was 57.3%, with average values at each visit ranging from 56.1 to 58.8%. The overall mean value for the MP%SIX in these SiH wearers was 59.7%, with average values at each visit ranging from 57.9 to 60.8%. Overall, this mid-peripheral data was slightly, yet significantly, higher than that noted for the central part of the corneal endothelium (p = 0.023). As can be seen from Table 5.4-1, no time-dependent change was found for the %SIX in either location. The net change in the C%SIX was 0.3% / year (r = 0.023) and -0.3% / year in the mid-peripheral region (r = -0.023). Neither of the calculated changes were statistically significant (p = 0.810, see also Table 5.4-1 in the appendix)

Endothelial morphometric inter-relationships

Out of the soft lens wearers who were refitted with SiH lenses, 18 subjects completed the study and thus had five measurements of endothelial morphometric parameters after the initial examination. Three subjects had four measurements and one had three measurements; hence, the total group size for SiH lens wearers was 22 for the correlation analyses.

At baseline, before refitting into SiH lenses, endothelial cell density showed no obvious relationship with the degree of polymegethism, in neither the central nor the mid-peripheral parts of the endothelium. Similarly, cell density showed no relationship to the percentage of 6-sided cells. See Table 5.4-2 in the appendix for correlation coefficients and probability values. Refitting into SiH lenses did not change these results (See Table 5.4-3 in the appendix).

On the other hand, the degree of polymegethism correlated strongly with the degree of pleomorphism, both in the central (r = -0.586 , p = 0.004) and mid-peripheral part (r = -0.783, p < 0.001) of the endothelium before refitting into SiH lenses. Higher COV values were found to be associated with lower %SIX values (see Figure 3.3-29 and Figure 3.3-30). After refitting into SiH lenses, the slope of the regression line that represented the CCOV vs. C%SIX steepened slightly, reflecting a central reduction in the degree of polymegethism (COV) and pleomorphism (i.e. *increase* of %SIX), however, the relationship remained strong (see Figure 3.3-29, solid line, r = 0.753, p < 0.001). In the mid-peripheral area the MPCOV vs. MP%SIX relationship remained largely unchanged after refitting into SiH lenses (Figure 3.3-30, r = 0.745, p < 0.001).

Strong, positive correlations were found between central and mid-peripheral morphometric parameters in this group of soft lens wearers before refitting into SiH lenses. For example, as shown in Figure 3.3-31, high CECD was associated with high MPECD (r = 0.634, p = 0.002). An individual in this group of soft lens wearers was more likely to have a higher MPECD than CECD before being refitted with SiH lenses (see Figure 3.3-31). Although the regression line moved slightly in the direction of a more evenly spread distribution, most individuals still had higher MPECD values after refitting into SiH lenses.

Similar to ECD, strong relationships were also found for the CCOV vs. MPCOV variables (Figure 3.3-32): the more polymegethism centrally, the more mid-peripherally. While more subjects had higher COV values in their central endothelium than mid-peripherally before refitting, an individual was more likely to have a close to even distribution of the degree of polymegethism after refitting into SiH lenses (Figure 3.3-32). The data were also less spread after refit.

In contrast to ECD and COV, the C%SIX only correlated significantly with the MP%SIX after refitting into SiH lenses (see Figure 3.3-33). Prior to refitting, most individuals had higher MP%SIX than C%SIX values, but the data were rather spread. After refitting, the data were less spread and more subjects had similar MP%SIX and C%SIX.

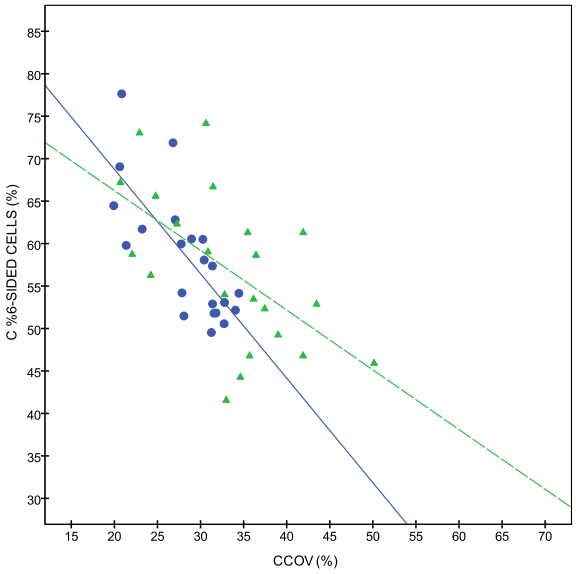


Figure 3.3-29
Scatter plots and linear regression analysis showing the relationship between mean CCOV and C%SIX before (triangles, dashed regression line, p = 0.004, r = -0.586) and after refitting with *SiH lenses* (closed circles, solid line , p < 0.001, r = -0.753). N = 22.

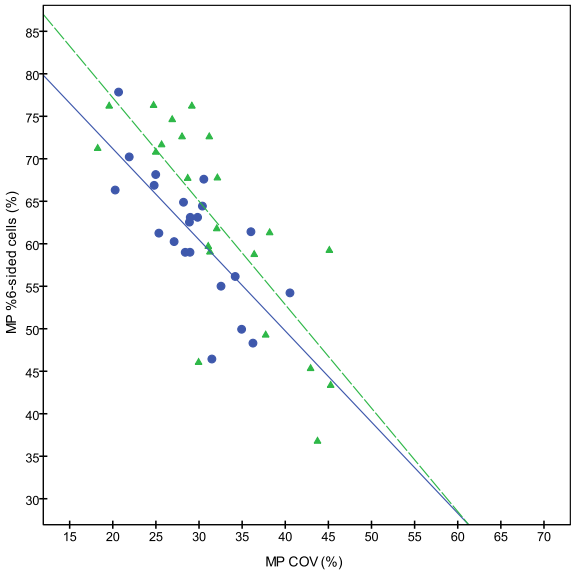


Figure 3.3-30
Scatter plots and linear regression analysis showing the relationship between mean MPCOV and MP%SIX before (triangles, dashed regression line, p < 0.001, r = -0.783) and after refitting with *SiH lenses* (closed circles, solid line , p < 0.001, r = -0.745). N = 22.

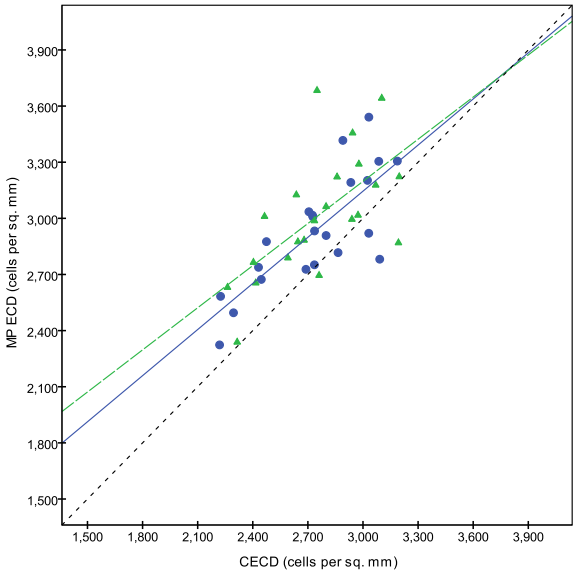


Figure 3.3-31
Scatter plots and linear regression analysis showing the relationship between mean CECD and MPECD before (triangles, dashed regression line, p = 0.002, r = 0.634) and after refitting with *SiH lenses* (closed circles, solid line , p < 0.001, r = 0.781). The dotted line shows the one:one correlation. N = 22.

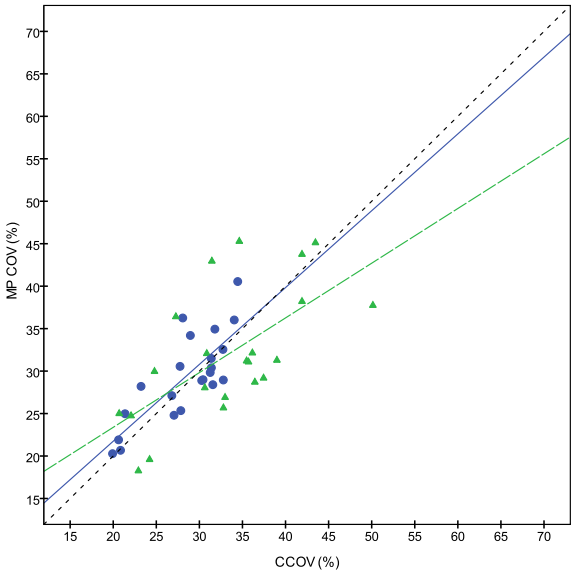


Figure 3.3-32
Scatter plots and linear regression analysis showing the relationship between mean CCOV and MPCOV before (triangles, dashed regression line, p = 0.001, r = 0.636) and after refitting with *SiH lenses* (closed circles, solid line , p < 0.001, r = 0.791). The dotted line shows the one:one correlation. N = 22.

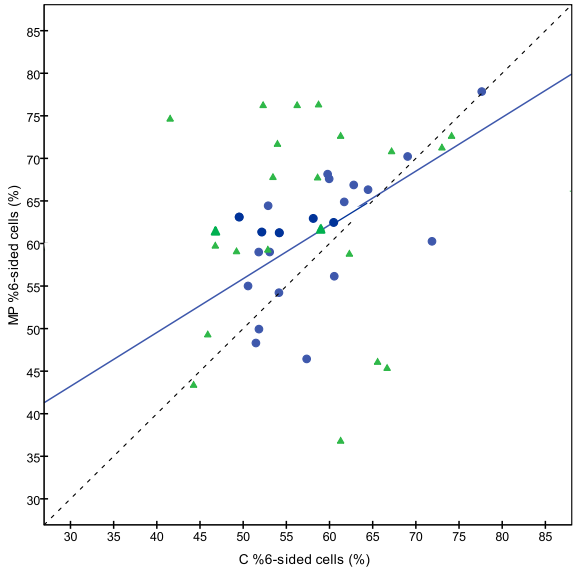


Figure 3.3-33
Scatter plots and linear regression analysis showing the relationship between mean C%SIX and MP%SIX before (triangles, p = 0.702, r = 0.086) and after refitting with *SiH lenses* (closed circles, solid line , p = 0.002, r = 0.627). The dotted line shows the one:one correlation. N = 22.

3.4 RESULTS FOR LASIK SUBJECTS

3.4.1 Group demographics and vision assessments

Of the 26 subjects successfully enrolled, 21 attended at all visits. However, for the five other subjects, 3 were not able to make the appointment at 3 months and another at 12 months. One subject did not meet for three appointments, i.e. 3, 18 and 24 months. Therefore, overall 21 subjects were studied for the entire study period, but the data of the other five subjects is included where appropriate (see Table 3.4-1).

The subjects who were going to have LASIK were aged 31.4 ± 5.4 years (mean ± SD, n = 26) at the first visit. The group comprised 12 women (46%) and 14 men (54%), and all were experienced contact lens wearers until some time before surgery. They had successfully used soft contact lenses for at least 2.5 years. On average, these subjects had worn contact lenses for 11.8 ± 4.6 years at the first visit, and no significant difference was found (p = 0.450, t-test) when just the subgroup of 21 subjects was considered (average 12.4 ± 4.5 years of soft contact lens wear).

Twenty-two of the 26 subjects who initially participated in the LASIK group of the study wore soft contact lenses every day, and most of them (85%) wore the lenses for 10 hours or more (see also Table 3.4-2). When asked to grade the *quality of vision* (when wearing their contact lenses) on a 100 mm long horizontal visual analogue scale, the average score for the contact lens wearers who were going to have LASIK performed was 79 ± 21 (mean ± SD), range 26 to 100.

Table 3.4-1
Subject details of LASIK group at baseline

	Initial assignment to the study	Completed study
Number of subjects	26	21
Age at the first visit (years)	31.35 ± 5.39	31.29 ± 5.69
Age range (years)	21 to 41	21 to 41
Gender (F:M)	12:14	8:13
Duration of lens wear at the first visit (years)	11.77 ± 4.64	12.36 ± 4.45
Refractive error (MSE)[a]	-4.38 ± 1.58	-4.40 ± 1.39

[a] Mean refractive error (MSE) in spherical equivalent power (DS). All other data are mean ± S.D.

Table 3.4-2
Contact lens-wear modality of the pre-LASIK subjects

Hours per day	Initial assignment N (%)	Completed study n (%)
5-10	4 (15)	2 (10)
10-15	11 (42)	11 (52)
> 15	11 (42)	8 (38)
Total	26 (100)	21 (100)

When asked about lens wear *comfort* ("How does your eye feel when wearing contact lenses?") the average score was 63 ± 27 (range 15 to 100). The average grading of vision quality and lens wear comfort were a little lower for this group of subjects than the other groups. Nevertheless, the between-group differences were not significant ($p > 0.1$, Kruskal-Wallis test) and in light of the wearing time, these subjects were all considered as successful contact lens wearers.
Nearly half the LASIK group subjects had replaced their lenses daily and the rest replaced their lenses regularly (Table 3.4-3). Two subjects, of those who reported replacing their lenses monthly, used SiH lenses on a continuous (30 days) wear basis and one replaced his soft hydrogel lenses after every 14 days of extended wear. The usage of various types of disinfection systems in this group reflected the lens wear modality. Table 3.4-4 shows that 15 subjects used no disinfection system at all and a cross tabulation (not shown) proved that these subjects either used daily disposables or some form of continuous or extended lens wear.

Table 3.4-3
Replacement schedule of soft contact lenses for the pre-LASIK subjects

	Initial assignment N (%)	Completed study n (%)
Planned replacement (6, 9 or 12 m)	2 (8)	2 (10)
Monthly replacement	12 (46)	11 (52)
Daily disposables	12 (46)	8 (38)
Total	26 (100)	21 (100)

Table 3.4-4
Disinfection systems used by the pre-LASIK subjects

	Initial assignment N (%)	Completed study n (%)
Chemical based	10 (38)	9 (43)
H_2O_2 based	1 (4)	1 (5)
None	15 (58)	11 (52)
Total	26 (100)	21 (100)

At baseline, mean spherical refractive error (MSE: spherical equivalent) for all the subjects who were going to have LASIK performed was -4.38 ± 1.58 DS, range -8.50 to -1.50 DS (N=26). Seven subjects had astigmatism but the cylinder did not exceed 1.00 DC in any cases. None of the subjects wore toric lenses. For the 21 subjects completing all visits, the results were very similar; their mean spherical refractive error was -4.40 ± 1.39 DS (Table 3.4-5).

The mean refractive error was +0.29 ± 0.44 DS at three months postoperatively. This change in refractive error was obviously statistically significant ($p < 0.0001$, Wilcoxon Signed Rank test). At the end of the study, all subjects were within ± 1.00 DS (MSE) of the target (plano), and 86% were within ± 0.50 DS. Furthermore, as shown in Figure 3.4-1, the MSE remained relatively stable throughout the rest of the study. Statistical comparisons, revealed no detectable differences between refractive error measures at each post-operative assessment ($p > 0.018$, Friedman test, Table 3.4-5).

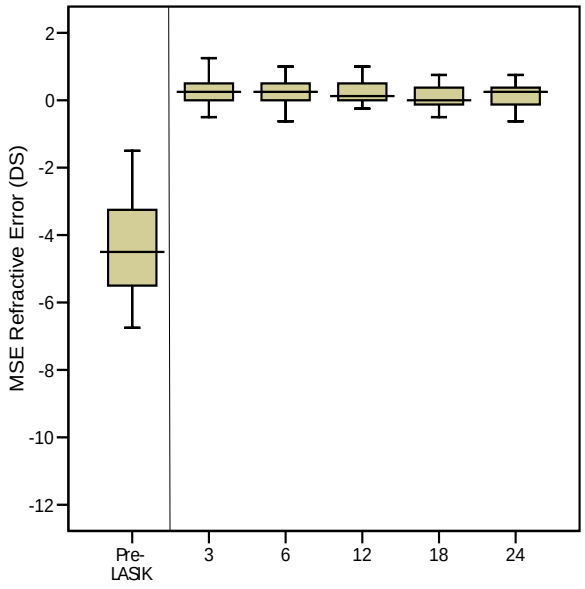


Figure 3.4-1
Box plot to show refractive error (MSE) before and during two years after LASIK (n = 21).

The visual acuity was only measured with best spectacle correction at baseline since most of the subjects had stopped wearing their contact lenses one to three weeks before the LASIK surgery. As detailed in Table 3.4-5, the BCHCVA averaged 0.00 ± 0.09 before surgery (baseline values). At the first post-operative assessment made at 3 months, the BCHCVA had improved (to a mean of -0.09 ± 0.07) and this improvement was statistically significant ($p = 0.002$, Wilcoxon signed rank test). Thereafter, the mean values and range of values for the BCHCVA remained largely stable (Table 3.4-5) with no overall substantial changes noted. One subject did however loose one line of best-corrected visual acuity, but for the remaining subjects either no change occurred or they actually gained one or two lines, probably due to the image size effect.

The LogMAR low contrast visual acuity was also measured and the mean values were typically 1-2 lines less than high contrast visual acuity. Pre-operatively the mean BCLCVA was 0.19 ± 0.10. After surgery, the average best-corrected LCVA improved significantly ($p = 0.002$, Wilcoxon signed rank test) and it continued to improve during the study (Table 3.4-5). However, although significantly different from the pre-operative value, no significant inter-visit differences were seen for the best-corrected low contrast VA. Low contrast VA with no correction was not measured pre-operatively. Post-operatively, the average LCVA measured 0.15 ± 0.11 log MAR units at 3 months, and no significant changes were observed at later visits ($p = 0.407$, Friedman test). Except at 6 months, the uncorrected LCVA was significantly poorer than the best corrected LCVA at all points of time ($p \leq 0.033$)

Table 3.4-5
Refractive error (MSE) and visual acuity measurements: LogMAR Best Corrected High- and Low Contrast Visual Acuity (BCHCVA and BCLCVA, respectively) and Habitual HCVA and LCVA of *LASIK subjects* (n = 21) over a period of two years. All values are mean ± 1SD with ranges in brackets.

	Time (months)					
	baseline	3	6	12	18	24
Refractive error[a]	-4.40 ± 1.39 (-6.75 to -1.50)	+0.29 ± 0.44 (-0.50 to 1.25)	+0.25 ± 0.38 (-0.63 to 1.00)	+0.20 ± 0.35 (-0.25 to 1.00)	+0.13 ± 0.36 (-0.50 to 0.75)	+0.14 ± 0.34 (-0.63 to 0.75)
BCHCVA	0.00 ± 0.09 -0.16 to 0.14	-0.09 ± 0.07 -0.20 to 0.04	-0.11 ± 0.06 -0.20 to 0.02	-0.10 ± 0.06 -0.20 to 0.02	-0.11 ± 0.07 -0.20 to 0.10	-0.12 ± 0.06 -0.20 to 0.00
Habitual HCVA	-	-0.03 ± 0.08 -0.18 to 0.12	-0.06 ± 0.10 -0.20 to 0.12	-0.06 ± 0.07 -0.20 to 0.06	-0.06 ± 0.08 -0.20 to 0.14	-0.09 ± 0.08 -0.20 to 0.04
BCLCVA	0.19 ± 0.10 0.06 to 0.38	0.11 ± 0.08 -0.08 to 0.22	0.11 ± 0.06 0.02 to 0.24	0.10 ± 0.06 -0.02 to 0.20	0.09 ± 0.06 0.00 to 0.27	0.08 ± 0.06 -0.02 to 0.20
Habitual LCVA	-	0.15 ± 0.11 -0.02 to 0.38	0.14 ± 0.11 0.02 to 0.50	0.16 ± 0.08 -0.02 to 0.38	0.14 ± 0.09 0.02 to 0.38	0.13 ± 0.08 -0.02 to 0.30

[a] Mean refractive error (MSE) in spherical equivalent power (DS)

3.4.2 Ocular comfort

These subjects were first assessed just prior to their refractive surgery. All had been soft contact lens wearers but had discontinued lens wear at the request of the surgical centre. Notwithstanding, nearly all subjects (25 out of 26) reported that they experienced one or more of the listed symptoms. Twenty-one subjects finished the entire study, and 20 of these reported symptoms at baseline (Figure 3.4-2).

Over time, however, the number of subjects reporting ocular symptoms decreased (Figure 3.4-2 and Table 3.4-6. The change appeared to be stepped, with a decrease evident at the 3 month visit (15 of 21, 71%), and with a further reduction evident in the second year. At the 24-month assessment, the number of subjects reporting ocular symptoms had dropped to 12 (57%).

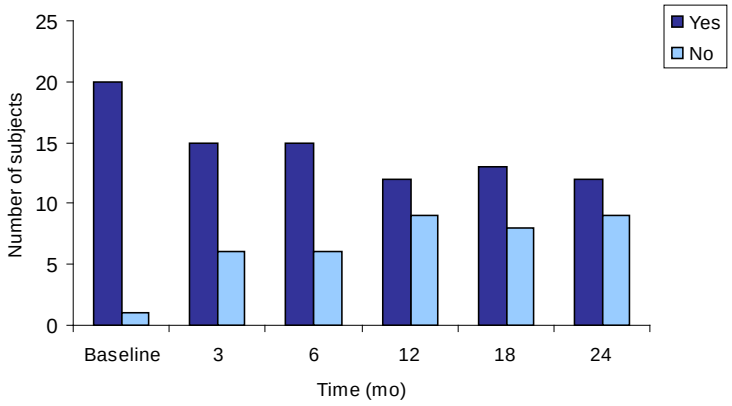


Figure 3.4-2
Frequency of pre- and post LASIK subjects (n = 21) reporting one or more ocular symptoms.

Table 3.4-6
Symptom frequency (count) in *LASIK subjects* who completed the study (n = 21).

Symptom frequency (n)	Time (months)					
	Baseline	3	6	12	18	24
Never	1	7	6	9	8	9
Sometimes	13	14	13	10	12	12
Often	7	0	2	2	1	0
Always	0	0	0	0	0	0

When asked about the frequency of symptoms, 72% of the symptomatic LASIK subjects reported to have these “sometimes” at baseline. Seven (28%) reported that they experienced symptoms “often”. Compared to baseline, the symptomatic subjects reported to have symptoms less frequently postoperatively (Table 3.4-6).

For the 20 symptomatic subjects who finished the study the mean grading of symptom severity was 35 ± 21 mm before surgery (range 2 to 76). This had declined to an average of 24 ± 15 (range 6 to 50) at 24 months (Table 3.4-7). However, as noted in Table 3.4-7, only five subjects in the LASIK

group reported to have symptoms at every visit. Subjects reporting no symptoms at any visit were not instructed to fill in the VAS scales and thus the eligible sample for symptom severity became too small for this group to draw any robust time dependent conclusions.

Table 3.4-7
Symptom severity assessed by VAS in symptomatic *LASIK subjects*. All values are given as group mean ± SD (minimum to maximum values in brackets).

Symptom severity (mm)	Time (months)					
	Baseline	3	6	12	18	24
Eligible sample (n = 5)*	40 ± 12 (21 to 49)	31 ± 19 (7 to 59)	27 ± 15 (14 to 52)	27 ± 20 (14 to 63)	32 ± 18 (12 to 52)	28 ± 15 (15 to 50)
Main sample	35 ± 21 (0 to 78)	27 ± 18 (0 to 59)	22 ± 12 (0 to 52)	29 ± 15 (12 to 63)	26 ± 17 (5 to 52)	24 ± 15 (6 to 50)
N	20	14	15	12	13	12

* I.e. those subjects who reported on their symptoms using the VAS at all visits.

3.4.3 Tear film characteristics

Twenty-one of the 26 originally enrolled subjects finished the study after having had LASIK surgery. However, some additional data are missing because occasionally the slit lamp that was normally used was not available (see Table 3.4-8). In addition, a few fluorescein-TBUT measurements were not taken due to a missing yellow Wratten filter.

At baseline, prior to the surgery, the average (± S.D) tear-volume as estimated by the average tear meniscus height (TMH) was 0.24 ± 0.09 mm, ranging from 0.15 to 0.50 mm, for the LASIK subjects who completed the study (Table 3.4-8). Over the span of the study, individual TMH measurements gave a wide range of values from 0.1 to 0.7 mm (Figure 3.4-3). Average values at any one time point ranging from 0.19 to 0.32 (Table 3.4-8) were not significantly different ($p = 0.091$, Friedman test). As shown in Figure 3.4-3, consistent with some of the other subject groups, the TMH values fluctuated somewhat and were notably variable at the 12 month assessment. The values at the final visit were not obviously different to those at baseline (average values of 0.22 vs. 0.24 mm). Overall, the TMH in post-LASIK subjects was 0.23 mm (95% CI = 0.20 – 0.26 mm).

The pre-LASIK baseline measures of average tear volume (as estimated by the phenol red thread test, PRT) were 18.1 ± 8.3 mm, with values from 6.0 to 31.5 mm. The average PRT values, which ranged from 15.5 to 18.7 mm, differed by less than 1.5 mm between subsequent visits (Table 3.4-8). At the first post-operative assessment at 3 months, the range of PRT values was unaltered (i.e. 6 to 32 mm). Both the mean (15.5 mm) and median value (13.0 mm) were notably lower at 12 months but, again, these values were not statistically lower ($p = 0.159$, Friedman test, Figure 3.4-4). The range of values at baseline, 3 months and 12 months were essentially the same. A scatter plot with a linear regression analysis indicated a very slight increase in the PRT results with time (with the slope being +0.96 mm / y) (Figure 3.4-5). However, this change was not statistically significant ($p = 0.409$, $r = 0.081$). Overall mean PRT (average of five measurements over a period of two years) for the group of 21 post-LASIK subjects was 17.5 mm (95% CI = 14.6 to 20.3 mm).

Table 3.4-8
Mean ± SD (range) of the tear film tests of the subjects that completed the study. *LASIK group* (N=21). For missing data, see text.

Tear film test	Time (months)						
	Valid n	Baseline	3	6	12	18	24
TMH (mm)	18	0.24 ± 0.09 (0.15 to 0.50)	0.19 ± 0.08 (0.10 to 0.40)	0.20 ± 0.08 (0.10 to 0.35)	0.32 ± 0.20 (0.10 to 0.70)	0.21 ± 0.07 (0.10 to 0.30)	0.22 ± 0.08 (0.15 to 0.40)
PRT (mm)	21	18.1 ± 8.3 (6.0 to 31.5)	17.1 ± 8.2 (6.0 to 32.0)	17.1 ± 7.0 (7.0 to 30.0)	15.5 ± 8.4 (5.0 to 30.0)	18.2 ± 7.4 (6.0 to 32.0)	18.7 ± 8.6 (7.0 to 35.0)
NIBUT (sec)	21	24.2 ± 21.7 (5.2 to 103.4)	29.5 ± 38.4 (8.3 to 160.5)	26.9 ± 27.8 (5.9 to 118.0)	37.3 ± 48.5 (9.7 to 233.0)	45.2 ± 50.3 (7.13 to 196.0)	35.0 ± 37.6 (7.0 to 176.0)
f-TBUT (sec)	18	26.0 ± 32.2 (5.0 to 130.0)	45.2 ± 115.9 (4.6 to 500.0)	31.0 ± 52.4 (4.7 to 201.0)	33.3 ± 53.9 (6.1 to 233.0)	31.5 ± 35.0 (6.4 to 120.0)	62.7 ± 77.2 (5.5 to 324.5)

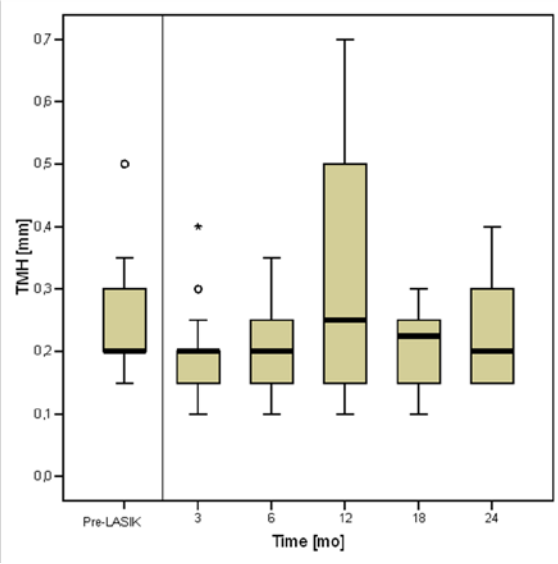


Figure 3.4-3
Box-plots of tear meniscus height (TMH) in the post-*LASIK subjects* (n = 18).

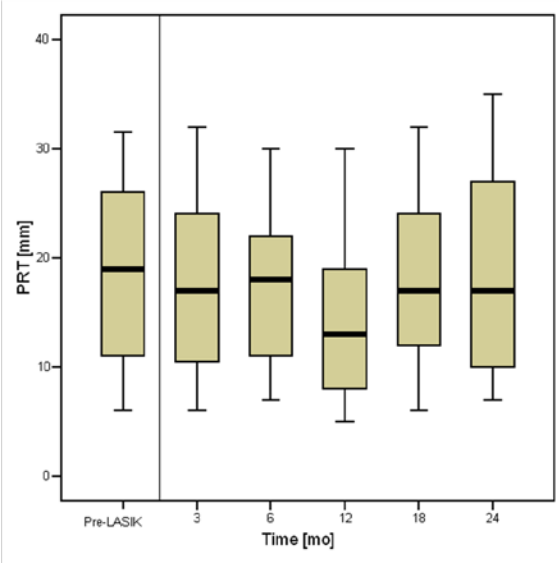


Figure 3.4-4
Box-plots to show time-related changes of phenol red thread (PRT) test in the post-*LASIK subjects* (n = 21).

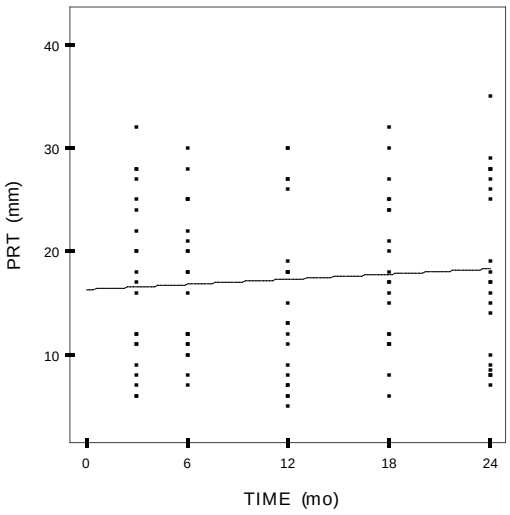


Figure 3.4-5
Scatter-plot to illustrate time-related changes of phenol red thread (PRT) test in post-*LASIK subjects*. The linear regression line shown indicates no statistically significant effect (slope = +0.96 mm/year, p = 0.409, Pearson's r = 0.081)

The tear quality as measured by non-invasive tear break up-time (NIBUT) averaged 24.2 ± 21.7 sec for the 21 pre-LASIK subjects who finished the study. The ranges varied considerably between visits (being from 98.1 to 223.3 sec.) as did the average values, which ranged from 24.2 sec at baseline to 45.2 sec at 18 months (Table 3.4-8). As Figure 3.4-6 indicates, the NIBUT at 18 months was significantly longer than at 6 months (Friedman test p = 0.019, Wilcoxon signed rank test p = 0.003). However, any increment in NIBUT with time was not evident when analysed by linear regression (not shown), and indeed the 24 months value did not differ from any of the other values. Overall, the NIBUT value in post-LASIK subjects was 33.1 sec. (95% CI = 18.9 to 47.0 sec).

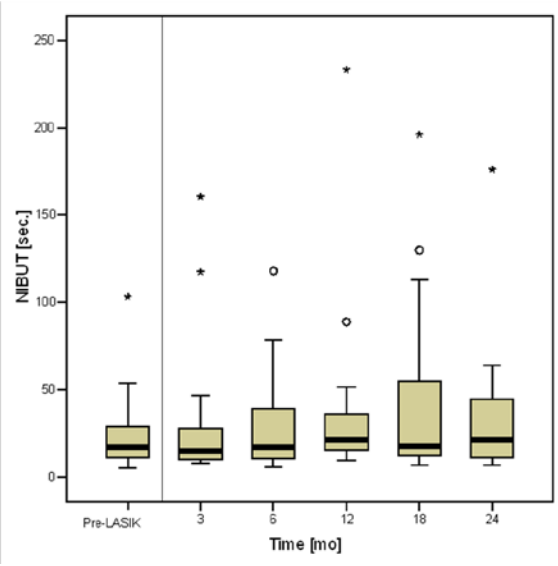


Figure 3.4-6
Box-plots showing time-related changes of non-invasive tear break-up time (NIBUT) in the post-*LASIK subjects* (n = 21).

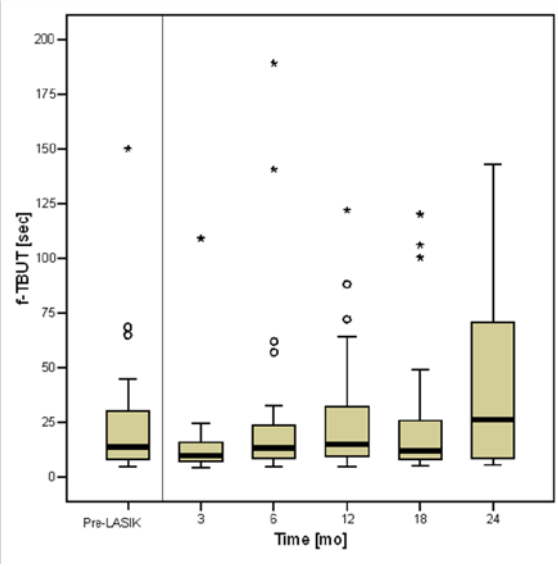


Figure 3.4-7
Box-plots showing time-related changes of fluorescein-tear break-up time (f-TBUT) in post-*LASIK subjects* (n = 18). Note that two subjects with extreme f-TBUT values (500s at 3 months and 325 sec at 24 months) are not visible on the plot but included in the analysis.

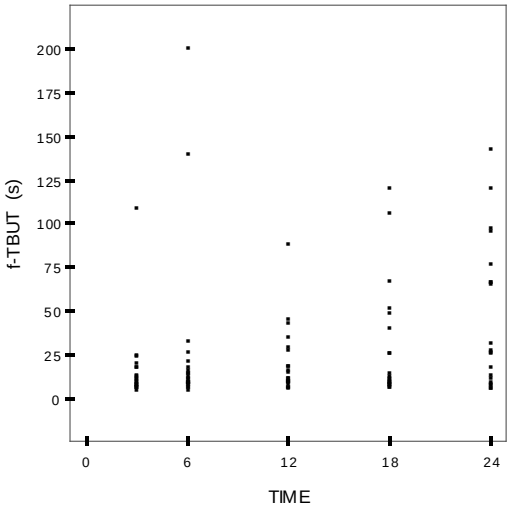


Figure 3.4-8
Scatter plot illustrating time related changes of fluorescein-tear break-up time (f-TBUT) in post-*LASIK subjects*. Non-parametric correlation analysis indicated a statistically significant effect (p = 0.019, Spearman's rho = 0.231). Note that two subjects with extreme f-TBUT values (500s at 3 months and 325 sec at 24 months) are not visible on the plot but included in the analysis.

The tear quality as assessed by fluorescein-tear break-up time (f-TBUT) was 26.0 ± 32.2 seconds (ranging from 5.0 to 130.0 seconds) for 18 LASIK subjects pre-operatively. Similar to the NIBUT and as indicated by the large SD, the f-TBUT measurements varied considerably with values from 4.6 to 500.0 s. Average values ranged from 26.0 sec at baseline to 62.7 sec at 24 months. Visit-to-visit analysis revealed no significant differences (p = 0.243, Friedman test). However, the gradual increase in the group mean values from 6 to 24 months gave the impression of a time-dependent change in f-TBUT, as indicated in Figure 3.4-7. A just statistically significant time-related relationship was noted by correlation analysis (Figure 3.4-8, p = 0.019, Spearman's rho = 0.231). Overall, the mean f-TBUT in post-LASIK subjects was 38.3 sec. (95% CI 14.3 to 62.3 sec).

3.4.4 Ocular surface characteristics

The eyelids and meibomian glands of the soft lens wearers, who underwent LASIK for myopia, were generally healthy and rather unremarkable. However, up to three subjects displayed moderate blepharitis at different visits (grade 1.6 to 2.0). Pre-operatively, the average eyelid and meibomian gland grading were 0.4 ± 0.7 and 0.6 ± 0.5, respectively (Table 3.4-9). Over the next 5 visits, changes in eyelid grading of up to 0.4 occurred, but no significant differences were detected ($p = 0.241$, Friedman test). Likewise, as seen from Table 3.4-9, only subtle changes were seen for the meibomian glands from visit to visit ($p = 0.142$, Friedman test).

Eye redness, in terms of bulbar hyperaemia and limbal redness averaged 1.5 ± 0.4 and 1.0 ± 0.7 before surgery. Three months after LASIK, bulbar redness increased a little to a mean of 1.7 ± 0.5 (range 0.8 to 2.3). A year after, average bulbar redness was back to baseline levels (Table 3.4-9) and no significant changes were observed ($p = 0.356$, Friedman test). Likewise, mean limbal redness remained at the same level throughout the study ($p = 0.737$, Friedman test).

Pre-operative mean grading of corneal vascularisation was 0.7 ± 0.6 (range 0.0 to 2.0). At the three first post-operative assessments, mean corneal vascularisation remained unchanged. Eighteen months after surgery, the mean level was only lowered by 0.2, which was not different from baseline ($p = 0.183$, WSR test). However, the degree of vascularisation at 18 and 24 months was 0.4 units lower than the average vascularisation at 0.9 ± 0.6 at 3 months ($p = 0.004$, WSR test) (Table 3.4-9).

In the LASIK group only eight out of the 21 subjects (38%) showed corneal staining before their surgery. After LASIK, less than half of the subjects had corneal staining with 24% (3 months) to 43% (at 6 months). However, the average level of staining (in the subjects who actually had staining) ranged from 0.8 ± 0.4 to 1.2 ± 0.5. In Table 3.4-9, the total group-mean grading of staining are given over the 6 visits ($n = 21$). At baseline, mean grading of staining was 0.5 ± 0.7 (range 0.0 to 2.5), whereas after LASIK the level of staining ranged from 0.2 ± 0.5 at 3, 18 and 24 months to 0.5 ± 0.7 at 6 months. However, no significant inter-visit differences in corneal staining levels were discovered by the Friedman test ($p = 0.542$).

Table 3.4-9
Ocular surface characteristics as graded with Efron's grading scale [0 - 4] in post-*LASIK subjects* who completed the study (n = 21, except for papillary conjunctivitis where n = 16). The numbers represents group mean ± SD and range (in brackets).

Ocular surface characteristic	Time (months)					
	baseline	3	6	12	18	24
Eyelids	0.4 ± 0.7 (0.0 to 2.0)	0.5 ± 0.8 0.0 to (2.0)	0.8 ± 0.8 0.0 to (2.0)	0.6 ± 0.6 (0.0 to 2.0)	0.6 ± 0.7 0.0 to (2.0)	0.7 ± 0.6 (0.0 to 2.0)
Meibomian glands	0.6 ± 0.5 (0.0 to 1.8)	0.7 ± 0.6 (0.0 to 1.8)	0.6 ± 0.5 (0.0 to 2.0)	0.4 ± 0.3 (0.0 to 1.0)	0.5 ± 0.4 (0.0 to 1.3)	0.6 ± 0.4 (0.0 to 1.5)
Bulbar hyperaemia	1.5 ± 0.4 (1.0 to 2.0)	1.7 ± 0.5 (0.8 to 2.3)	1.7 ± 0.4 (0.9 to 2.5)	1.6 ± 0.4 (0.9 to 2.3)	1.5 ± 0.4 (0.9 to 2.0)	1.6 ± 0.3 (0.8 to 2.1)
Limbal redness	1.0 ± 0.7 (0.0 to 2.2)	0.9 ± 0.5 (0.0 to 2.0)	1.2 ± 0.7 (0.0 to 2.3)	1.1 ± 0.6 (0.0 to 2.0)	1.0 ± 0.4 (0.0 to 1.7)	0.9 ± 0.6 (0.2 to 2.0)
Corneal vascularisation	0.7 ± 0.6 (0.0 to 2.0)	0.9 ± 0.6 (0.0 to 2.0)	0.7 ± 0.5 (0.0 to 1.7)	0.7 ± 0.5 (0.0 to 1.8)	0.5 ± 0.5 (0.0 to 1.7)	0.5 ± 0.4 (0.0 to 1.0)
Corneal staining*	0.5 ± 0.7 (0.0 to 2.5)	0.2 ± 0.5 (0.0 to 1.5)	0.5 ± 0.7 (0.0 to 2.5)	0.3 ± 0.5 (0.0 to 1.5)	0.2 ± 0.4 (0.0 to 1.4)	0.2 ± 0.4 (0.0 to 1.4)
Conjunctival staining	1.1 ± 1.1 (0.0 to 3.0)	1.0 ± 0.9 (0.0 to 2.5)	1.2 ± 0.9 (0.0 to 3.0)	0.5 ± 0.6 (0.0 to 1.8)	0.1 ± 0.2 (0.0 to 0.8)	0.2 ± 0.4 (0.0 to 1.2)
Papillary conjunctivitis	1.4 ± 0.4 (0.5 to 2.0)	1.7 ± 0.4 (1.0 to 2.5)	1.7 ± 0.5 (1.0 to 2.7)	1.7 ± 0.4 (0.5 to 2.3)	1.5 ± 0.4 (0.8 to 2.0)	1.4 ± 0.5 (0.3 to 2.2)

* Corneal staining was graded using CCLRU's grading scales [0-4]. See methods chapter for details.

At baseline, 62% of the soft lens wearers who later underwent LASIK had conjunctival staining. After LASIK, the percentage of subjects displaying conjunctival staining changed substantially; from 62% and 76% at 3 and 6 months to only 19% at 18 and 24 months. Likewise, the level of conjunctival staining varied from 1.1 to 1.2 at baseline, 3 and 6 months to levels of 0.1 and 0.2 at 18 and 24 months ($p \leq 0.004$ vs. baseline, WSR test).

Before surgery, the mean grading of papillary conjunctivitis was 1.4 ± 0.4. After surgery, papillary conjunctivitis was still graded to moderate levels. At 3, 6 and 12 months the mean grading was 1.7, but at 18 and 24 months it was a little lower at 1.5 ± 0.4 and 1.4 ± 0.5. No significant inter-visit differences were detected by Friedman test, however ($p = 0.160$).

3.4.5 Corneal thickness and corneal curvature

Prior to their refractive surgery, the mean CCT in the LASIK subject group was 530 ± 28 µm. Their mean mid-peripheral corneal thickness values, again prior to the surgery, averaged 618 ± 34 µm at baseline (Table 3.4-10). The CCT and MPCT values were thus the same as those seen in the soft lens group (Table 3.2-10), slightly higher than those recorded in the spectacle wearing myopes (Table 3.1-7) and slightly less than those in the soft lens wearers who were refitted into SiH lenses (Table 3.3-10).

Table 3.4-10
Mean ± 1SD central and mid-peripheral corneal thickness (CCT and MPCT) and central corneal radius of curvature (K), in *LASIK subjects* (n = 20). Minimum and maximum values are given in brackets.

	Time (months)					
	Baseline	3	6	12	18	24
CCT (µm)	530 ± 28 (471 to 584)	448 ± 39 (370 to 510)	450 ± 39 (372 to 525)	450 ± 37 (372 to 521)	456 ± 41 (375 to 524)	456 ± 39 (375 to 531)
MPCT (µm)	618 ± 34 (540 to 673)	593 ± 40 (515 to 666)	594 ± 45 (525 to 665)	603 ± 38 (540 to 666)	609 ± 43 (517 to 666)	613 ± 43 (527 to 668)
Central K (mm)	7.90 ± 0.32 (7.56 to 8.47)	8.73 ± 0.39 (8.29 to 9.77)	8.71 ± 0.39 (8.29 to 9.74)	8.71 ± 0.36 (8.31 to 9.73)	8.70 ± 0.36 (8.30 to 9.70)	8.63 ± 0.38 (8.20 to 9.70)

Following successful LASIK procedures, these subjects were assessed at 3.1 ± 0.4 months after their operation (range 2.5 to 4.2 months). At this 3-month visit, the mean central CT was 448 ± 39 µm, whilst the mid-peripheral thickness values were 593 ± 40 µm. The changes in both the central and mid-peripheral thickness values were statistically significant ($p \leq 0.020$, pairwise comparison, Bonferroni correction). At the ensuing assessments over the next 21 months, scheduled to closely coinciding with the assessment times for the other subject groups, the mean thickness values obtained at central corneal sites fluctuated only slightly with differences from the first set of post-operative values of only 8 µm (Table 3.4-10). However, for the mid-peripheral sites, differences from the first post-operative assessment were up to 20 µm. Therefore, the maximum differences in mean values were 1.8% for central sites and 3.3% for mid-peripheral sites.

A box plot to show the CCT values over time is given in Figure 3.4-9, showing the overall consistency of most CCT values after the operation, but that there were two individuals with substantially thinner corneas (see outliers at 12 months in Figure 3.4-9). The mean central thickness values at the final assessment at 24 months were marginally higher than at the first post-operative assessment (i.e. 448 µm vs. 456 µm). This 1.8% increase was consistent, with the 95% CI being 0.9 to 15.8 µm and was statistically significant ($p = 0.022$, pairwise comparison, Bonferroni correction).

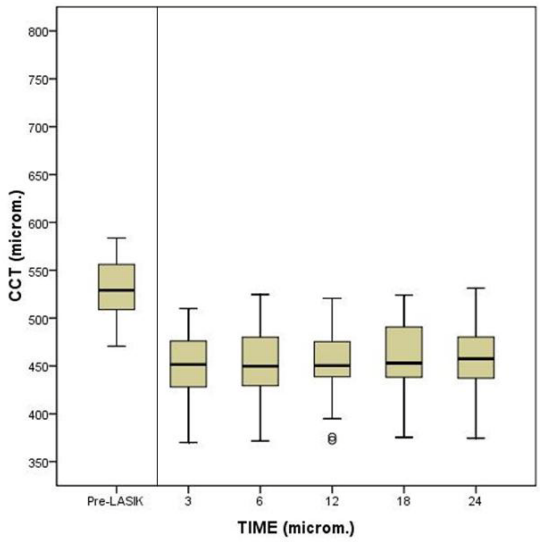


Figure 3.4-9
Box plot to show central corneal thickness over time in *LASIK subjects* (n = 20).

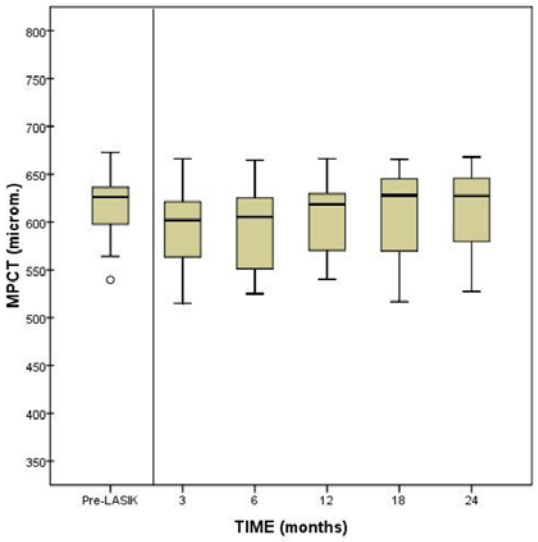


Figure 3.4-10
Box plot to show mid-peripheral corneal thickness over time in *LASIK subjects* (n = 20).

A slightly different change to that in the central cornea was indicated for the mid-peripheral sites where it appeared that a slight progressive thickening occurred. The mid-peripheral parts were close to the edge of the ablation zone, which ranged from 5.5 to 7.0 mm in diameter. Nevertheless, the MPCT values were 2.6% higher at 18 months after the operation and 3.3% higher at 24 months, compared to the first post-operative assessment (Table 3.4-10). Repeated ANOVA indicated significant between-visit differences ($p = 0.006$). However, post-hoc analysis of the 20 µm increment in MPCT observed at 24 months was only close to being statistically significant ($p = 0.080$, pairwise comparison, Bonferroni correction). A box plot to show the mid-peripheral thickness values over time is given in Figure 3.4-10.

If the overall mean thickness values over the 5 post-operative visits are taken as a reference value (i.e. 452 µm and 603 µm), then the maximum differences (fluctuations) in thickness values can be calculated to be just ± 0.9% (range -0.9 to + 0.9%) and ± 1.6% (-1.6 to + 1.7%) for the central and mid-peripheral sites, respectively.

Linear regression analyses for the post-operative CCT values revealed no detectable progressive change over time ($p = 0.406$, $r = 0.084$, Figure 3.4-11). Linear regression analyses for the post-operative MPCT values again indicated no progressive time-related change ($p = 0.064$, $r = 0.186$, Figure 3.4-12) despite the fact that both the average values (Table 3.4-10) and median values (Figure 3.4-10) slowly increased over time. Similarly, regression analysis over the period between 6 months and 24 months post-operatively showed the same results (i.e. no change in CCT and a slight increase in MPCT) but neither was statistically significant ($p \geq 0.146$, not shown).

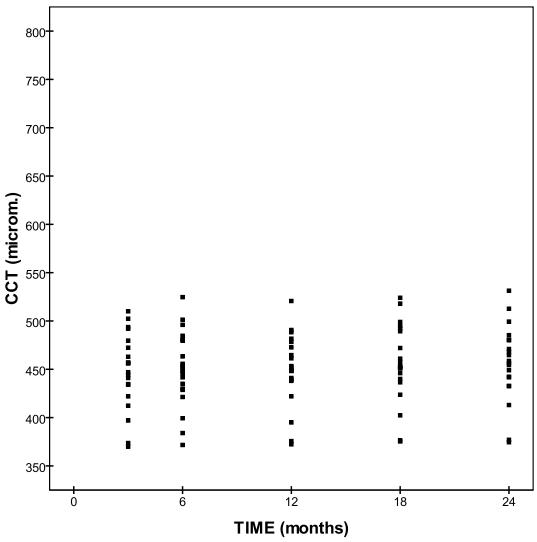


Figure 3.4-11
Regression analysis to show time-related changes of central corneal thickness (µm) in post-*LASIK subjects* (n = 20). The linear regression line indicates no statistically significant effect (p = 0.084, r = 0.406).

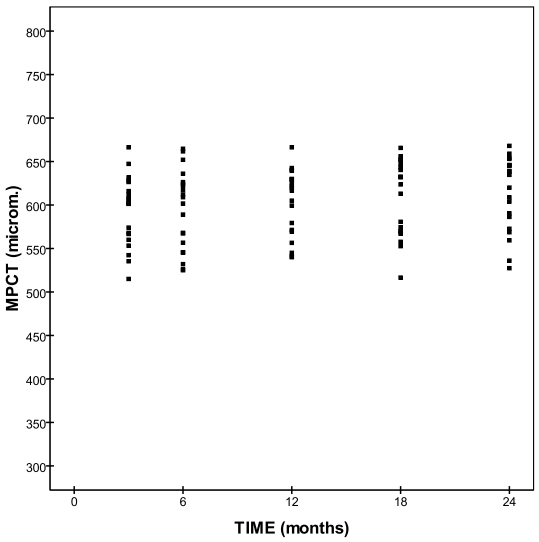


Figure 3.4-12
Regression analysis to show time-related changes of mid-peripheral corneal thickness (µm) in post-*LASIK subjects* (n = 20). The linear regression line indicates no statistically significant effect (p = 0.064, r = 0.186).

OrbScan data was not available for all subjects, and so for these analyses only data from 14 subjects, whose Orbscan data was accessible at all visits, was used Table 3.4-10).

For this group of subjects who were soft lens wearers prior to surgery, the mean anterior curvature radius at baseline was 7.94 ± 0.31 mm (range 7.56 to 8.47; Table 3.4-10). One subject had a very flat pre-operative cornea (K at 8.47 mm, with a refractive error of -5.50 DS). Because of the LASIK surgery, the central mean radius of curvature was significantly larger at all post-operative visits. At 3 months post-operatively the mean central K was 8.73 ± 0.39 mm; i.e. 0.83 mm flatter than pre-LASIK (p = 0.001, Wilcoxon Signed Rank test). Over time, a small re-steepening seemed to take place. Inter-variability and visit-to-visit changes in central K, represented by the median and inter-quartile range, are illustrated in Figure 3.4-13.

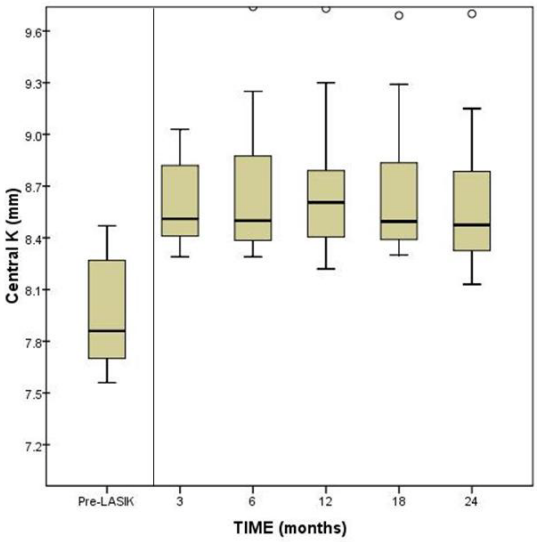


Figure 3.4-13
Box plot to show central corneal radius of curvature values over time (mm) in post-*LASIK subjects* (n = 14).

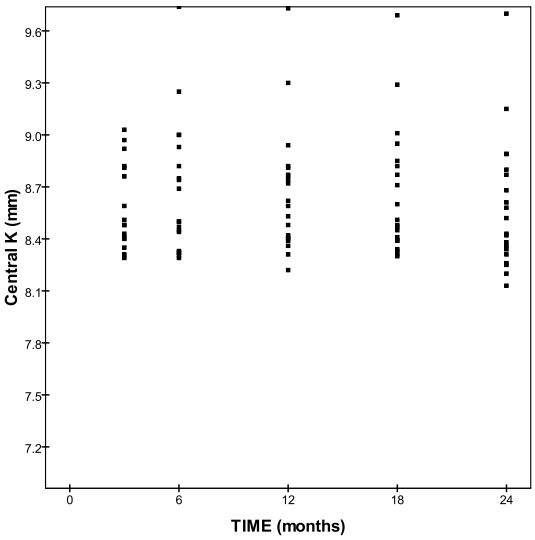


Figure 3.4-14
Scatter plot to illustrate time-related changes in central corneal radius of curvature values (mm) over time in post-*LASIK subjects* (n = 14). Non-parametric correlation analysis indicates no statistically significant effect (p = 0.281, Spearman's rho = -0.131).

The subject with the very flat pre-operative cornea had a remarkably flatter cornea than the rest of the test-group at the 3-month assessment (K = 9.77 mm). Non-parametric correlation analysis (Figure 3.4-14) showed no negative relationship between the post-operative anterior corneal surface curvature and time (p = 0.281, Spearman's rho = -0.131). Nevertheless, at 24 months, mean central K was 8.63 ± 0.38, which was significantly smaller than at every (apart from baseline) preceding occasion (p ≤ 0.003, Wilcoxon Signed Rank test). The overall group mean (of five measurements over a period of two years) radius of curvature after LASIK was 8.77 mm (95% CI = 8.55 – 8.99 mm).

3.4.6 Corneal endothelial cell morphometry

Endothelial cell density –ECD

At baseline, the average central endothelial cell density (CECD) in these contact lens wearers was 2635 ± 370 cells / mm^2 (Table 3.4-11). One subject had notably high CECD values (seen as outliers on Figure 3.4-15). As also shown, the CECD values changed very little from visit to visit, with the range and ± 25% inter-quartile intervals being modest and similar across all visits. The median values showed no obvious trend. The largest difference in average CECD values between two assessments was 46 cells / mm^2 (95% CI = -41 to 132 cells / mm^2) and this was not statistically significant (p = 0.609, repeated ANOVA).

Table 3.4-11
Cell density (mean ± 1SD) of the central (CECD) and mid-peripheral (MPECD) corneal endothelium before and at five occasions (over a two-year period) after *LASIK* (n = 20). Numbers in brackets represent the minimum and maximum observations. A paired t-test was used to explore any regional differences and repeated ANOVA for time related changes (α = 0.05).

ECD	Time (months)						p (ANOVA)
	Pre-LASIK	3	6	12	18	24	
CECD (cells/mm^2)	2635 ± 370 (2041 - 3559)	2642 ± 328 (2102 - 3447)	2633 ± 303 (2106 - 3350)	2653 ± 340 (2146 - 3614)	2607 ± 301 (2063 - 3425)	2615 ± 299 (2200 - 3324)	0.609
MPECD (cells/mm^2)	2913 ± 274 (2519 - 3673)	2850 ± 215 (2538 - 3302)	2820 ± 286 (2363 - 3494)	2799 ± 198 (2413 - 3259)	2765 ± 249 (2394 - 3521)	2739 ± 221 (2417 - 3219)	0.001
p (paired t-test)	0.001	0.001	<0.001	0.018	0.004	0.023	
MPECD:CECD ratio	1.117 ± 0.115 (0.785 - 1.277)	1.087 ± 0.091 (0.823 - 1.230)	1.075 ± 0.076 (0.894 - 1.207)	1.064 ± 0.088 (0.853 - 1.171)	1.067 ± 0.091 (0.928 - 1.279)	1.053 ± 0.081 (0.862 - 1.160)	0.036

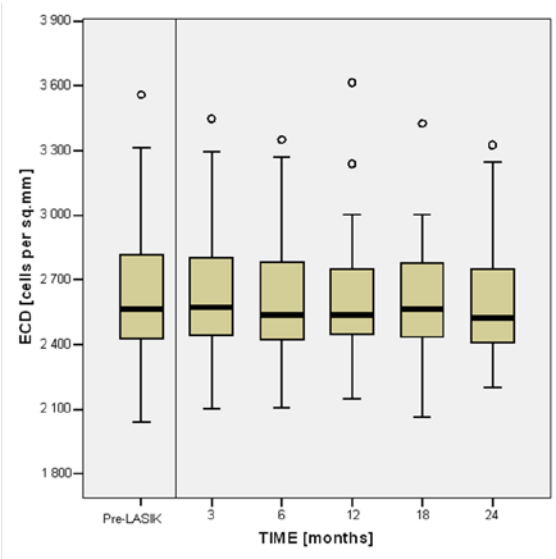


Figure 3.4-15
Box plots of the endothelial cell density in the central region of the corneas (CECD) before and at five occasions (over a two-year period) after *LASIK* (n = 20).

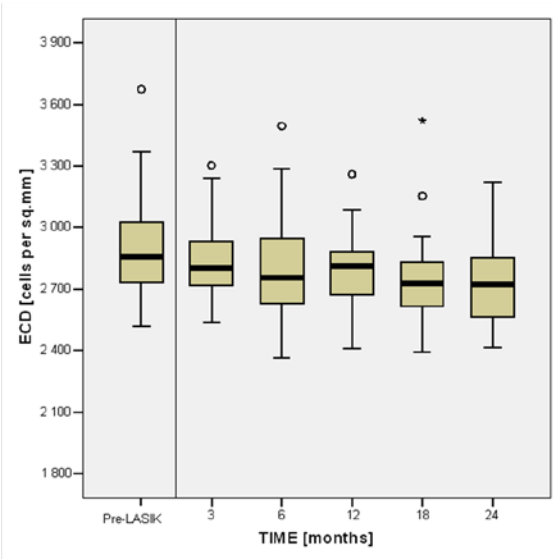


Figure 3.4-16
Box plots of the endothelial cell density in the mid-peripheral region of the corneas (MPECD) before and at five occasions (over a two-year period) after *LASIK* (n = 20).

In the mid-peripheral region of these corneal endothelia, the average cell density (MPECD) at baseline was 2913 ± 274 cells / mm^2, which was 10.6% higher than the average CECD values, and this difference was statistically significant (p = 0.001, paired t-test). Again, the single subject had rather higher MPECD values (Figure 3.4-16). The MPECD values at later assessments were consistently lower. Within 3 months, the average MPECD value was noted to be 2.2% lower, and within 6 months after LASIK surgery, the average MPECD was 3.2% lower than at baseline. However, the changes noted at 3 and 6 months post-operatively were not statistically significant (p = 0.941 and 0.200, respectively, Bonferroni correction). While the median MPECD value at 12 months was slightly higher than at the previous two assessments (Figure 3.4-16), the average MPECD values were lower at 12, 18 and 24 months (Table 3.4-11). At 24 months, the apparent net reduction from the average pre-operative value was 6.0% (95% CI = 1.3 to 10.7%) and this was statistically significant (p = 0.006, Bonferroni correction).

The overall mean CECD, in these soft lens wearers who then underwent LASIK surgery, was 2631 cells / mm^2, with average values at each visit ranging from 2607 to 2653 cells / mm^2. As might be expected from the box plot (Figure 3.4-15), no statistically significant time-related change in CECD was revealed by linear regression analysis (Figure 3.4-17). The slope of the regression line was slightly negative (at close -13.9 cells / mm^2 / year), but the calculated slope had a very substantial variance (of ± 47.2 cells/ mm^2 / year; r = 0.031), and was not statistically significant (p = 0.737, see also Table 5.5-1 in the appendix).

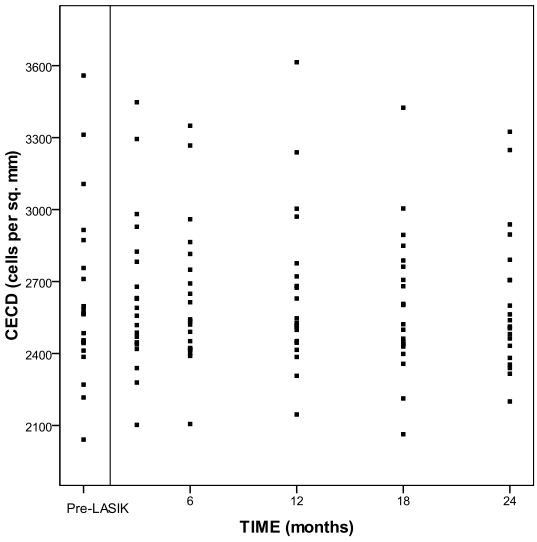


Figure 3.4-17
Linear regression analysis of the central cell density (CECD) and time before and at five occasions over 24 months after *LASIK* (n = 20, p = 0.737, r = -0.031).

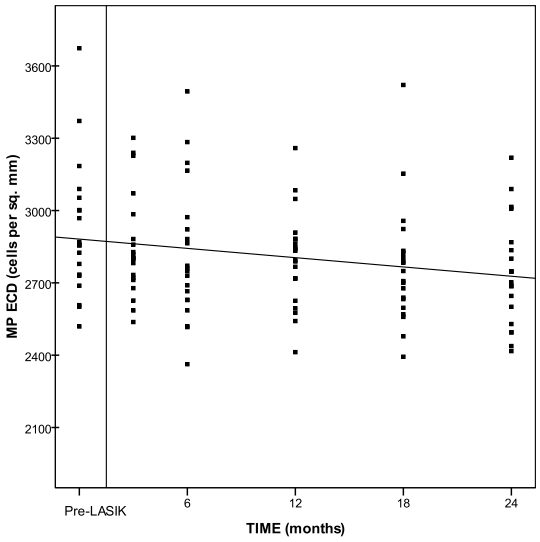


Figure 3.4-18
Linear regression analysis of the mid-peripheral cell density (MPECD) and time before and at five occasions over 24 months after *LASIK* (n = 20, p = 0.014, r = -0.223).

The overall mean MPECD value was 2814 cells / mm^2 with the range of average values being from 2739 to 2913 cells / mm^2. This set of MPECD values was significantly higher than the overall CECD values (p < 0.0001). The MPECD not only showed a tendency to decrease over time (Figure 3.4-16), but a statistically significant decline in MPECD could be detected by a linear regression analysis (see Figure 3.4-18 and Table 5.5-1 in the appendix). The net slope was -77 cells / mm^2 / year and although the variance in the slope estimate was rather substantial

(at ± 60.3 cells / mm^2 / year), this negative slope was still statistically significant (p = 0.014; r = -0.223). While the average data values and the median values (as shown in Figure 3.4-16) do not obviously indicate a stepped change, the most substantial change appeared to be in the first 3 months. So, if the baseline values were not included in the linear regression analysis, no further significant time-dependent change in the mid-peripheral ECD after LASIK could be detected (p = 0.097, r = -0.167).

The MPECD:CECD ratio for this group of soft lens wearers prior to LASIK was 1.111 with the overall mean values for MPECD being 2913 cells / mm^2 and that for the CECD being 2635 cells / mm^2. There were MPECD:CECD ratios as high as +1.277, and most of the pre-LASIK subjects showed positive MPECD:CECD ratios (Figure 3.4-19). However, a single individual had a substantially negative MPECD:CECD ratio of 0.785. After the LASIK surgery, analysis of the post-operative group mean MPECD:CECD ratio revealed a slight negative shift (Figure 3.4-20), i.e. the modal value was now shifted to the left. Overall, a possible change in the MPECD:CECD ratio as a consequence of the surgery was noted. If the baseline data and the post-operative data were compared, an analysis for any detectable time-related changes of the MPECD:CECD ratio revealed a slight, but statistically significant result. As already described, the MPECD was slightly lower at the first post-operative visit and lower again at 6 months whilst there was no equivalent change in the CECD values. As a result, the MPECD:CECD ratio significantly declined over time (p = 0.014, Figure 3.4-21). However, the correlation was weak (r = -0.204) and, if analysed slightly differently, to assess whether or not this change was evident only in the post-operative period, then no statistically significant change in the MPECD:CECD ratio could be detected (p = 0.221, r = -0.124).

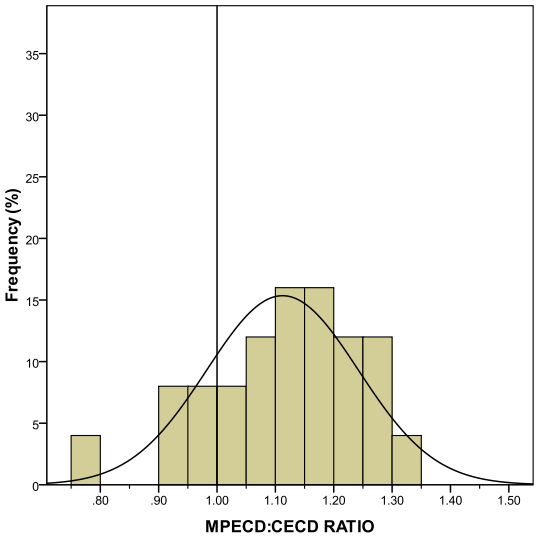


Figure 3.4-19
Histogram showing the frequencies of individual endothelial MPECD:CECD ratios at a single occasion (baseline) before *LASIK*. The reference line shows the level of no difference between MPECD and CECD. N = 25.

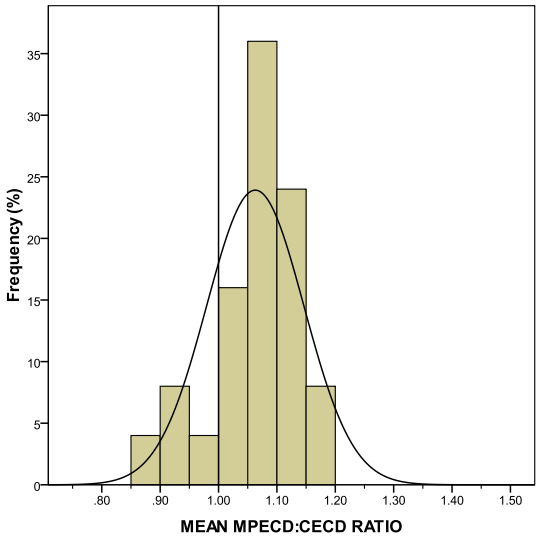


Figure 3.4-20
Histogram showing the overall frequencies of individual mean endothelial MPECD:CECD ratios in a group of post-*LASIK subjects* (n = 25) from five occasions over a period of two years. The reference line shows the level of no difference between MPECD and CECD.

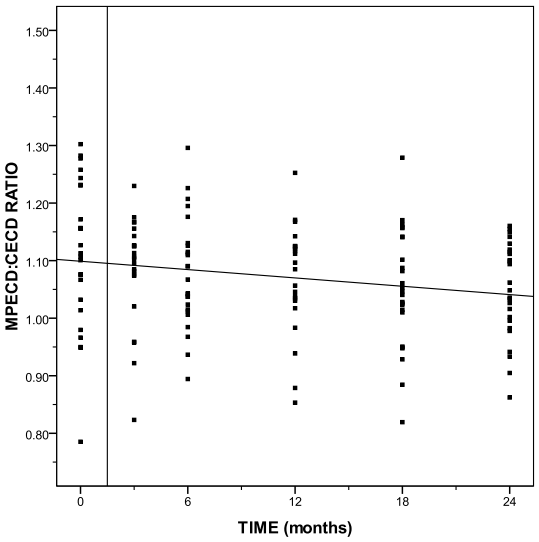


Figure 3.4-21
Regression analysis of the endothelial MPECD:CECD ratio in LASIK subjects over a period of two years (p = 0.014, r = -0.204). The reference line indicates the intervention of *LASIK*

For the post-LASIK group, endothelial cell densities were also assessed after correcting for the possible effects of image magnification changes that arise since the central and mid-peripheral corneal thickness is significantly altered by the surgery (Table 3.4-11). It has been concluded, partly based on theoretical considerations, the estimates of ECD in a thin cornea will be over-estimated by the IMAGEnet software (Isager *et al.*, 1996). However, adjusting for magnification errors did not significantly affect the main results (see the appendix, section 5.5.1).

Endothelial polymegethism -COV

At baseline (i.e. pre-operatively), the degree of polymegethism in the central region of the endothelium (CCOV) in the LASIK subjects averaged 34.3 ± 5.4%, with the individual values ranging from 27.3 to 47.8% (Table 3.4-11). Three months after surgery, a very noticeable reduction in the CCOV was noted, with the average CCOV then being 30.1 ± 5.7%. This net reduction of 4.2% (95% CI =1.4 to 7.0%) was statistically significant (p = 0.001, Bonferroni correction). Further small reductions in CCOV were noted at the 6, 12 and 24-month assessments (Table 3.4-11), although the median CCOV values fluctuated slightly rather than consistently going down (Figure 3.4-22). These later inter-visit changes, of 0.3 to 1.1%, were not statistically significant (p > 0.25, Bonferroni correction). After 2 years, the mean CCOV was 5.7% lower than at baseline (95% CI = 2.3 to 9.1%) and this reduction was also highly statistically significant (p < 0.001, Bonferroni correction).

Table 3.4-12
Polymegethism (mean ± 1SD of the coefficient of variation in cell area; COV) of the central (CCOV) and mid-peripheral (MPCOV) corneal endothelium before and at five occasions (over a two-year period) after *LASIK* (n = 20). Numbers in brackets represent the minimum and maximum observations. A paired t-test was used to explore any regional differences and repeated ANOVA for time related changes (α = 0.05).

COV	Time (months)						p (ANOVA)
	Pre-LASIK	3	6	12	18	24	
CCOV (%)	34.3 ± 5.4 (27.3 - 47.8)	30.1 ± 5.7 (19.8 - 41.3)	29.8 ± 5.6 (19.9 - 40.9)	28.7 ± 5.2 (22.9 - 42.9)	28.3 ± 5.1 (18.9 - 36.6)	28.6 ± 5.0 (19.6 - 37.7)	<0.001
MPCOV (%)	33.5 ± 8.1 (20.0 - 61.4)	32.1 ± 6.7 (23.3 - 53.8)	29.6 ± 5.0 (20.5 - 39.5)	30.6 ± 5.4 (21.3 - 43.4)	28.7 ± 5.1 (19.8 - 41.7)	29.8 ± 5.8 (22.0 - 43.2)	<0.001
p (paired t-test)	0.528	0.076	0.847	0.042	0.708	0.285	
MPCOV:CCOV ratio	0.976 ± 0.163 (0.660 - 1.285)	1.075 ± 0.146 (0.785 - 1.304)	1.007 ± 0.153 (0.791 - 1.334)	1.077 ± 0.150 (0.796 - 1.398)	1.034 ± 0.196 (0.749 - 1.491)	1.049 ± 0.173 (0.758 - 1.377)	0.193

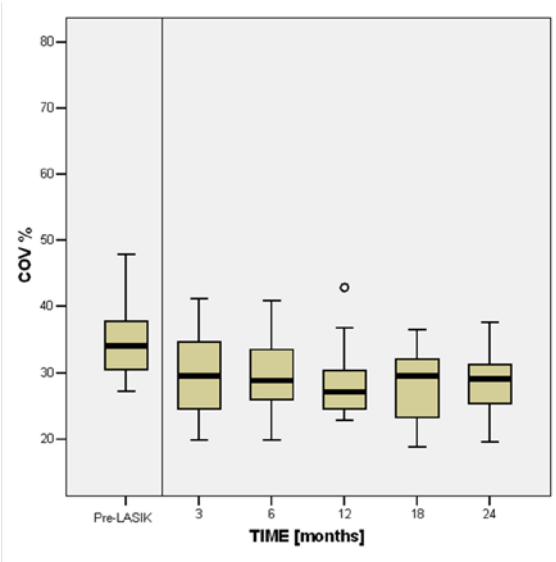


Figure 3.4-22
Box-plots of the degree of endothelial polymegethism in the central cornea (CCOV) before and at five occasions over 24 months after *LASIK* (n = 20).

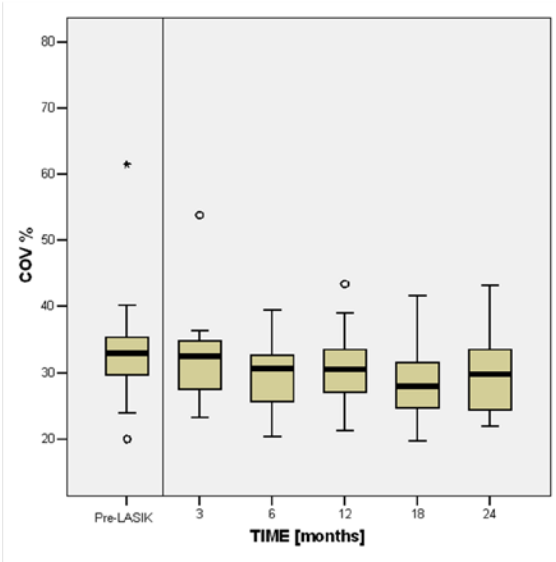


Figure 3.4-23
Box-plots of the degree of endothelial polymegethism in the mid-peripheral corneas (MPCOV) before and at five occasions over 24 months after *LASIK* (n = 20).

The polymegethism of the mid-peripheral parts of the corneal endothelium (MPCOV) averaged 33.5 ± 8.1% before surgery, with individual endothelia values ranging from 20.0 to 61.4% (Table 3.4-11). The very high MPCOV of 61.4% was clearly an outlier (Figure 3.4-23). As with the CCOV, the MPCOV values declined within the first three months after the LASIK surgery, but only by 1.4% and the median MPCOV value changed even less than this (Figure 3.4-23). Thereafter, the decline in MPCOV was even less, and a significant further reduction was not detected between assessments by Bonferroni post-hoc analyses (p > 0.09).

Overall, the mean CCOV value, in the post-LASIK subjects over the 6 visits, was 30.0% with average values ranging from 28.6 to 34.3%. Overall, the mean MPCOV in these post-LASIK subjects was 30.7% (with average values from 28.7 to 33.5%). Specific analyses for any time-related change in CCOV value revealed a statistically significant decline of -2.2% / year (p = 0.002), but with a rather large variance on the slope estimate (of ± 1.7% / year), the r-value

was only fair (r = -0.278; see Table 5.5-1 in the appendix and Figure 3.4-24). The main effect appeared to be in the first 3 months, for if baseline values were left out from the analysis, no net time-related changes in COV could be detected (r = -0.12, p = 0.240). Similar to the CCOV values, a statistically significant but even weaker time-related change was identified by linear regression analyses for mid-peripheral sites (Table 5.5-1). The slope of the linear regression line was only -0.15 ± 0.21 (r = -0.199), but still significantly different from zero (p = 0.029, see Figure 3.4-25).

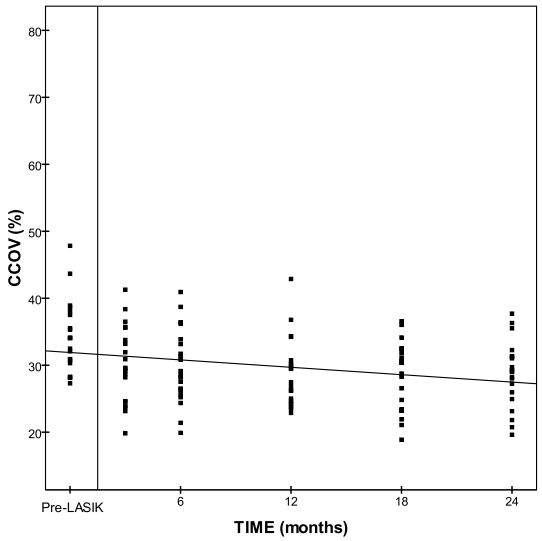


Figure 3.4-24
Scatter plots to show changes in the degree of endothelial polymegethism (COV) in the central cornea of *post-LASIK subjects* (n = 20). P = 0.002, r = -0.278.

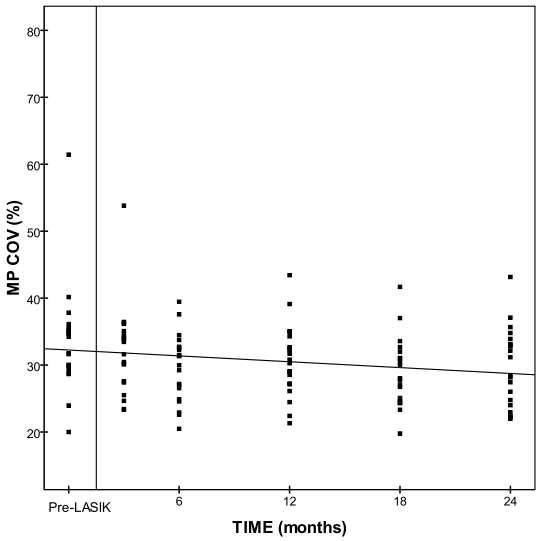


Figure 3.4-25
Scatter plots to show changes in the degree of endothelial polymegethism (COV) in the mid-peripheral cornea of in *post-LASIK subjects* (n = 20). P = 0.029, r = -0.199

Endothelial pleomorphism - %SIX

Analyses of the pleomorphism (%SIX) of the LASIK group revealed some unexpected differences and changes. Before the LASIK surgery, this group of contact lens wearers had only a mean ± 1SD C%SIX of 50.5 ± 9.3%. There was also a very wide range of values, from 32.3 to 74.6% (Table 3.4-13). At the 3 months' assessment after the successful LASIK surgery, examination of the endothelial images indicated that the C%SIX 56.8 ± 10.6%, i.e. had notably increased from pre-surgery (baseline) values) even though a similarly wide range of values (of 33.0 to 75.0%) was still present. This change was statistically significant (p = 0.036, Bonferroni correction). The C%SIX continued to increase at later assessments (Figure 3.4-26), but no significant inter-visit changes could be detected later in the study. At the end of the study, the mean C%SIX was still 6.0% higher than at baseline. However, this difference was not statistically significant (p = 0.342, Bonferroni correction).

Table 3.4-13
Percentage of six-sided cells (%SIX) (mean ± 1SD) of the central (C%SIX) and mid-peripheral (MP%SIX) corneal endothelium before and at five occasions (over a two-year period) after *LASIK* (n = 20). Numbers in brackets represent the minimum and maximum observations. A paired t-test was used to explore any regional differences and repeated ANOVA for time related changes (α = 0.05).

%SIX	Time (months)						p (ANOVA)
	Pre-LASIK	3	6	12	18	24	
C%SIX (%)	50.5 ± 9.3 (32.3 - 74.6)	56.8 ± 10.6 (33.3 - 75.0)	56.0 ± 8.9 (32.8 - 73.2)	58.4 ± 10.7 (29.2 - 80.7)	59.2 ± 9.9 (39.0 - 76.4)	56.5 ± 8.8 (38.9 - 72.4)	0.006
MP%SIX (%)	57.0 ± 11.8 (37.0 - 80.0)	58.1 ± 8.8 (35.6 - 73.0)	62.8 ± 9.4 (45.6 - 81.5)	58.5 ± 11.2 (35.8 - 83.1)	61.7 ± 7.3 (44.6 - 73.7)	63.6 ± 10.0 (45.9 - 79.0)	0.004
p (paired t-test)	0.011	0.614	0.002	0.946	0.249	0.015	
MP%SIX:C%SIX ratio	1.144 ± 0.215 (0.791 - 1.500)	1.057 ± 0.251 (0.712 - 1.632)	1.136 ± 0.155 (0.737 - 1.402)	1.021 ± 0.195 (0.744 - 1.474)	1.061 ± 0.167 (0.840 - 1.347)	1.145 ± 0.213 (0.783 - 1.553)	0.172

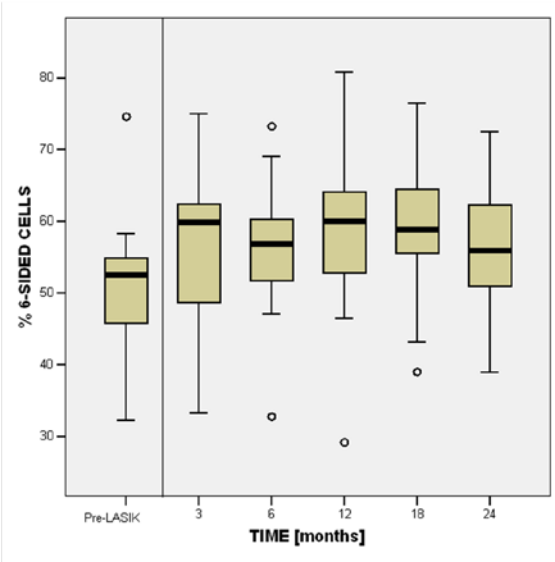


Figure 3.4-26
Box-plots of the degree of endothelial pleomorphism in the central cornea (C%SIX) before and at five occasions over 24 months after *LASIK* (n = 20).

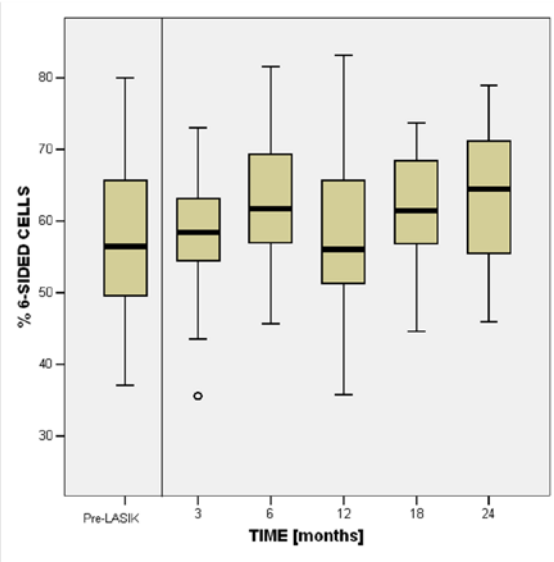


Figure 3.4-27
Box-plots of the degree of endothelial pleomorphism in the mid-peripheral corneas (MP%SIX) before and at five occasions over 24 months after *LASIK* (n = 20).

Before surgery, the MP%SIX was larger than C%SIX at 57.0 ± 11.8% vs. 50.5 ± 9.3%, respectively (p = 0.011). Similar regional differences were also noted at later occasions (Table 3.4-13). The MP%SIX did also increase after LASIK, however at a slower rate than it did centrally. A moderate increase was first seen after 6 months when mean MP%SIX was 62.8 ± 9.4% (p = 0.064, Bonferroni correction) (Figure 3.4-27). After 6 months, the MP%SIX did not change much, and at 24 months the mean MP%SIX in post-LASIK subjects was 63.6 ± 10.0%.

The overall mean C%SIX in LASIK subjects over the 6 visits was 56.2% (with group mean values from 50.5 to 59.2%). MP%SIX was 4.1% larger (60.3%). The difference was significant, p < 0.001. A regression analysis revealed no linear relationship between time and the C%SIX (r = 0.171, p = 0.061). On the other hand, a weak but statistically significant time-related positive correlation was found for MP%SIX (r = 0.182, p = 0.047; see also Table 5.5-1 in the appendix).

Endothelial morphometric inter-relationships

In soft lens wearers who underwent LASIK for their myopia, 20 subjects completed the study and thus had five post-operative measurements of endothelial morphometric parameters. Four subjects had four measurements and one subject had three measurements; hence, the total group size for post-LASIK subjects was 25 for the correlation analyses.

At baseline, before LASIK, endothelial cell density showed no obvious relationship with the degree of polymegethism, in neither the central nor the mid-peripheral parts of the endothelium. Similarly, cell density showed no relationship to the percentage of 6-sided cells. See Table 5.5-2 in the appendix for correlation coefficients and probability values. The event of LASIK did not change these results (See Table 5.5-3 in the appendix).

On the other hand, the degree of polymegethism (COV) correlated strongly with the degree of pleomorphism (%SIX), both in the central ($r = -0.660$, $p < 0.001$) and mid-peripheral part ($r = -0.690$, $p < 0.001$) of the endothelium prior to surgery. Endotheliae with higher COV values had lower %SIX values (see Figure 3.4-28 and Figure 3.4-29). After LASIK, the slope of the regression line that represented the COV vs. %SIX steepened slightly, reflecting a reduction in the degree of polymegethism (COV) and pleomorphism (i.e. increase of %SIX), however, the relationship remained strong (see Figure 3.4-28, $r = 0.734$, $p < 0.001$). In the mid-peripheral area this relationship also remained stable after LASIK (Figure 3.4-29, $r = 0.872$, $p < 0.001$).

Strong, positive correlations were found between central and mid-peripheral morphometric parameters before LASIK. For example, as shown in Figure 3.4-30, high CECD was associated with high MPECD ($r = 0.479$, $p = 0.015$). This relationship remained strong after LASIK too. Furthermore, an individual in this group of soft lens wearers was more likely to have higher MPECD values than CECD values before LASIK (see Figure 3.4-30). After LASIK, the regression line moved slightly in the direction of a more evenly spread distribution. In spite of this shift, most individuals still had higher MPECD values after LASIK.

Similar strong relationships were also found for the COV and %SIX cells variables (Figure 3.4-31 and Figure 3.4-32): the more polymegethism and pleomorphism centrally, the more mid-peripherally.
While more subjects had higher CCOV values than MPCOV values before LASIK, an individual was more likely to have a close to even distribution of the degree of polymegethism after LASIK. Two thirds of the subjects had higher MP%SIX than C%SIX whereas the remaining third had similar, or slightly higher C%SIX before surgery (Figure 3.4-32). However, after LASIK, the data was closer to the regression line, which shifted in the direction of a one:one relationship.

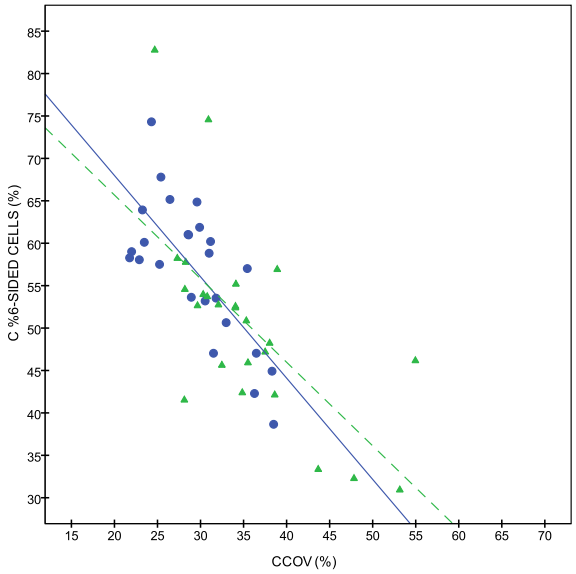


Figure 3.4-28
Scatter plots and linear regression analysis showing the relationship between mean CCOV and C%SIX before (triangles, dashed regression line, $p < 0.001$, $r = -0.660$) and after *LASIK* (closed circles, solid line , $p < 0.001$, $r = -0.734$). N = 25.

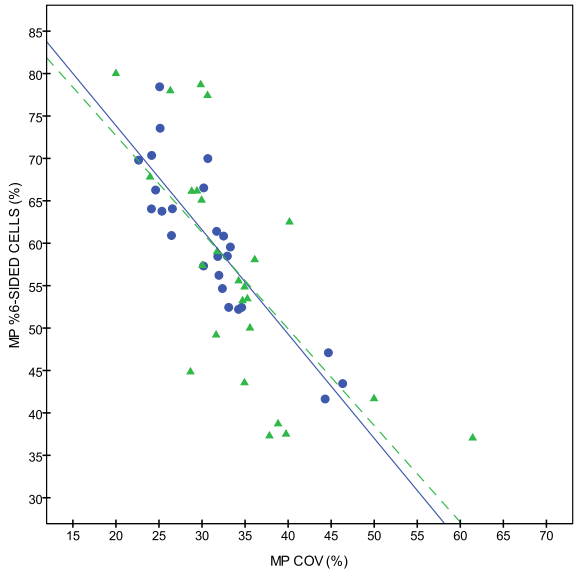


Figure 3.4-29
Scatter plots and linear regression analysis showing the relationship between mean MPCOV and MP%SIX before (triangles, dashed regression line, $p < 0.001$, $r = -0.690$) and after *LASIK* (closed circles, solid line , $p < 0.001$, $r = -0.872$). N = 25.

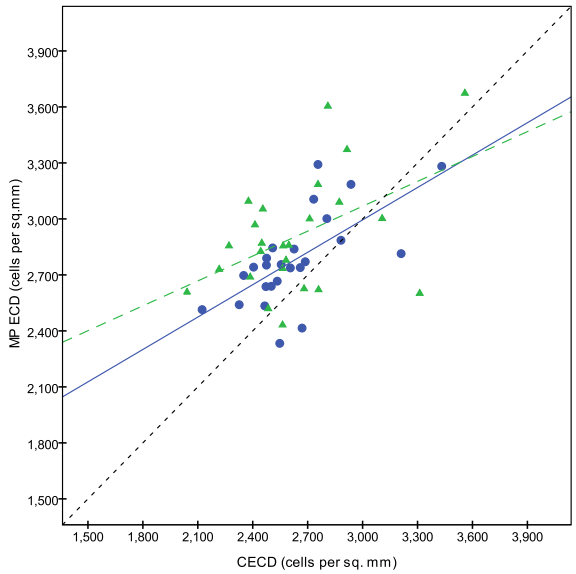


Figure 3.4-30
Scatter plots and linear regression analysis showing the relationship between mean CECD and MPECD before (triangles, dashed regression line, $p = 0.015$, $r = 0.479$) and after *LASIK* (closed circles, solid line , $p < 0.001$, $r = 0.652$). The dotted line shows the one:one correlation. N = 25.

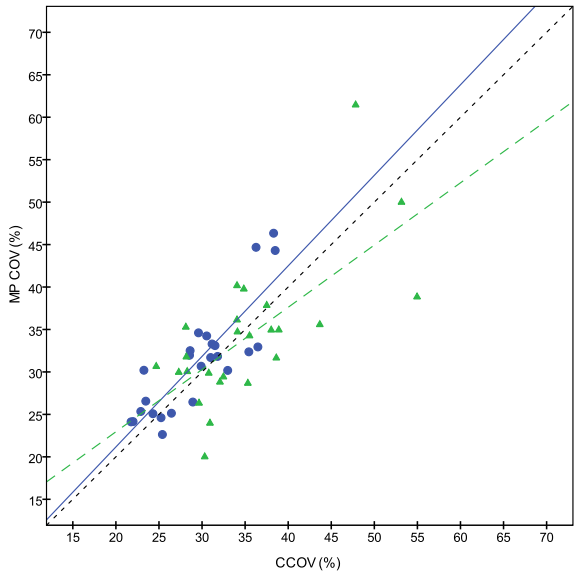


Figure 3.4-31
Scatter plots and linear regression analysis showing the relationship between mean CCOV and MPCOV before (triangles, dashed regression line, $p < 0.001$, $r = 0.683$) and after *LASIK* (closed circles, solid line , $p < 0.001$, $r = 0.847$). The dotted line shows the one:one correlation. N = 25

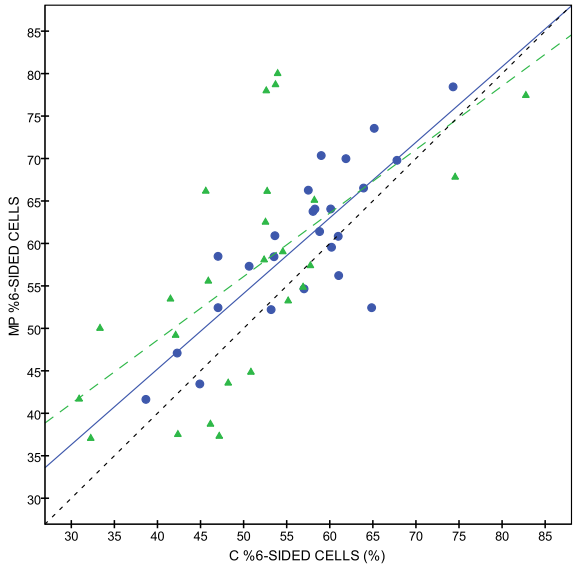


Figure 3.4-32
Scatter plots and linear regression analysis showing the relationship between mean C%SIX and MP%SIX before (triangles, dashed regression line, p = 0.001, r = 0.631) and after *LASIK* (closed circles, solid line , p < 0.001, r = 0.816). The dotted line shows the one:one correlation. N = 25

3.5 RESULTS FOR BETWEEN-GROUP COMPARISONS

This section summarises the main results of the four preceding studies. Comparisons between groups are illustrated by time lines. Because of the large variability in the data (especially for the tear film measurements), error bars have been omitted from the graphs. For complete information of the variability in the data, the reader is referred to the preceding sections.

3.5.1 Group demographics and vision assessments

Age, gender and contact lens wear

The mean age of the 92 successfully enrolled subjects was 29.6 ± 5.7 years. Kruskal-Wallis test revealed a significant difference between the age distribution of the four groups (p = 0.010). The subjects who were refitted with SiH lenses were aged 26.8 ± 6.0 years and so were significantly younger than both the pre-LASIK subjects at 31.3 ± 5.4 years (p = 0.007, Mann-Whitney U (MWU) test) and the spectacle wearers at 32.3 ± 5.8 years (p = 0.010, MWU, Table 3.5-1).

The distribution of men and women in the four test-groups was very similar (see Table 3.5-1). More men than women participated in the spectacle group (57%) and LASIK group (54%) and vice versa in the soft lens (44%) group and SiH group (39%). However, the between-group differences were not significant (p = 0.548, chi sq. test).

The originally enrolled contact lens wearers (N = 77) had worn lenses for 10.0 ± 5.0 years (range 2.5 to 23.0 years). A Kruskal-Wallis test revealed that the length of wear differed significantly between groups (p = 0.005). On average, those lens wearers who were refitted with SiH lenses had worn lenses 4 years less than pre-LASIK subjects had and 2.8 years less than soft lens wearers (p ≤ 0.03, MWU). See also Table 3.5-1. The distribution of contact lens type and disinfection systems was equal between groups (p ≥ 0.3, MWU).

Table 3.5-1
Gender distribution, mean age and years of lens wear (±1SD) for all test groups at baseline.

	SPX	SCL	SIH	LASIK
N	21	25	26	26
Men / Women (%)	57 / 43	44 / 56	39 / 61	54 / 46
Age (years)*	31 ± 6	29 ± 4	27 ± 6	32 ± 5
Years of lens wear*	NA	11 ± 4	8 ± 4	12 ± 5
Refractive error (MSE)[a*]	-2.11 ± 1.72	-3.66 ± 1.40	-3.68 ± 2.37	-4.38 ± 1.58

* Statistically significant differences between groups. See text for details.
[a] Mean refractive error (MSE) in spherical equivalent power (DS).

Refractive error

The mean refractive error for the enrolled subjects was -3.71 ± 1.87 DS (mean spherical equivalent; MSE). Kruskal-Wallis test revealed that MSE differed significantly between groups at baseline (p = 0.013, Table 3.5-1). Spectacle wearers had the lowest MSE of -2.11 ± 1.71 DS which was 2.27 DS less myopia than the MSE in pre-LASIK subjects at -4.38 ± 1.58 DS (p = 0.003, MWU). The other two test groups had slightly less myopia at -3.66 ± 1.40 DS and -3.68 ± 2.37 DS than did the pre-LASIK subjects, but were not significantly different from any group.

After intervention, obviously LASIK subjects had significantly lower refractive error. For all test groups, mean spherical equivalent remained stable through the study (see Figure 3.5-1).

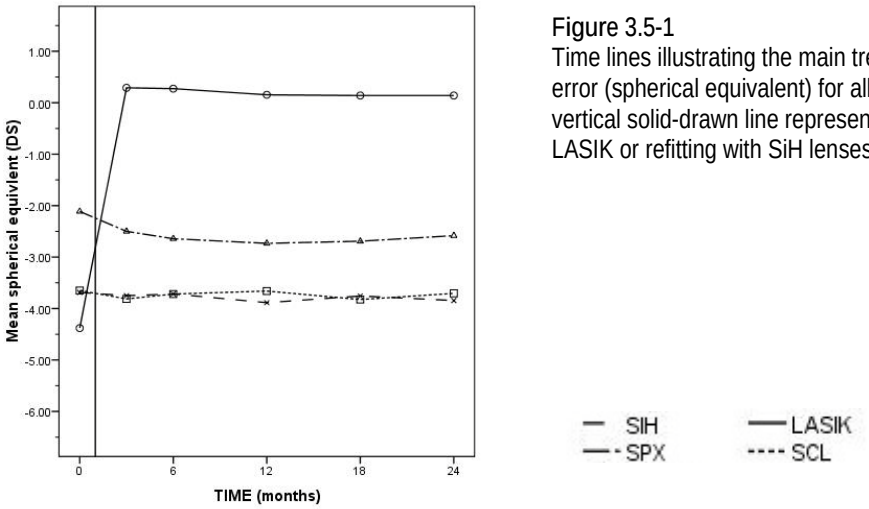


Figure 3.5-1
Time lines illustrating the main trends of mean refractive error (spherical equivalent) for all subject groups. The vertical solid-drawn line represents the intervention of LASIK or refitting with SiH lenses.

Best Corrected Visual Acuity - BCVA

Overall best-corrected *high contrast* visual acuity (BCHCVA) for the 92 enrolled subjects was -0.07 ± 0.12 LogMAR units at baseline. Total mean best-corrected *low contrast* visual acuity (BCLCVA) was 0.11 ± 0.11 logMAR. Statistically significant differences between groups were found for both BCHCVA and BCLCVA at baseline ($p < 0.001$, K-W). Post-hoc analyses, using the Mann-Whitney U test, revealed that both BCHCVA and BCLCVA in pre-LASIK subjects (at -0.01 ± 0.09 and 0.18 ± 0.11 logMAR) were close to one VA line poorer than for the other three groups ($p \leq 0.001$, Figure 3.5-2 A and B). These had BCHCVA measures ranging from -0.08 ± 0.07 logMAR (soft lens wearers) to -0.10 ± 0.09 logMAR (spectacle wearers) and BCLCVA measures ranging from 0.07 ± 0.11 logMAR (pre-SiH lens wearers) to 0.11 ± 0.10 logMAR (spectacle wearers).

After LASIK, no significant differences in BCHCVA or BCLCVA were found between the LASIK subjects and the other three groups. However, statistically significant differences were found between BCHCVA in SiH wearers and SCL wearers at 18 months ($p = 0.002$, MWU) and at 24 months ($p = 0.001$, MWU). The differences were only around 0.05 (i.e. half VA line) and not clinically significant (see Figure 3.5-2A). Furthermore, SiH lens wearers had significantly better BCLCVA than SCL wearers and post-LASIK subjects at 12 and 24 months ($p \leq 0.001$, K-W, Figure 3.5-2B).

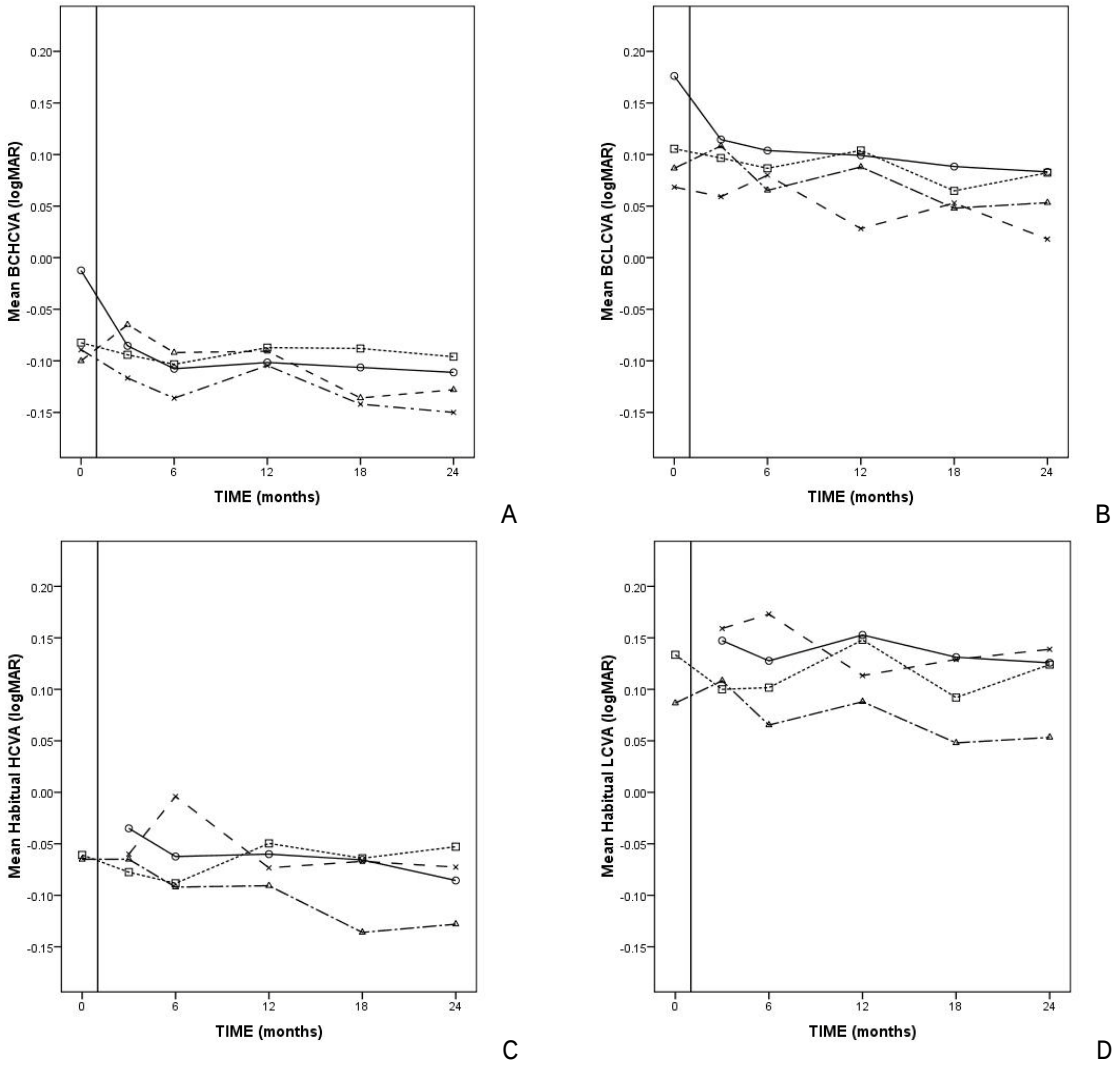


Figure 3.5-2
Time lines illustrating the main trends of mean logMAR BCHCVA (A), BCLCVA (B), *habitual* HCVA (C) and *habitual* LCVA (D) for all subject groups. The vertical solid-drawn lines represent the intervention of LASIK or refitting with SiH lenses.

Habitual Visual Acuity

Because many pre-LASIK subjects and soft lens wearers who were going to be refitted with SiH lenses did not wear their habitual lenses at the first visit (baseline), habitual VA measurements were only obtained from spectacle wearers and SCL wearers. No between-group differences was found at baseline for habitual HCVA ($p = 0.075$, MWU) or habitual LCVA ($p = 0.017$, MWU). During the first year after LASIK / refitting, no differences in habitual LCVA were found between the four test groups. However at 18 and 24 months, spectacle wearers had close to one line better habitual HCVA than SCL wearers ($p < 0.01$, MWU). Similarly, spectacle wearers tended to have better habitual LCVA than the other tree test groups, and the differences of up to one VA line were statistically significant at 18 months ($p < 0.01$, MWU). See also **Error! Reference source not found.**C and D.

3.5.2 Ocular comfort

At the beginning of this study, the proportion of subjects reporting at least one dry eye symptom was similar for the four groups of subjects. Between 80 and 90% of the subjects reported having one or more ocular symptom. The symptom severity was also very similar in all four groups at baseline ($p = 0.780$, Kruskal-Wallis test). At the last visit, 24 months later, the proportion of subjects reporting symptoms post-LASIK was only 60% while in spectacle wearers and contact lens wearers 80 to 85% reported symptoms occurring at least sometimes. Moreover, the mean values for later visits indicated that the symptom severity in the SiH lens wearers and post-LASIK subjects was slightly lower (see Figure 3.5-3), but the reduction was not statistically different compared to other two test groups ($p \geq 0.190$, Kruskal-Wallis test).

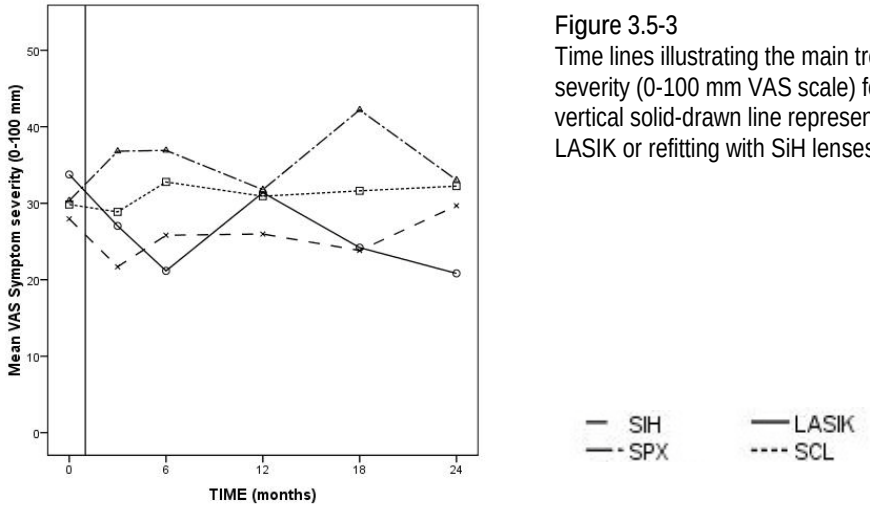


Figure 3.5-3
Time lines illustrating the main trends of mean symptom severity (0-100 mm VAS scale) for all subject groups. The vertical solid-drawn line represents the intervention of LASIK or refitting with SiH lenses.

3.5.3 Tear film characteristics

Tear meniscus height

TMH measurements were obtained from 88 of the 92 originally enrolled subjects. At baseline, the total mean TMH was 0.23 ± 0.08 mm (range 0.1 to 0.6). The group mean TMH measures differed from each other with maximum 0.05 mm (p = 0.394, Kruskal-Wallis test, see also Figure 3.5-4). Similarly, no statistically significant difference was detected between lens wearers and non-lens wearers (p = 0.904, Mann-Whitney U test).

In contrast to the situation at baseline, the TMH measures differed significantly between the groups at 3 months (p = 0.015, K-W). Post-LASIK subjects had a smaller average TMH value than did the spectacle wearers and SiH lens wearers. A Mann-Whitney U test revealed that the difference of 0.03 mm between mean TMH values in SiH lens wearers and post-LASIK subjects was significant at a level of 0.01. However, at later visits the between-group similarities were even more than it was at baseline: At the end of the study, the difference in mean TMH measures was 0.02 mm, which was clearly not significant (p = 0.430, K-W). No difference was found when comparing subjects who had had LASIK performed with those who did not (p = 0.176, MWU) or when comparing post-LASIK subjects with lens wearers (p = 0.188, MWU). In total, mean TMH was 0.22 ± 0.07 mm (range 0.08 to 0.40) at 24 months. The relatively large discrepancy between the TMH measurements of subjects examined in Oslo (SCL and LASIK subjects) and those examined in Kongsberg (SIH and spectacle wearers) seen at 12 months (see Figure 3.5-4) were due to a missing graticule gauge that should be attached to one of the ocular eyepieces on the slit lamp biomicroscope. Instead, TMH measurements were estimated using the slit width scale.

Phenol red thread test

PRT measures were obtained from all 92 enrolled subjects. The overall mean PRT was 18.6 ± 8.1 mm and each group mean PRT measure differed from each other by only 0.01 to 2.8 mm. At baseline, soft lens wearers had the lowest mean PRT measure at 16.6 ± 8.3 mm, whereas the three other groups had mean measures of 19.2, 19.3 and 19.4 mm (Figure 3.5-4). However, the inter-group difference of nearly 3 mm was not statistically significant (p = 0.478, K-W). As for TMH values, the PRT measures were found to differ significantly between groups at 3 months (p = 0.003, K-W). Post-LASIK subjects had a lower measure at 16.9 ± 8.0 mm compared to the subjects who had not gone through surgery (p = 0.006, M-W). The latter groups had average PRT measures that ranged from 19.5 to 25.5 mm. However, the differences were not solely due to a reduction in PRT values in post-LASIK subjects but just as much due to increased PRT values in SiH lens wearers (see Figure 3.5-4). SiH lens wearers had significantly larger PRT values than post-LASIK subjects and SCL wearers (p ≤ 0.01, MWU) both at the 3 and 6 month's assessments. Later, the PRT test values were more similar for the four groups. At 24 months, group average PRT measures differed by only up to 2.8 mm (between SCL wearers and post-LASIK subjects), which was not statistically significant (p = 0.478, K-W).

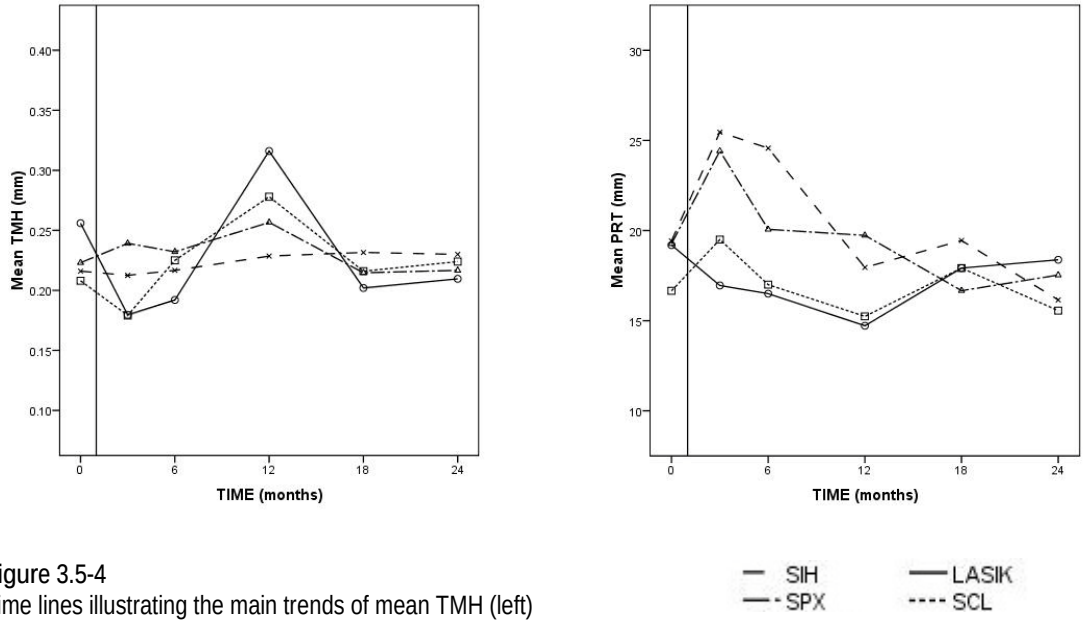


Figure 3.5-4
Time lines illustrating the main trends of mean TMH (left) and PRT values (right) for all subject groups. The vertical solid-drawn lines represent the intervention of LASIK or refitting with SiH lenses.

Non-invasive tear break-up time

NIBUT measures were obtained from all 92 enrolled subjects at baseline and the overall NIBUT was 28.2 ± 25.6 seconds. For all four subject groups, the non-invasive tear break up time (NIBUT) values varied considerably at all occasions and no statistically significant inter-group differences could be identified at baseline or at most later occasions ($p \geq 0.307$, K-W). An exception was seen at 12 months ($p = 0.001$, K-W) where SiH lens wearers had 16.4 and 18.6 seconds shorter mean NIBUT than -LASIK subjects and post spectacle wearers, respectively ($p < 0.01$, MWU). See also Figure 3.5-5.

Fluorescein-tear break-up time

Fluorescein-TBUT measures were obtained from 87 of the 92 enrolled subjects at baseline and the overall f-TBUT was 18.3 ± 23.8 seconds. Similar to NIBUT measures, considerable intra-group variations were also noted for f-TBUT. However, the distributions of measures varied significantly between the groups. Non-parametric tests revealed statistically significant between-group differences at all occasions ($p \leq 0.024$, K-W). Post-hoc analyses revealed that the LASIK and soft contact lens groups generally had longer f-TBUT measures than the spectacle and SiH contact lens group ($p \leq 0.01$, MWU, Figure 3.5-5). For example, at three months the mean f-TBUT value for post-LASIK and SCL wearers were 39.7 ± 107 and 23.7 ± 21.7 seconds, respectively whereas in SiH-lens wearers and spectacle wearers the respective values were 10.8 ± 7.4 and 9.6 ± 6.7 seconds.

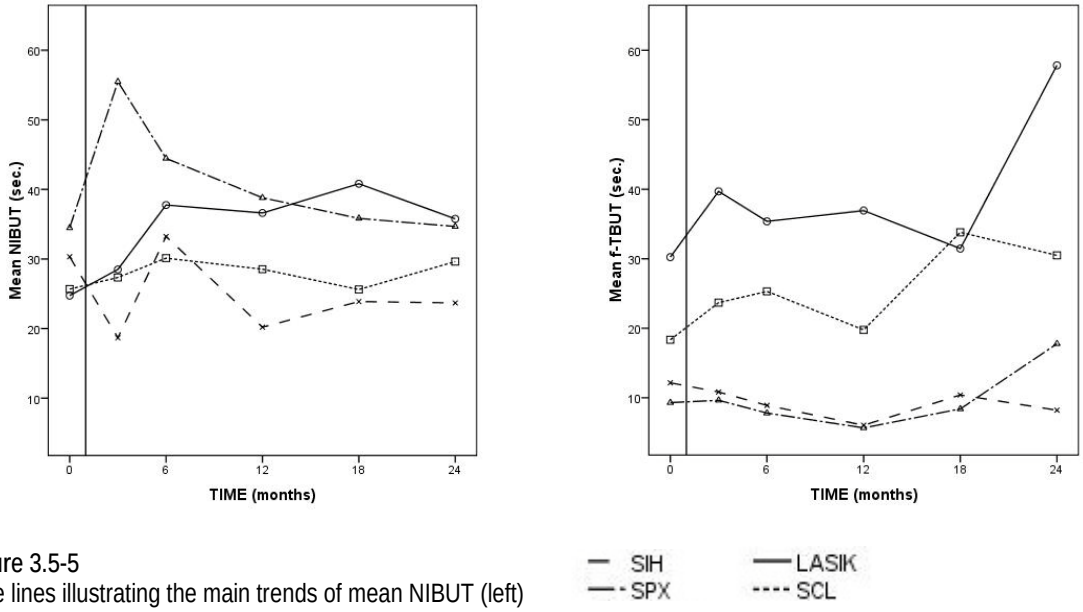


Figure 3.5-5
Time lines illustrating the main trends of mean NIBUT (left) and f-TBUT values (right) for all subject groups. The vertical solid-drawn lines represent the intervention of LASIK or refitting with SiH lenses.

3.5.4 Ocular surface characteristics

Eye redness

The overall grading of conjunctival hyperaemia was 1.3 ± 0.6 at baseline. Inter-group differences were evident by Kruskal-Wallis test ($p < 0.001$). Soft lens wearers had 0.3 to 1.0 grading-units higher visible conjunctival redness than did the other groups ($p \leq 0.002$, M-W U), which is also a clinically significant difference. Statistically significant inter-group differences were also found at later occasions. However, these were in the order of ≤ 0.3 units and not considered clinically significant. See also Figure 3.5-6 A.

At baseline, overall mean limbal redness was 1.0 ± 0.5. Similar to conjunctival redness at baseline, limbal redness differed significantly between groups ($p < 0.001$, K-W). Average limbal redness was graded 0.4 to 0.6 grading-units higher in soft lens wearers than the average of 0.6 ± 0.2 found in spectacle wearers ($p \leq 0.012$, MWU). Three months after intervention, mean limbal redness was slightly lower in post-LASIK subjects and SiH lens wearers and these two groups did no longer differ from spectacle wearers ($p \geq 0.224$, MWU). At the end of the study, limbal redness was very similar in all four groups and no differences were detected ($p = 0.420$, K-W). See also Figure 3.5-6 B

At baseline, no inter-group differences of the degree of corneal vascularisation could be detected by Kruskal-Wallis test ($p = 0.166$). Overall, corneal vascularisation was graded very mildly to a mean of 0.7 ± 0.4. At 3 months significant inter-group differences in corneal vascularisation grading were found ($p = 0.005$, K-W). Corneal vascularisation had increased in post-LASIK subjects to a mean of 0.9 ± 0.5, which was significantly higher than in spectacle wearers (0.4 ± 0.2, $p = 0.004$, MWU). Soft lens wearers had a mean vascularisation grading of 0.8 ± 0.4, and this was also significantly higher than in spectacle wearers ($p = 0.003$) but not different from SiH lens wearers (0.6 ± 0.3, $p = 0.085$, MWU). At the end of the study, no inter-group differences in corneal vascularisation grading were detected ($p = 0.053$, K-W). See also Figure 3.5-6 C

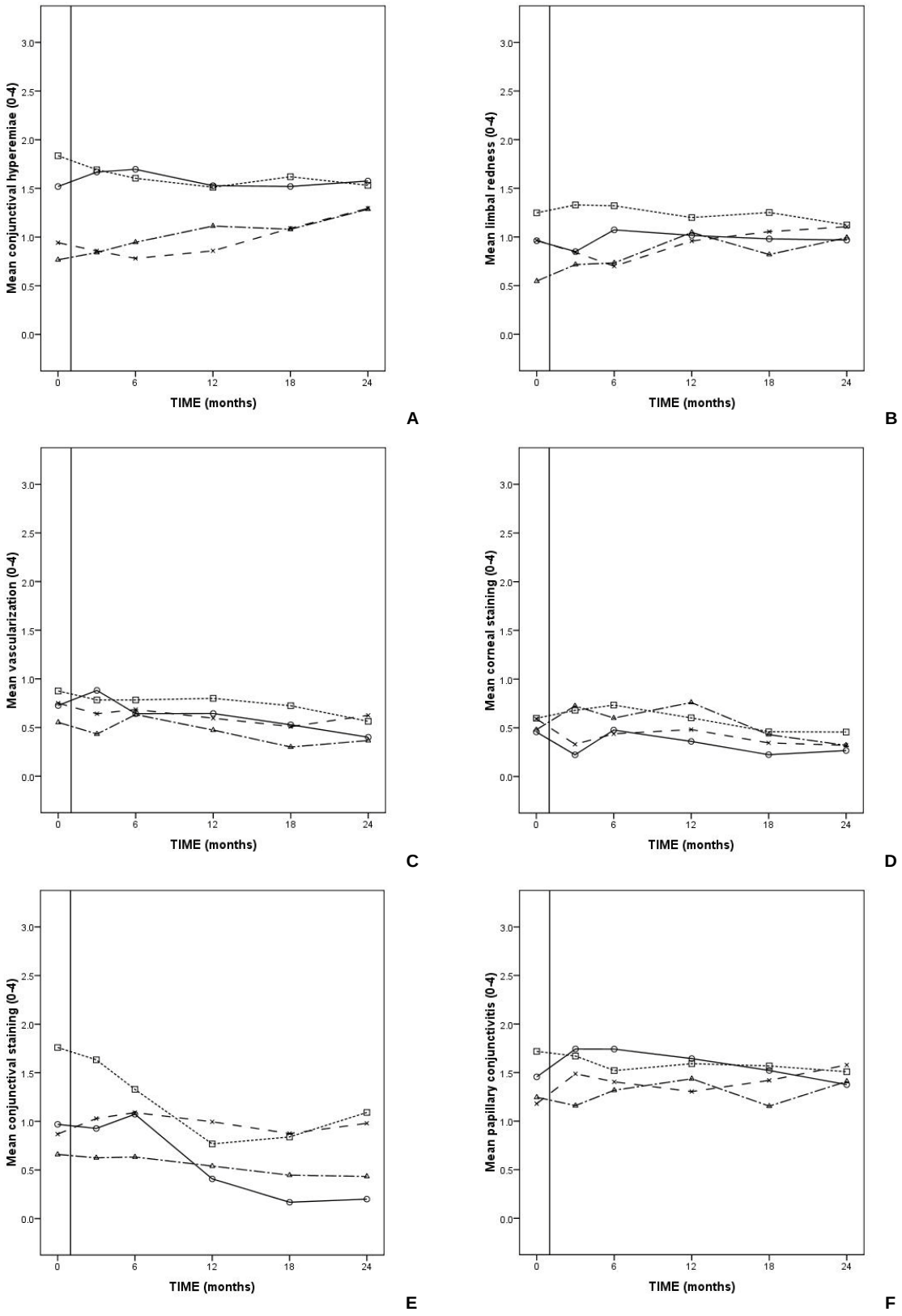


Figure 3.5-6 (A-F)
Time lines illustrating the main trends of mean grading of ocular surface characteristics using Efron's printed grading scales (0-4) for all subject groups. The vertical solid-drawn lines represent the intervention of LASIK or refitting with SiH lenses.

Fluorescein staining of the ocular surface

The overall mean grading of corneal fluorescein staining at baseline was 0.5 ± 0.6 and no significant differences was found between the four test groups (p = 0.625, KW). Similarly, at later occasions no significant between-group differences were found (p ≥ 066, KW). See also Figure 3.5-6 D. An exception was seen at the 3 month's assessment where spectacle wearers had the highest mean grading of corneal staining at 0.7 ± 0.8, which was significantly greater than the grading of staining for post-LASIK subjects at 0.2 ± 0.5 (p < 0.01, MWU). It should be noted, however, that the 3-month's assessment of spectacle wearers took place in the pollen season and over 15% had staining grading ≥ 2, which rather strongly influenced the mean value. The other three groups were seen just before or much later in the pollen season. The prevalence of seasonal allergies was 30 to 50% and was not different amongst the groups.

Conjunctival staining

At baseline the overall mean grading of conjunctival fluorescein staining was 1.1 ±1.0. Between-group differences was found (p = 0.008, KW) and post-hoc analyses revealed that spectacle wearers had significantly lower grading of conjunctival staining at 0.66 ± 0.66) than did the SCL wearers (at 1.76 ± 1.13, p <0.01, MWU). See also Figure 3.5-6 E.

After the intervention of LASIK or refitting (except from the 12 month's assessment), spectacle wearers consistently had significantly lower mean conjunctival staining grading (ranging from 0.43 to 0.66) than the two lens wearing groups which mean grading ranged from 0.78 to 1.63 (p < 0.01, MWU). See also Figure 3.5-6 E. Furthermore, conjunctival staining gradually reduced over time in post-LASIK subjects (Figure 3.5-6 E). During the second year after surgery the mean grading of conjunctival staining was significantly lower for LASIK subjects (ranging from 0.16 to 0.40) compared to SCL and SiH lens wearers whose mean grading ranged from 0.77 to 1.10 (Figure 3.5-6 E).

(Contact lens induced) papillary conjunctivitis

At baseline, significant inter-group differences in mean (contact lens induced) papillary conjunctivitis were revealed by the Kruskal-Wallis test (p = 0.001). The mean CLPC grade in soft lens wearers of 1.7 ± 0.4 was significantly larger than in pre-SiH lens wearers (1.2 ± 0.5) and spectacle wearers (1.3 ± 0.4, p ≤ 0.006 M-W U). In pre-LASIK subjects, mean CLPC was 1.5 ± 0.4 and did not differ significantly from any other group. Significant inter-group differences of mean CLPC were also present shortly at the 3 months' assessment (p = 0.004, K-W). Spectacle wearers had significantly less papillary conjunctivitis with a mean grade of 1.2 ± 0.3 compared to post-LASIK subjects (1.7 ± 0.5), soft lens wearers (1.7 ± 0.5) and SiH lens wearers (1.5 ± 0.3, p ≤ 0.009, M-W U). At the last assessment, no inter-group differences in CLPC grades were detected by Kruskal-Wallis test (p = 0.247). The mean CLPC grade was 1.4 ± 0.2, 1.5 ± 0.5, 1.6 ± 0.3 and 1.4 ± 0.4 in spectacle wearers, soft lens wearers, SiH lens wearers and post-LASIK subjects, respectively. See also Figure 3.5-6 F.

3.5.5 Corneal thickness and corneal curvature

Central corneal thickness measurements were obtained from 98 subjects, including the extra six spectacle wearers (see section 1.1.1 for details). At baseline, the total mean CCT was 530 ± 36 µm, ranging from 518 ± 41µm (spectacle wearers) to 538 ± 39 µm (SiH group). No statistically significant between-group differences was found (p = 0.323, ANOVA). However, there was a tendency of thicker CCT in soft lens wearers (p = 0.090, t-test): Mean CCT in subjects wearing contact lenses (N = 77, including soft lens wearers who later underwent LASIK or changed into SiH lenses) was 533 ± 35 µm, which was 2.9% more than the mean CCT in spectacle wearers. The differences in mean values between the lens-wearing groups were much smaller, in the order of 2 to 5 µm, i.e. < 0.01%. This tendency was also apparent at later visits, since CCT did not change over time for either group (see the respective previous sections on prospective analyses). At all later occasions, the central cornea was naturally significantly thinner for post-LASIK subjects. See also Figure 3.5-7

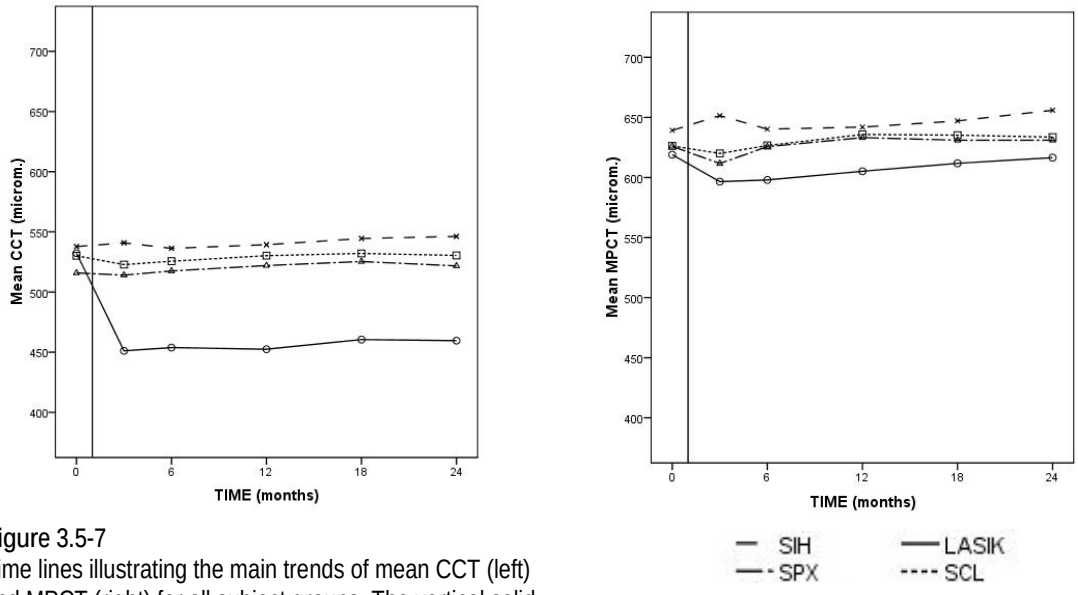


Figure 3.5-7
Time lines illustrating the main trends of mean CCT (left) and MPCT (right) for all subject groups. The vertical solid-drawn lines represent the intervention of LASIK or refitting with SiH lenses.

As for CCT, mid-peripheral corneal thickness measurements were obtained from 98 subjects at baseline, and the overall mean MPCT was 626 ± 48 µm. No statistically significant between-group differences were detected (p = 0.405, ANOVA). In contrast to CCT, no tendencies of differences in MPCT between lens wearers and spectacle wearers were found (p = 0.483, t-test). At three months, the SiH group had significantly thicker MPCT than spectacle wearers and soft lens wearers (p = 0.040, ANOVA). However, post-hoc analysis failed to show any statistically significant difference (Bonferroni test, p = 0.100). This tendency of thicker mid-peripheral corneal thickness in SiH lens wearers was transient and at the end of the study, no such tendency was evident (p = 0.421, ANOVA). As for CCT, MPCT was significantly thinner in post-LASIK subjects three

months after surgery. However, due to a small re-thickening with time (see section 3.4.5) the difference was no longer significant after two years (p = 0.142, ANOVA). See also Figure 3.5-7

Central corneal curvature measurements were obtained from 86 subjects at baseline, and the overall K was 7.88 ± 0.24 mm. No statistically significant between-group difference was found (p = 0.433, Kruskal-Wallis test). Similar results were obtained at 3 and 24 months when comparing spectacle wearers with the two contact lens wearing groups. At later occasions, central corneal curvature was obviously significantly flatter for post-LASIK subjects both at the first pre-operative assessment and at the end of the study (p < 0.001, Bonferroni correction). See also Figure 3.5-8.

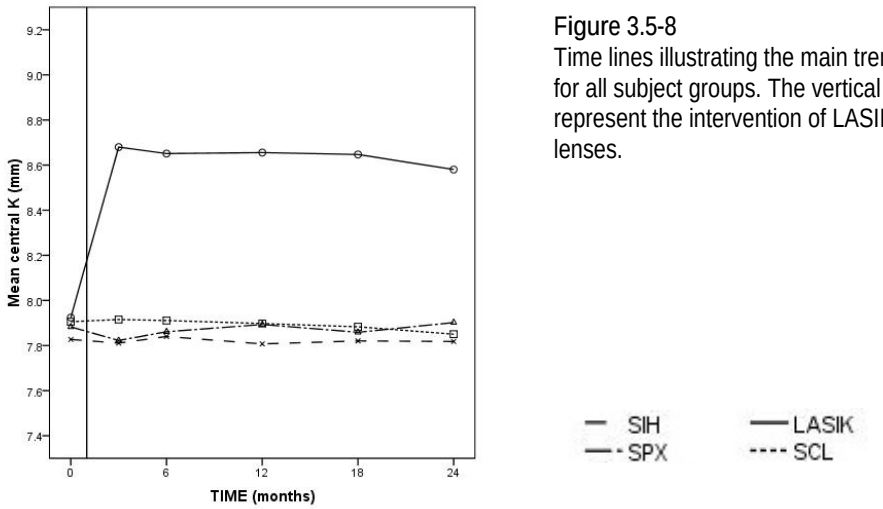


Figure 3.5-8
Time lines illustrating the main trends of mean central K for all subject groups. The vertical solid-drawn lines represent the intervention of LASIK or refitting with SiH lenses.

3.5.6 Corneal endothelial cell morphometry

Endothelial cell density - ECD

No between-group difference was evident for the CECD values (p = 0.593, one-way ANOVA), which were obtained from all 98 subjects, including the extra six spectacle wearers at baseline (see section 1.1.1 for details). At baseline, the total mean CECD was 2706 ± 281 cells/mm^2 ranging from 2643 ± 332 cells/mm^2 (pre-LASIK subjects) to 2747 ± 275 cells/mm^2 (soft lens wearers before refitting with SiH lenses). For all four test-groups, the central cell loss per year was modest and no significant time-dependent changes were noted (see the respective previous sections on prospective analyses). Hence, no between-group differences in CECD were evident at the first visit after the interventions (p = 0.856, ANOVA) or at the end of the study (p = 0.424, ANOVA). See also Figure 3.5-9.

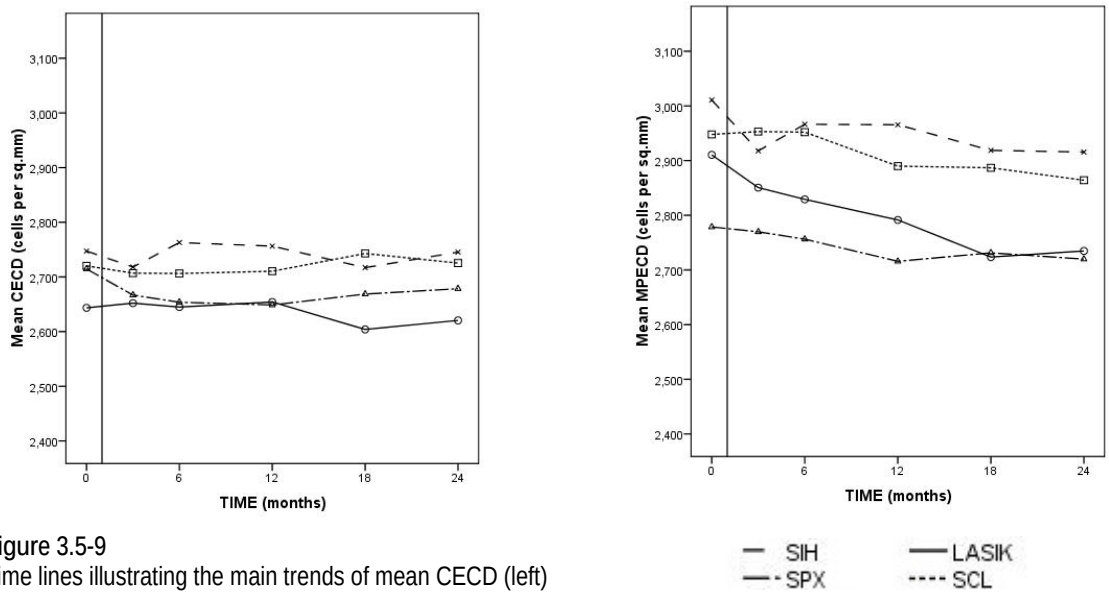


Figure 3.5-9
Time lines illustrating the main trends of mean CECD (left) and MPECD (right) for all subject groups. The vertical solid-drawn lines represent the intervention of LASIK or refitting with SiH lenses.

Before the intervention, the total mean MPECD for all subjects (N = 98) was 2918 ± 311 cells/mm^2, ranging from 2779 ± 283 cells/mm^2 in spectacle wearers to 3011 ± 329 cells/mm^2 in soft lens wearers (prior to refitting with SiH lenses). Due to large intra-group variability, ANOVA test revealed no statistically significant differences between the four test-groups (p = 0.077). However, the mean values suggested a difference in MPECD between lens wearers and spectacle wearers and was thus further analysed (see below). After the interventions, no between-group differences in MPECD was found, neither at the 3-months assessment (p = 0.279, ANOVA) nor at 24 months (p = 0.058, ANOVA). However, similar to baseline, the two lens wearing groups tended to having higher mean MPCD values at 2864 ± 297 cells/mm^2 (SCL) and 2916 ± 262 cells/mm^2 (SiH), whereas post-LASIK subjects and spectacle wearers had slightly lower but very similar mean MPECD values at 2735 ± 253 cells/mm^2 and 2720 ± 246 cells/mm^2, respectively. See also Figure 3.5-9.

For all four test-groups, strong, positive linear relationships were found for CECD and MPECD, both before and after the intervention: High CECD was associated with high MPECD. However, some differences were noted between spectacle wearers and soft lens wearers. Inspections of the scatter plots and accompanying linear regression analyses of CECD vs. MPECD (Figure 3.2-28, Figure 3.3-31 and Figure 3.4-30) suggested that an individual who wore soft lenses was more likely to have higher MPECD values than CECD values compared to spectacle wearers. Spectacle wearers showed a more even distribution of cells; i.e. the linear regression line was closer to the one:one correlation line (Figure 3.1-28).

If the difference between MPECD and CECD was expressed as the MPCD:CECD ratio, a significant between-group difference was found at baseline ($p = 0.032$, one-way ANOVA, Table 3.5-2). Soft lens wearers who later underwent LASIK for their myopia had MPECD:CECD ratio of 1.111, which was significantly higher than the MPECD:CECD ratio of 1.024 in spectacle wearers ($p = 0.036$, Bonferroni correction).

Table 3.5-2
Baseline endothelial cell densities (cells / mm^2) in the central (C) and mid-peripheral (MP) cornea, and the MPECD expressed as the difference from CECD (in %) in contact lens wearers (and sub-groups of these) versus spectacle wearers. All values are given as mean ± 1SD with range (min to max) in brackets.

	N	CECD	MPECD	Sign. (paired t-test)	MPECD:CECD ratio
SPECTACLE WEARERS	21	2715 ± 161 (2264 to 3014)	2779 ± 283 (2220 to 3270)	0.220	1.024 ± 0.085 (0.840 to 1.197)
ALL LENS WEARERS	77	2703 ± 307 (1890 to 3559)	2957 ± 309 (2338 to 3683)	<0.001	1.100 ± 0.109* (0.785 to 1.406)
Soft lens wearers	25	2720 ± 313 (1890 to 3223)	2948 ± 292 (2453 to 3380)	<0.001	1.091 ± 0.113 (0.940 to 1.406)
Pre-SiH lens wearers	26	2747 ± 274 (2262 to 3197)	3011 ± 329 (2338 to 3683)	<0.001	1.099 ± 0.090 (0.899 to 1.339)
Pre-LASIK subjects	26	2643 ± 332 (2041 to 3559)	2911 ± 307 (2432 to 3673)	<0.001	1.111 ± 0.125** (0.785 to 1.302)
TOTAL	98	2706 ± 281 (1890 to 3559)	2918 ± 311 (2220 to 3683)	<0.001	1.084 ± 0.109 (0.785 to 1.406)

* Significantly different from MPECD:CECD ratio in spectacle wearers ($p=0.001$, unpaired two-sided t-test)
**Significantly different from MPECD:CECD ratio in spectacle wearers ($p=0.032$, one-way ANOVA; $p=0.036$, Bonferroni post-hoc test)

After intervention, the MPCECD vs. CECD regression line shifted in the direction of a more even distribution for the post-LASIK subjects whereas a smaller shift was observed after refitting with SiH lenses (Figure 3.3-31 and Figure 3.4-30). Similarly, the modal values of the frequency curves for MPECD:CECD ratios shifted to the left, closer to a ratio of one, after LASIK (Figure 3.4-19 - Figure 3.4-20), and, to a lesser extent, after refitting into SiH lenses (Figure 3.3-20 - Figure 3.3-21). For spectacle wearers and soft lens wearers who continued to wear their habitual lenses, no such shift was seen. (See Figure 3.1-19 - Figure 3.1-20 and Figure 3.2-19 - Figure 3.2-20).

In conclusion, the difference between mid-peripheral and central cell densities, as expressed by the MPECD:CECD ratio did differ significantly between myopic soft lens wearers and spectacle wearers. It should be noted, however, that the ranges of MPECD:CECD ratios were substantial for

all four groups. About 15-20% of the contact lens wearers did not show a greater MPECD. A simple correlation analysis was performed to explore whether other variables could offer an explanation. Age and contact lens wear have previously been suggested to influence the distribution of cells (see introduction, section 1.1.4). Central corneal curvature (K) and central and mid-peripheral corneal thickness were also included to elucidate whether any geometrical factors could be associated with the MPECD:CECD ratio. Last, refractive error was included, since this parameter indirectly reflects the thickness (and thus transmissibility) of the soft lenses, which materials had similar Dk values.

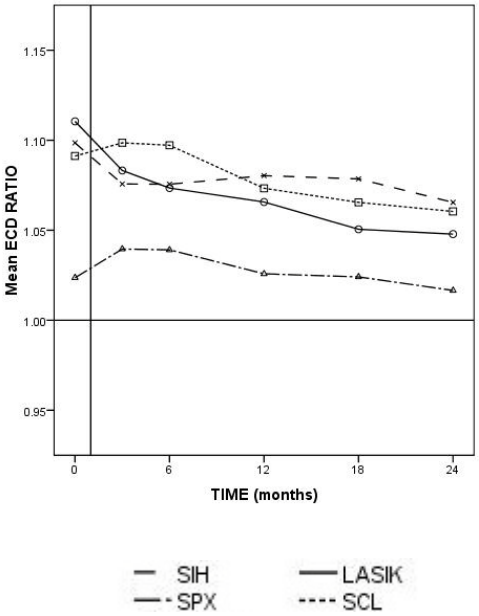


Figure3.5-10
Time lines illustrating the main trends of mean ECD ratio for all subject groups. The vertical solid-drawn line represents the intervention of LASIK or refitting with SiH lenses. The horizontal solid-drawn line represents ECD ratio = 1; i.e. no difference between central and mid-peripheral cell density.

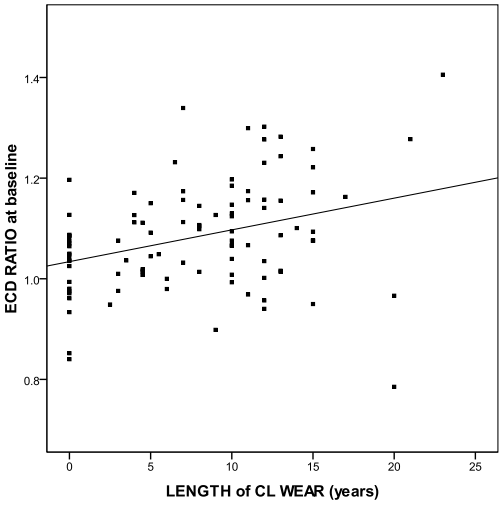


Figure 3.5-11
Scatter plot (of all subjects) with linear regression line showing an association of higher ECD ratios with higher number of years of lens wear at baseline. Note the assumption that has been made that spectacle wearers have equal endothelial characteristics as soft lens wearers with 0 years of lens wear. R=0.333, p=0.001.

Ocular measures and refractive error was not found to be associated with MPECD:CECD ratio (See appendix, Table 5.6-1 and Table 5.6-2). Furthermore, age was not found to be associated with the difference in MPECD to ECD, neither in the spectacle group (n = 21, r = -0.44, p = 0.851) nor in the soft lens wearers at baseline (n = 77, r = 0.119, p = 0.303). However, it should be noticed that the correlation coefficients for lens wearers and spectacle wearers differed substantially. Age was strongly associated with length of lens wear. Partial correlation analysis, controlling for length of lens wear, changed the correlation coefficient to be more similar to what was found for spectacle wearers (r = -0.007, p = 0.951). Furthermore, the MPECD:CECD ratio was not found to be significantly associated with length (years) of lens wear at baseline (n = 77, r = 0.200, p = 0.080). However, if assuming that spectacle wearers have the same endothelial characteristics as a lens wearer who has worn lenses for 0 years, this trend (i.e. an individual could be expected to have a greater MPECD:CECD ratio the longer he or she had worn soft lenses) was found to be statistically significant (n = 98, r = 0.333, p = 0.001).

Endothelial polymegethism - COV

Central corneal morphometry evaluations were obtained from 94 subjects. At baseline, the total mean CCOV was 31.8 ± 7.5 % ranging from 26.6 ± 5.9 % (spectacle wearers) to 34.9 ± 7.9 % (pre-LASIK group). Statistically significant between-group differences was found (p = 0.001, ANOVA). Spectacle wearers had significantly lower CCOV values than soft lens wearers (N = 74, including soft lens wearers who later underwent LASIK or changed into SiH lenses). Mean CCOV in subjects wearing soft contact lenses was 33.3 ± 7.2 % , which was 6.7% (percentage points) more than the mean CCOV in spectacle wearers (p < 0.001, t-test). At the first assessment after intervention, there was still a significant between-group difference in COV values (p = 0.036, ANOVA), however mean CCOV values in SiH lens wearers (28.6 ± 5.0 %) and post-LASIK subjects (30.8 ± 6.0) were no longer significantly different from spectacle wearers (28.6 ± 4.5 %, p = 1.000, Bonferroni correction). Similarly, at 24 months, no statistically significant difference in mean CCOV values was found between spectacle wearers (27.8 ± 4.9 %), SiH lens wearers (28.7 ± 4.8 %) or post-LASIK subjects (29.0 ± 5.4 %, p = 1.000, Bonferroni correction).

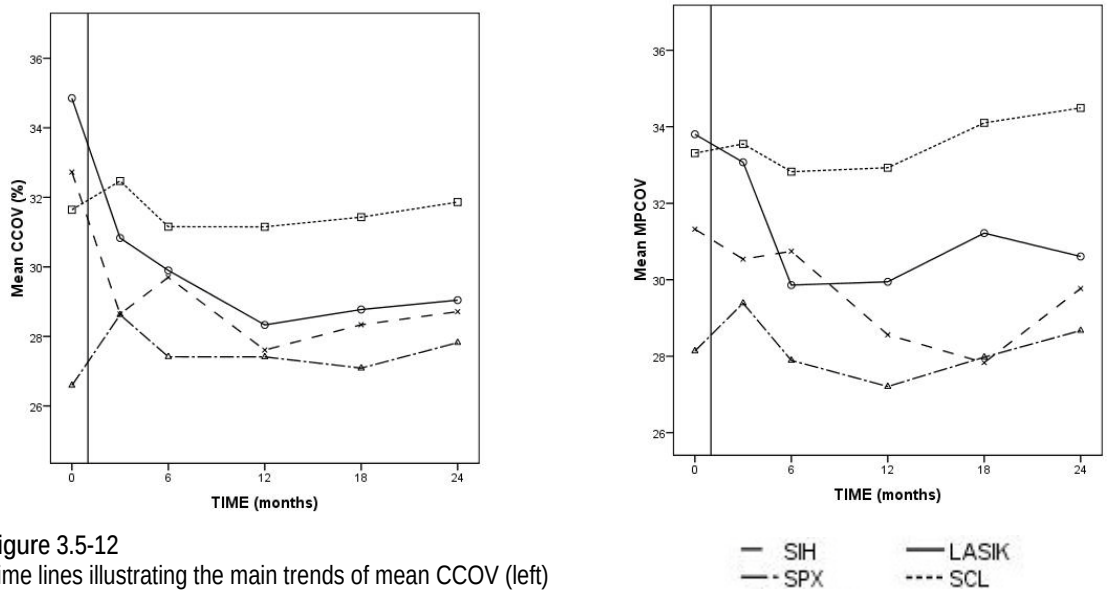


Figure 3.5-12
Time lines illustrating the main trends of mean CCOV (left) and MPCOV (right) for all subject groups. The vertical solid-drawn lines represent the intervention of LASIK or refitting with SiH lenses.

At baseline, the total mean MPCOV was 33.8 ± 8.3 % (N = 94) ranging from 26.1 ± 5.7 (spectacle wearers) to 34.4 ± 10.1 % (soft contact lens group). Statistically significant between-group differences was found (p = 0.046, ANOVA). Spectacle wearers had significantly lower (mean difference of 5%, p = 0.014, t-test) MPCOV values than soft lens wearers (N = 74, including soft lens wearers who later underwent LASIK or changed into SiH lenses). At the first assessment after intervention, no statistically significant between-group differences in MPCOV values were detectable (p = 0.160, ANOVA), in spite of the group-mean values being similar to baseline values for all groups. At the end of the study, the results were similar to baseline values (p = 0.029, ANOVA) with the soft lens wearers having lower %6-sided cells in the mid-peripheral parts of the endothelium than did the spectacle wearers (p = 0.052, Bonferroni correction). However, the mean

MPCOV values in SiH lens wearers and post-LASIK subjects were not significantly different from spectacle wearers (mean differences of 1.9 and 0.8, p = 1.000, Bonferroni correction).

Comparable to the results for ECD, strong, positive linear relationships between central and mid-peripheral values were also found for COV in all four test-groups: High levels of polymegethism centrally predicted high levels of polymegethism mid-peripherally. The results for the post-LASIK subjects were very similar to the lens wearers who were refitted with SIH lenses. While some more subjects had higher COV values in their central endothelium than mid-peripherally before the intervention, an individual was more likely to have a close to even distribution of the degree of polymegethism after LASIK (Figure 3.4-31, p171) or refitting with SiH lenses (Figure 3.3-32, p146). The data were also less spread after refit and the pattern became closer to what was seen for spectacle wearers (se Figure 3.1-29, p95). However, the group mean MPCOV was not significantly different from the CCOV in any of the groups (p ≥ 0.083, paired t-tests, see Table 3.5-3), and expressing the MPCOV:CCOV ratio did not reveal the same differences between soft lens wearers and spectacle wearers as did the MPECD:CECD ratio.

Table 3.5-3
Baseline endothelial polymegethism, COV (%) in the central (C) and mid-peripheral (MP) cornea, and the MPCOV:CCOV ratio in contact lens wearers (and sub-groups of these) versus spectacle wearers. All values are given as mean ± 1SD with range (min to max) in brackets.

	N	CCOV	MPCOV	Sign. (paired t-test)	MPCOV:CCOV ratio
SPECTACLE WEARERS	15	27.4 ± 6.3 (16.7 to 39.3)	28.0 ± 5.7 (18.0 to 39.6)	0.544	1.035 ± 0.137 (0.834 to 1.322)
SPECTACLE WEARERS	21	26.6 ± 5.9 (16.7 to 39.3)	28.1 ± 5.7 (18.0 to 39.6)	0.083	1,071 ± 0,144 (0,834 to 1,322)
ALL LENS WEARERS	77	33.1 ± 7.2 (20.7 to 55.0)	32.8 ± 8.5 (18.3 to 63.9)	0.710	1.002 ± 0.199 (0.660 to 1.745)
Pre-LASIK subjects	26	34.9 ± 7.9 (22.8 to 55.0)	33.8 ± 8.3 (20.0 to 61.4)	0.400	0.981 ± 0.166 (0.660 to 1.285)
Soft lens wearers	25	31.6 ± 6.2 (22.3 to 42.7)	33.3 ± 9.8 (20.8 to 63.9)	0.288	1.056 ± 0.238 (0.812 to 1.745)
Pre-SiH lens wearers	26	32.7 ± 7.3 (20.7 to 50.1)	31.3 ± 7.3 (18.3 to 45.3)	0.247	0.971 ± 0.186 (0.752 to 1.365)
TOTAL	98	31.7 ± 7.4 (16.7 to 55.0)	31.8 ± 8.1 (18.0 to 63.9)	0.868	1.017 ± 0.190 (0.660 to 1.745)

Endothelial pleomorphism - %SIX

At baseline, the total mean C%SIX was 57.3 ± 11.4% ranging from 51.2 ± 11.7% (pre-LASIK group) to 64.0 ± 10.4% (spectacle wearers). Statistically significant between-group differences was found (p = 0.001, ANOVA). Spectacle wearers had significantly higher number of six-sided cells than soft lens wearers (N = 74, including soft lens wearers who later underwent LASIK or changed into SiH lenses): The mean C%SIX in subjects wearing soft contact lenses was 55.3 ± 11.0 %, which was 8.7% less than the mean C%SIX in spectacle wearers (p = 0.002, t-test). At 3 and 24 months after intervention, no statistically significant between-groups differences in the C%SIX were

found (p ≥ 0.251, ANOVA). Although the C%SIX increased significantly in post-LASIK subjects (see section 3.4.6) and no changes over time were observed for soft lens wearers or SiH lens wearers, the (non-significant) reduction of six-sided cells found in spectacle wearers (see section 1.1.1) caused the absence of a statistically significant between-group differences after intervention.

At baseline, the total mean MP%SIX was 61.3 ± 11.8 % and no significant between-group differences were evident (p = 0.083, ANOVA). Although ANOVA analyses suggested statistically significant between-group differences at 3 months (p = 0.033), post-hoc analysis using the Bonferroni test did not reveal any statistically significant differences (p ≥ 0.052). In accordance with the prospective analyses, the MP%SIX was similar to previous assessments and no inter-group differences were found at 24 months (p = 0.101, ANOVA).

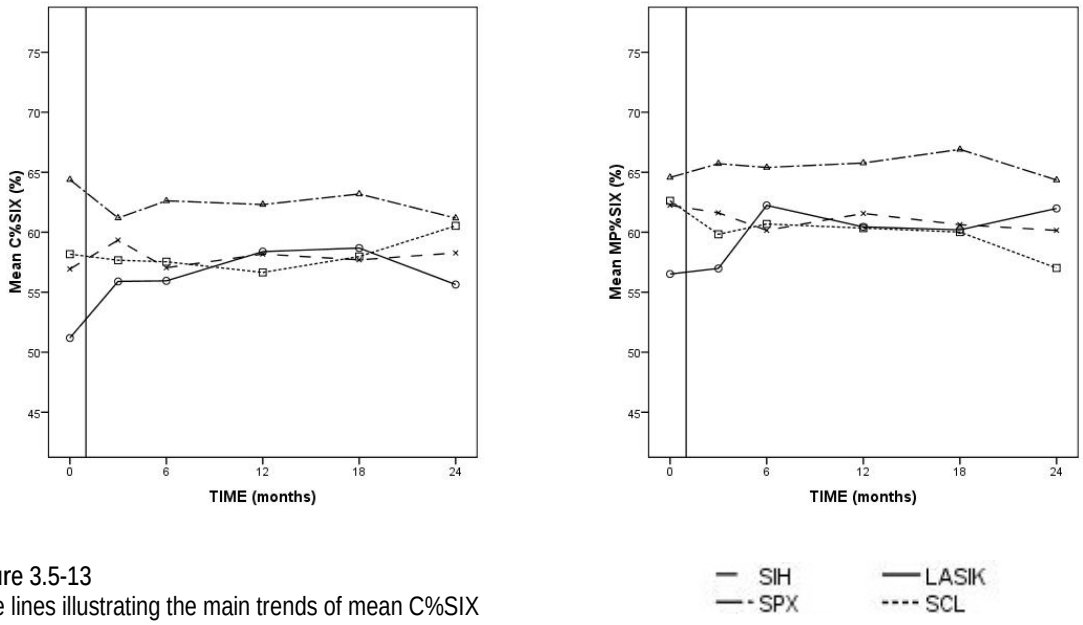


Figure 3.5-13
Time lines illustrating the main trends of mean C%SIX (left) and MP%SIX (right) for all subject groups. The vertical solid-drawn lines represent the intervention of LASIK or refitting with SiH lenses.

Similar to the results for ECD and COV, strong, positive linear relationships between central and mid-peripheral values were also found for %SIX in all four test-groups: High levels of pleomorphism centrally predicted high levels of pleomorphism mid-peripherally. However, the data for %SIX were more spread than the ECD and COV data. Furthermore, the data for %SIX were more spread for the lens-wearing subjects than for spectacle wearers. Inspections of the graphs suggested that around two thirds of the subjects wearing contact lenses had higher %6-sided cells in the mid-peripheral endothelium than in the central endothelium whereas the remaining third had similar, or slightly higher *C%SIX* (Figure 3.4-32). This impression was only partly confirmed when the mean MP%SIX was compared to mean C%SIX (see Table 3.5-4): Soft lens wearers who later underwent LASIK had significantly higher MP%SIX than C%SIX whereas the other two groups of soft lens wearers showed no (p = 0.097), or only borderline (p = 0.056), significant differences between MP%SIX and C%SIX. On the contrary, there were very clearly no differences between MP%SIX and C%SIX for spectacle wearers (p = 0.721, paired t-test). Nevertheless, if the difference between

MP%SIX and C%SIX was expressed as the MP%SIX:C%SIX ratio, no between-group differences were revealed by the ANOVA test (p = 0.619). After LASIK, the data for %SIX was less spread, similar to the change seen in SiH wearers (compare Figure 3.4-32 with Figure 3.3-33), . However, no significant between-group differences in the mean MP%SIX:C%SIX ratio could be detected after the interventions of LASIK or refitting into SiH lenses (one-way ANOVA, p = 0.888).

Table 3.5-4
Baseline endothelial pleomorphism, %SIX, in the central (C) and mid-peripheral (MP) cornea, and the MPSIX to CSIX ratio in contact lens wearers (and sub-groups of these) versus spectacle wearers. All values are given as mean ± 1SD with range (min to max) in brackets.

	N	C%SIX	MP%SIX	Sign. (paired t-test)	%SIX ratio
SPECTACLE WEARERS	15	64.4 ± 11.7 (41.8 to 82.4)	64.6 ± 11.2 (49.2 to 80.3)	0.962	1.030 ± 0.235 (0.645 to 1.559)
SPECTACLE WEARERS	21	64.0 ± 10.4 (41.8 to 82.4)	65.0 ± 10.1 (49.2 to 80.3)	0.721	1.035 ± 0.199 (0.645 to 1.559)
ALL LENS WEARERS	77	55.4 ± 11.1 (30.9 to 82.8)	60.4 ± 12.0 (36.8 to 80.0)	0.001	1.118 ± 0.265 (0.600 to 2.371)
Pre-LASIK subjects	26	51.2 ± 11.7 (30.9 to 82.8)	56.5 ± 13.3 (37.0 to 80.0)	0.023	1.123 ± 0.229 (0.791 to 1.500)
Soft lens wearers	25	58.2 ± 12.0 (31.2 to 78.9)	62.6 ± 10.3 (45.3 to 80.0)	0.097	1.118 ± 0.317 (0.762 to 2.371)
Pre-SiH lens wearers	26	56.9 ± 8.5 (41.5 to 74.1)	62.2 ± 11.5 (36.8 to 76.3)	0.056	1.114 ± 0.253 (0.600 to 1.796)
TOTAL	98	57.2 ± 11.5 (30.9 to 82.8)	61.4 ± 11.7 (36.8 to 80.3)	0.001	1.101 ± 0.254 (0.600 to 2.371)

For all four test-groups, strong negative linear relationships were found for the degree of polymegethism (COV) and pleomorphism (%SIX), both in the central and mid-peripheral part of the endothelium. As might be expected, highly polymegethous endotheliae were found to be associated with a lower percentage of 6-sided cells. After LASIK or refitting into SiH lenses, the slope of the regression line that represented the central degree of polymegethism vs. pleomorphism steepened slightly, reflecting a reduction in the degree of polymegethism and pleomorphism (i.e. increase of %6-sided cells), however, the relationship remained strong for both groups. In the mid-peripheral area this relationship also remained stable after interventions.

CHAPTER 4 – DISCUSSION

4.0 Introduction

The results obtained through the clinical observations undertaken in this study indicate that postoperative changes following LASIK surgery on the anterior segment of the myopic eyes examined in the present study are modest. These findings do not support the view in ophthalmic literature that refractive excimer laser surgery can potentially cause long-term damage to the cornea. However, numerous novel observations were made in the current study. These observations, which have not been previously reported, have arguably significant clinical applications and will be addressed successively.

4.1 Endothelial morphometry

4.1.1 Polymegethism reduces after LASIK or lens-change

This study sought to evaluate if and how LASIK, compared with lens change into SiH lenses, may alter endothelial cell density and the degree of endothelial polymegethism and pleomorphism caused by previous long-term soft contact lens wear.

No detrimental effects of LASIK on the corneal endothelium were evident shortly or up to two years after surgery. Some of the results imply that LASIK reduces or even reverse some of the changes in the endothelial mosaic followed by long-term soft contact lens wear.

First, this study revealed no significant effect of LASIK on the central cell density (CECD) in previous soft lens wearers. At baseline, the mean CECD was 2635 ± 370 cells / mm^2. In accordance with previous reports (see section 1.4.6, p44), this figure was not different from the CECD in non-lens wearers or the other soft contact lens wearers. Over the study period of two years, central cell density values changed very little from visit to visit with an average reduction of 0.4% per year. This result is comparable with previously reported natural cell loss (Bourne *et al.*, 1997) and within the range reported in the majority of other long-term studies of endothelial cell density after PRK or LASIK (see section 1.5.6, p53). However, a few studies reported an *increased* CECD up to two years after PRK (Stulting *et al.*, 1996; Trocme *et al.*, 1996) or six months after LASIK (Perez-Santonja *et al.*, 1997). The authors hypothesized that cessation of contact lens wear and subsequent increased corneal oxygen availability had caused the more peripheral endothelial cells to migrate (back) towards the central parts, implying that contact lens wear causes central cells to migrate towards the more peripheral parts. Their theory may imply that a masked central cell loss took place in the current study as well as in previous studies that did not find any increase in CECD after excimer laser surgery. Further discussion of possible migration of endothelial cells in response to contact lens wear and subsequent LASIK surgery continue under section 4.1.2 on regional differences in endothelial cell characteristics.

Second, the extent of central polymegethism and pleomorphism significantly decreased after LASIK surgery in the present study. Pre-operative CCOV and C%SIX values were 34.3 ± 5.4% and 50.5 ± 9.3% respectively, which is consistent with values reported in the literature (see section 1.4.6, p44). These figures were also not different from the respective figures in the other contact lens wearing groups but as expected significantly different from spectacle wearers. However, over the two years after LASIK surgery the CCOV values significantly decreased by as much as 5.7% (percentage points), giving a group mean CCOV value not significantly different from the CCOV value in spectacle wearers at the two-year visit. Likewise, the C%SIX increased significantly by 6% within the first three months after LASIK. Thereafter no substantial changes in the C%SIX occurred.

Polymegethism and pleomorphism are indicators of endothelial stress and is closely associated with hypoxia and subsequent hypercapnia following contact lens wear. A total or partial recovery of such changes would clinically be regarded as solely positive. In the ophthalmic literature, it has often been insinuated or even stated that most of the subjects were contact lens wearers before they underwent PRK or LASIK (see section 1.5.6, p53). In spite of this, few studies have assessed polymegethism and pleomorphism in soft lens wearers after PRK and even fewer after LASIK. Most of these studies also found a reduction of the CCOV value both shortly and up to three years after PRK or LASIK within a range similar to the present study (see section 1.5.6, p53)

A few studies have indicated that contact lens related alterations of the corneal endothelial characteristics may, at least partly, recover after excimer refractive surgery such as PRK (Stulting *et al.*, 1996; Trocme *et al.*, 1996; Perez-Santonja *et al.*, 1997). Therefore the present study included one control group of soft contact lens wearers, who continued wearing their habitual lenses, and another group of soft contact lens wearers who were refitted with a contact lens that seemingly eliminates development of hypoxia related complications: the silicone-hydrogel lens.

Soft contact lens wear did not alter CECD to a significant extent in the present study. For all soft contact lens wearers at baseline, the mean CECD was 2703 ± 306 cells / mm^2, which is in accordance with previous reports and not different from the CECD in spectacle wearers (see section 1.4.6, p44). Small non-significant changes within the reported ranges of natural cell loss in CECD were found for both soft- and SiH contact lens wearers and spectacle wearers over a period of two years. These results merely reflect that the subjects were successful lens wearers and that refitting into SiH lenses, as expected, did not alter the CECD beyond natural changes.

Similar to CECD, CCOV and C%SIX did not change in the soft lens wearers who stayed in their habitual lenses over the two-year period. However, for the subjects refitted with SiH lenses changes very similar to those seen for the LASIK group were found: The mean CCOV reduced significantly by 5.5% within the first three months of SiH lens wear. After 2 years of SiH lens wear the mean CCOV in these subjects was 28.7 ± 4.8%, which was not significantly different from spectacle wearers at 27.8 ± 4.9% (p=0.6). However, contrary to post-LASIK subjects, the percentage of six sided cells did not change significantly over time in SiH wearers.

These results generally contribute to current theories on the endothelial cell layer's morphometric transformations in response to environmental changes and specifically to current theories on the reversibility of polymegethism. Clear differences between spectacle wearers and soft lens wearers in the present study confirm the established fact that hypoxia and hypercapnia during soft lens wear is associated with changes in endothelial morphometric features. It has not been previously shown to the same extent that polymegethism may be reversible if oxygen availability increases. The results from the present study support and extend the results from previous small scale studies or case-series of wearers of contact lenses with no or low oxygen transmissibility who temporarily terminated their lens wear or were refitted with contact lenses with higher Dk, where the degree of polymegethism reduced at least partially (see section 1.4.6, p44).

The changes in the endothelial polymegethism for the soft lens wearers who were refitted with SiH lenses were also very similar to the changes found in LASIK subjects. Similar to what other research groups have suggested, it is not unreasonable to attribute these changes to the cornea's increased accessibility to oxygen (Stulting *et al.*, 1996; Trocme *et al.*, 1996; Perez-Santonja *et al.*, 1997). The endothelial cells have a great capacity of changing their diameter and shape in response to environmental changes and in the processes of wound healing. However, the increased %SIX found in the LASIK subjects was not found for SiH wearers. Generally, an increase in the number of six-sided cells is a clear indication of improved cell metabolism. That this improvement was not seen for the SiH lens wearers may suggest that even a minimal reduction in the corneal oxygen availability influence corneal endothelial metabolism. A recent study did observe short-term endothelial changes (blebs) in response to SiH lens wear, although the changes were smaller than for soft lens wear (Brennan *et al.*, 2008). Bleb-response is associated with corneal oxygen availability. Another possibility is that the lack of change in the percentage of six-sided cells is caused by a mild inflammation. As further discussed under section 4.1.2, the mechanical irritation and subsequent epithelial changes may well cause changes in the appearance of the endothelial cell layer.

Arguably, the present study not only supports but also strengthens previous conclusions that LASIK does not cause significant central endothelial cell loss. Although the number of subjects was limited, the regular and detailed evaluation of the endothelial cell layer over a prolonged period after surgery gives credibility to the result of a gradual, consistent but very small reduction in CECD after LASIK, which was apparent at each of the six assessments over the study period of two years. Moreover, all subjects were previous long-term soft contact lens wearers. Evaluation of endothelial characteristics and changes in soft contact lens wearers after LASIK was the aim of the current study, whereas this issue has most often only been discussed and hypothesized in previous studies where few, if any, details of contact lens wear have been given. In addition, the present study included control groups of soft contact lens wearers who either continued wearing their habitual lenses or were refitted with SiH lenses. Such control groups have generally not been included in previous studies and the results found for these groups of subjects supports the findings made for the post-LASIK subjects. The fact that similar improvements of central

endothelial morphometric features were found for both intervention groups (SiH wearers and LASIK subjects), contributes to our current understanding of how oxygen availability influences this cell layer.

4.1.2 Between-group differences in ECD distribution

This study further sought to evaluate the regional differences in endothelial cell characteristics in long-term successful soft hydrogel contact lens wearers as compared to spectacle wearers and how these regional differences may change after LASIK or refitting with SiH lenses.

Mid-peripheral endothelial cell density (MPECD) was found to be higher than central cell density (CECD) for most subjects and the difference between MPECD and CECD, expressed as the MPECD:CECD ratio, was found to be linked to soft lens contact lens wear. The larger MPECD:CECD ratio in soft contact lens wearers reduced after LASIK but not after refitting with SiH lenses. This novel way of presenting cell distribution gives new knowledge on how soft contact lens wear influences the distribution of endothelial cells. Furthermore, the results raise questions regarding the aetiology of some of the morphometric changes in the corneal endothelium seen in soft contact lens wearers.

Most subjects evaluated in the current study had noteworthy higher MPECD compared to CECD. That this was observed confirms a number of previous studies and clearly shows that an assessment of the corneal endothelium that just considers the central region will only give a limited perspective. For the subjects evaluated in the present study, the overall mean difference (for all subjects) between the mid-peripheral cell density (MPECD) and the central cell density (CECD), as a percentage, was 8.4%. This value is higher than the overall average of 3.9% difference that has been noted from other published studies including both contact lens wearers and non-wearers. However, 10.2% of the subjects in the present study actually had MPECD values that were *lower* than the CECD values. This possibility appears to have largely been overlooked in the literature. It is considered important since significant difference between-group average MPECD and CECD values may not have arisen if a similar proportion of the subjects assessed had lower and higher MPECD values compared to CECD values. A conclusion was drawn from an Italian study that the MPECD was not statistically higher or lower than CECD in young adults (aged 20 to 44 years) but that MPECD was lower than CECD in older adults (aged 45 to 70 years) (Roszkowska *et al.*, 2004). Details of the proportions of subjects with lower or higher MPECD values were not provided however, and it should also be noted that the mid-peripheral values were actually an average from all four quadrants. All the subjects in the present study were young adults. A similar higher value for the difference between MPECD CECD of 6.8% was reported in another preoperative study for refractive surgery in younger patients (Trocme *et al.*, 1996). In common with the younger subjects reported in a later study (Amann *et al.*, 2003), a notable number of subjects were contact lens wearers. In neither of these reports were details provided of the years of contact lens wear.

For the entire group of contact lens wearers at baseline, the mean MPECD:CECD ratio was 1.100 with most of them (84%) having MPECD:CECD ratios >1. While their CECD values were not different from spectacle wearers (i.e. group mean CECD values of 2703 and 2715 cells/mm^2, respectively), their endothelia were clearly very different from spectacle wearers. For the spectacle wearers in the present study, the mean MPECD:CECD ratio was just 1.024, with 13 of 21 (62%) actually having MPECD:CECD ratio >1. If this is taken as a reference perspective, then the findings in this study on the contact lens wearers are not only noteworthy but reveal some largely new aspects of the corneal endothelium and its dynamic behaviour. This result also shows the importance of choosing the appropriate reference group for studies on the endothelium. The 10% difference between MPECD and CECD was found from pooling all data from the contact lens wearers, and so it should be noted that the MPECD:CECD ratios were 1.111 in the pre-LASIK group (soft lens wearers who later underwent LASIK), 1.091 in the soft lens group (who stayed in soft lenses) and 1.099 in the SiH lens group (who were previously daily soft lens wearers). An analysis of whether the years of soft contact lens wear could have a predictable effect on MPECD:CECD ratio values revealed a tendency of a positive linear relationship. The MPECD:CECD ratio was higher the longer a subject had worn soft lenses. The relationship was significant if spectacle wearers (who may be regarded as soft lens wearers who have worn lenses for 0 years) were included in the analysis (see 3.5.6, p186).

Before considering why such an effect could occur, this result shows that it is important to carefully match subjects in parallel cohorts to a level not previously considered. For example, in trying to compare the outcome of refractive surgery on previous contact lens wearers (i.e. soft lens wear has been discontinued) with continuing contact lens wear, the present study implies that it will be important to match the years of lens wear carefully. The same applies to comparisons where one group of subjects are refitted into new lenses compared to staying in the older lens material. In the present studies, the originally enrolled contact lens wearers (N = 77) had worn lenses for 10.0 ± 5.0 years (range 2.5 to 23.0 years). However, the length of wear differed significantly between groups ($p = 0.005$, Kruskal-Wallis test). On average, those lens wearers who were refitted with SiH lenses had worn lenses 4 years shorter than pre-LASIK subjects had and 2.8 years shorter than the soft lens wearers ($p \leq 0.03$, Mann-Whitney U test). This same issue is even more important in trying to ascertain what the age-related changes in the corneal endothelium might be. In many published studies on age-related changes in CECD, contact lens wearers have been excluded (Bourne *et al.*, 1997; Doughty *et al.*, 2000; Sanchis-Gimeno *et al.*, 2005) while in other studies it is unclear if any of the subjects were actually contact lens wearers because no clear statement was given (Roszkowska *et al.*, 2004). The present studies indicate that it is not only important to identify if contact lens wearers were included in a cross-sectional analysis of endothelial changes with age, but that it would also be useful to know the years of lens wear. Further research is clearly needed to assess whether the age-related changes in endothelial cell density are the same or different in contact lens wearers.

Some previous studies maintained the point of view that regional differences in endothelial cell density are age-related. However, there is not enough evidence, at this time, to support a simple

age-related difference in mid-peripheral versus central cells, since published data on older subjects has been contradictory (Amann *et al.*, 2003; Muller *et al.*, 2004; Roszkowska *et al.*, 2004). For the present studies, a statistically significant positive relationship was still found between years of soft lens wear and the MPECD:CECD ratio when controlling for age, MPCT:CCT ratio, refractive error and central corneal curvature. Therefore, the variable left is that of differences in stress to the endothelial cells, but it is still unclear as to how this may result in higher MPECD values to CECD values.

The finding of a higher mid-peripheral than central cell density is perhaps not surprising. Histological studies of some flat mounted corneas showed that the endothelial cell density was higher in the far peripheral parts than in the central part (Schimmelpfennig, 1984). Indications of the existence of human endothelial stem cells situated at the junctional region where the corneal endothelial cells meet the trabecular meshwork have been suggested (Whikehart *et al.*, 2005). Moreover, some biochemical studies suggested that human endothelial cells in the peripheral region have a greater potential of cell division than cells in the central region (Mimura and Joyce, 2006) and that more human endothelial cells in the central region are in a state of senescence compared to peripheral cells. (Paull and Whikehart, 2005; Konomi and Joyce, 2007). However, biochemical reasons cannot be generally applied since some individuals do not show a higher MPECD. Evidence for the capacity of any substantial endothelial cell proliferation have not been provided and these studies cannot explain why the mid-peripheral to central cell density ratio was found to be larger in soft contact lens wearers than in spectacle wearers in the present study.

The results of the current study indicate that a prolonged history of contact lens wear can have an effect on the MPECD:CECD ratio. However, it could be argued that this may not be a characteristic of the endothelium but perhaps a result of how the data are reported. Calculations of endothelial cell density is most commonly based on the average cell area (i.e. cell density = 1000000 / average cell area). Therefore, if only 25 to 50 cells are analyzed, the estimated cell density might be lower (if just the larger cells were measured) or higher (if mainly the smaller cells had been measured) than the central values. The risk of over- and under estimation of endothelial cell density in contact lens wearers will thus be larger since these individuals generally have a larger variation on cell area sizes (COV values). However, in the present study, 100 cells were measured from each location, a number that should give estimates of cell densities to within $\pm$ 2% or better (Doughty *et al.*, 2000).

The present studies provide data that support an idea that differences in oxygen availability to the corneal endothelium could play an important part in determining the MPECD:CECD ratio. It was the higher mid-peripheral densities that caused the increased MPECD:CECD ratio, not lower central cell densities, if the lens wearers were compared to the spectacle wearers. In view of the association between MPECD:CECD ratio in relation to years of soft contact lens wear, it is tempting to suggest that oxygen availability to the endothelium is a likely factor determining the differences in cell density. The soft hydrogel contact lens materials used in the present study typically had a calculated oxygen permeability (Dk) in the order of 15 to 30 x 10^{-11}(cm^2/sec) (ml

O_2[ml mmHg]) when using the equation developed by Morgan and Efron (1998): $Dk = 1.67e^{(0.0397 \times \text{water content})}$. Using lens thickness values given by the manufacturer for a minus 3.00 D lens, the central oxygen transmissibility (Dk/t) ranged from 21 to 26 x 10^{-9} (cm/sec) (ml O_2 [ml mmHg]). However, towards the periphery of soft hydrogel lenses of medium oxygen transmissibility the Dk/t can be expected to be from 30% to more than 50% lower than centrally for a spherical minus 3.00 D lens (Bruce, 2003). Trocmé and colleagues (1996) proposed that the reduced corneal oxygen availability in myopic contact lens wearers caused central cells to migrate towards the more peripheral parts of the endothelial cell layer, where the oxygen availability is even lower. Such a theory is appealing, since the endothelial cell layer's capacity for migration in wound healing processes and in response to stress is thoroughly documented. However, in light of this hypothesis one would expect the CECD in soft contact lens wearers to be lower than in spectacle wearers. This was not found to be the case in the present study or in other recent studies of soft lens wearers. Still, the phenomenon of migration may explain regional differences in the distribution of endothelial cells. Although the current study did not assess far peripheral ECD, the possibility cannot be excluded that soft contact lens wear may trigger far peripheral endothelial cells to migrate in central directions and consequently increasing the mid-peripheral ECD. The results seen for the soft contact lens wearers that underwent LASIK give support to this hypothesis.

For the lens wearers who had LASIK, significant reductions in the MPECD were observed 3 months after surgery, while only subtle changes were evident centrally. Consequently, the MPECD:CECD ratio reduced too. A further gradual reduction in MPECD:CECD ratio occurred over time with the mean ratio almost halved by 24 months. Any iatrogenic damage or stress to these corneas from the laser ablation itself seems an unlikely explanation since this would logically have caused central cell loss, which was not evident in the present study. As has been suggested by others, it seems more likely that the lens cessation was the reason with the cells no longer suffering from hypoxia but having essentially the same access to oxygen as a non-contact lens wearing eye (Stulting *et al.*, 1996; Trocme *et al.*, 1996). This suggested migration of peripheral cells towards the centre after PRK may have masked a central cell loss (since central cell density remained unchanged). However, this theory has little, if any, support in the current literature of short term studies of excimer laser surgery on the central endothelial cell density fifteen minutes or one month post-operatively (Kim *et al.*, 2001).

In the present study, spherical equivalent power was not found to be related to the MPECD:CECD ratio for soft lens wearers. Since lens power (i.e. thickness) of a certain material is proportional to the oxygen transmissibility of the lens, this result indicates that oxygen availability may not be the single reason for the increased MPECD:CECD ratio seen in many soft lens wearers. Moreover, the results from the soft lens wearers that changed into SiH lenses do not support that increased oxygen availability is the single reason for the changes in the MPECD:CECD ratio seen for post-LASIK subjects.

If the increased oxygen availability is the reason for the changes seen in post-LASK patients' endotheliae, then it would be expected to see similar changes in subjects who changed their soft

hydrogel lenses to lenses with much higher oxygen transmissibility. Silicone-hydrogel lenses (Lotrafilcon A) transmit much more oxygen than do soft hydrogel lenses used in the present study (Morgan and Efron, 1998; Efron *et al.*, 2007). Although a gradient can be expected for SiH lenses too, the peripheral Dk/t is still high; between 60 and 140 x 10^{-9} (cm/sec) (ml O_2 [ml mmHg]) for the SiH lenses (Focus Night&Day) used in the present study (Bruce, 2003).

In the present study, as in post-LASIK subjects, the degree of polymegethism decreased after lens change for the soft lens wearers who were refitted with SiH lenses. However, the reduction in MPECD seen in post-LASIK subjects was not seen for the SiH wearers. The reason for this finding is uncertain. If one accepts that migration of peripheral cells cause a greater MPECD:CECD ratio in soft lens wearers, it is not remarkable that changes in this relationship occur when lens wear ceases, as seen for the post-LASIK subjects in the present study. However, increased oxygen availability alone, by changing from soft hydrogel lens into SiH lens wear did not significantly change the MPECD:CECD ratio or the percentage of six-sided cells (see previous section, 4.1.1).

Moreover, lens change into SiH lenses did not change corneal thickness, which could be expected since daily soft lens wear is also associated with stromal oedema. It is however unclear whether the three groups of soft contact lens wearers had any oedema at baseline. No substantial or statistically significant difference between central or mid-peripheral corneal thickness in soft contact lens wearers and spectacle wearers was found, a finding consistent with other long term studies (see 1.4.5, p42). When long-term extended soft hydrogel lens wearers are refitted with lenses transmitting more oxygen than their habitual lenses, a thinning of the central cornea can be expected to occur (Holden *et al.*, 1985b; Bourne *et al.*, 1999b; Liu and Pflugfelder, 2000; Nourouzi *et al.*, 2006). Chronic oedema may lead to loss of stromal matrix, which becomes evident when the oedema resolves (Holden *et al.*, 1985b). In the present study, no change in central corneal thickness was observed over the study period of two years. The lack of thinning on switching to SiH wear might have occurred because the long term soft lens (daily) wearers did not have any notable oedema. This assumption is not unreasonable, since the corneal thickness in the soft lens wearers was not statistically significantly different from corneal thickness in spectacle wearers. Alternatively, the three groups soft lens wearers in the present study had a low level of oedema at baseline that the present study may not have detected. A non-significant 2.9% difference in corneal thickness in contact lens wearers and spectacle wearers was evident at baseline (see section 3.5.5, p184) and similar differences were observed at all later visits. Longitudinal changes within each group were much smaller (around 1% for central corneal thickness, see chapter 3, section 3.1.5, 3.2.5, 3.3.5 and 3.4.5). Although, the smallest detectable difference in corneal thickness for the present study was around 4% for between-group comparisons and 3% for prospective analyses (power = 0.8, unpublished data), the reported group-mean figures strongly indicate that no changes in corneal thickness occurred over time in either spectacle wearers or contact lens wearers. If any small degree of oedema was present in contact lens wearers, this was less than 4% when compared with spectacle wearers and it did not change over time in either group. In other words: a substantial increase in the oxygen transmissibility (refitting of soft contact lens wearers with SiH lenses) did not cause stromal thinning or eliminate any oedema, if at all present.

Although SiH lens wear alleviates some of the common low-Dk soft contact lens related complications, such as limbal redness and stromal oedema, the mechanical presence of a contact lens, especially when worn continuously, will probably stimulate the release of inflammatory mediators (Kallinikos *et al.*, 2006). A recent meta-analysis found that the risk for inflammatory events is twice as high in 30 days continuous SiH wear compared to seven days of extended low Dk soft contact lens wear (Szczotka-Flynn and Diaz, 2007). The study could not reveal whether the increased risk was linked to the lens material or the lens wear schedule. A large, recent Australian study found the annualized incidence of keratitis to be unchanged for overnight wear of SiH lenses when compared to overnight wear of low Dk soft hydrogel lenses (Stapleton *et al.*, 2008). The inflammatory corneal response is thus probably not linked to the soft lens material (i.e. corneal oxygen availability) but more likely to the combination of mechanical irritation and accumulation of tear proteins and conjunctival bacterial toxins during sleep. The results from these studies can be further interpreted as mechanical irritation of contact lens wear causes a mild inflammatory response which also have been hypothesized to induce a reduction, possibly by migration, in the posterior keratocyte population in SiH wearers (Kallinikos *et al.*, 2006). Increased variation in cell size and decreased number of six-sided cells to the same level as in soft contact lens wearers, and without any significant cell loss, have been demonstrated in subjects with previous unilateral herpetic keratitis (Hirose *et al.*, 1988). Endothelial morphometry changes have also been observed in much milder inflammatory conditions such as dry eyes or just superficial epithelial exposure staining (Brooks *et al.*, 1989). Interestingly, the unilateral morphometric changes observed in the Japanese study were persistent up to several years after the attack and the authors hypothesized that this “may be related to subclinical inflammation in the cornea and / or in the anterior chamber” (Hirose *et al.*, 1988). The results from these studies and the present study suggest that changes in endothelial morphometry and morphology in soft (hydrogel or silicone-hydrogel) contact lens wearers may not only be a consequence of reduced oxygen accessibility but that a persistent mild corneal inflammation or the presence of inflammatory mediators may be a triggering or at least contributing factor. The lack of corneal thinning in SiH wearers supports this theory, since inflammation is also associated with oedema.

It is tempting to conclude that the re-organization of endothelial cells seen in post-LASIK subjects occur due to lens removal and subsequent disappearance of inflammation. However, an alternative theory is that the refractive change of corneal shape itself caused an apparent reduction in mid-peripheral cell densities. However, one recent study found that flatter overall corneal curvature was related to higher mid-peripheral endothelial cell densities, and not lower (Muller *et al.*, 2004). The present study found no clear relationships between central corneal curvature and endothelial cell densities (unpublished results). It was considered beyond the scope of this thesis to explore this theory any further, but for future studies it would be very interesting to see if the corneal shape, and especially the shape of the posterior corneal surface, have any influence on the endothelial cell mosaic. Many studies have suggested that central parts of the posterior cornea shifts forward after LASIK or PRK (Yoshida *et al.*, 2003; Hashemi and Mehravaran, 2007). Other researchers have suggested that a mid-peripheral backward bulging takes place (Grzybowski *et al.*, 2005). However,

no significant posterior protrusion has been reported (Ciolino *et al.*, 2007), and it is also not clear how such changes may change the apparent or real local endothelial cell density or cell shapes.

4.1.3 Inter-relationships in endothelial cell morphometry

The current study revealed differences in the morphometry in the central and mid-peripheral corneal endothelium. Therefore, to further explore these differences, the cell density, cell shape and variation in cell size were systematically assessed to look for any particular pattern that could illuminate if the differences were consistent and if they may be related to soft contact lens wear, lens change or following LASIK (see also chapter 3, section 3.5.6).

The shape of polymegethous cells, small or enlarged, deviate from the most common endothelial cell shape, which have six-sides (Doughty, 1998a). Smaller cells can be four-sided or five-sided, while larger cells tend to have more than six sides, i.e. seven or eight sides (Doughty *et al.*, 1993; Doughty, 1998b; Muller *et al.*, 2000). Thus, the negative relationship that was found for the COV and %SIX for all groups subjects was not unexpected: Large degree of polymegethism was found to be associated with lower percentages of six sided cells, both in the central and mid-peripheral part of the corneal endothelium. The relationship was highly significant and the correlation coefficients were similar for all groups at around -0.50. This relationship has also been reported by a recent study using a similar method of cell analysis (Loukotova *et al.*, 2007). The relationship between the %SIX and COV did not change after LASIK or lens change and thus seems to be strong and not dependent on oxygen availability.

The COV value was not found to predict ECD when analysed by regression analysis, in any of the investigated groups of subjects. There is a risk of underestimation of the cell density when the degree of polymegethism is large (Hirst *et al.*, 1989), especially if a frame method is used, i.e. the number of cells within a fixed frame is counted, and the number of counted cells are few. The fact that the current study did not find a relationship between CED and COV is perhaps not surprising, since the method of cell density estimation has been shown to give little variance (Doughty *et al.*, 2000). One hundred contiguous cells were counted and the cell density was based on the sum of total cell areas divided by the number of cells. Likewise, ECD and %SIX did not correlate. This might be expected; since COV did not correlate with ECD, neither did %SIX correlate to ECD.

For all four test-groups, strong, positive linear relationships were found between central and mid-peripheral morphometric parameters, both before and after the intervention. For example, high levels of CCOV were associated with high levels of MPCOV. By inspection, the slopes of the regression lines in soft lens wearers were different from spectacle wearers at baseline. After LASIK or refitting into SiH lenses, the slopes of the regression lines moved in the direction of the slope for spectacle wearers for all morphometric parameters (see 3.5.6). One interpretation of these observations might be that the endothelial cell layer slowly re-organizes after lens cessation in the direction of the appearance of endothelia in spectacle wearers. For LASIK subjects, an additional theory is distortion of the cell shapes if the specular microscope was not perpendicularly adjusted

(Doughty, 1989). Although the SP2000 have features that intend to overcome this source of error (by implementing focus LEDs), it cannot be ignored that such errors may have happened, especially after LASIK when the corneal shape is changed. On the other hand, trends towards a shift in these relationships were noticed both after LASIK and after lens change into SiH lenses. Hence, it seems reasonable to suggest that increased oxygen availability partially reverses the changed morphometry pattern seen for soft lens wearers.

4.2 Dry eye symptoms and tear film characteristics

4.2.1 Dry eye symptoms in post-LASIK subjects persist

Compared to baseline values, a tendency of reduced prevalence, frequency and severity of dry eye symptoms were present for LASIK subjects three months and over a two-year period after surgery. Ninety-five percent of the subjects recruited for the LASIK group experienced one or more symptoms of dry eyes at least 'sometimes' at baseline. Although the majority of subjects who underwent LASIK still experienced dry eye symptoms three months (66.7%), and up to two years (57.1%), after surgery, this represented a clear tendency of fewer subjects reporting symptoms. Furthermore, while 33.3% reported symptoms occurring 'often' or 'always' at baseline, only up to 9.5% reported this (at 12 and 18 months) post-operatively. Since very few subjects reported symptoms at every of the six visits, any statistical time dependent comparisons of symptom severity could not be done, but again a tendency of reduced mean VAS values were noted (see Table 3.2-8).

On the other hand, when compared to contact lens wearers, the prevalence, frequency and severity of dry eye symptoms in the LASIK group was *not* found to be significantly different at baseline or at (most) later visits. Although the number of subjects who still experienced dry eye symptoms reduced after surgery, these figures were not found to be statistically significantly different from the corresponding figures for soft contact lens wearers or SiH lens wearers at the two-year assessment. Similarly, the symptom severity was not significantly different for LASIK subjects when compared to contact lens wearers at baseline or at any of the five post-operative assessments. The proportion who reported symptoms occurring 'often' or 'always' was only 11% and 15% and did not change significantly over two years, neither in the SCL group nor in the SiH group. Although slightly fewer of the post-LASIK subjects reported symptoms occurring 'often' or 'always', it was not significantly different from contact lens wearers. A peculiar result was that the prevalence, frequency and severity of dry eye symptoms in contact lens wearers and post-LASIK subjects were also not significantly different from spectacle wearers.

For contact lens wearers (including pre-LASIK subjects), the present study found a higher prevalence of one or more dry eye symptoms at baseline than previous studies: over 90% versus around 50% (see chapter 1, section 1.4.2). The overall mean VAS symptom severity in the present study (of 32.6) is consistent with previously reported contact lens wear related dryness in successful full-time contact lens wearers (see section 1.4.2). The proportion who reported symptoms occurring 'often' or 'always' did not change significantly over two years, neither in the SCL group nor in the SiH group, which is similar to the results from other studies of successful contact lens wearers (see chapter 1, section 1.4.2).

Dry eye symptoms are a common complication occurring shortly after LASIK and many studies have reported significant increase of both frequency and severity of dryness symptoms when compared to pre-operative values. Even up to five years after LASIK, around 50% can be expected to report one or more dry eye symptom. The present results are in general agreement with the literature in that around 50% can be expected to report one or more dry eye symptom at least up to five years after LASIK (see chapter 1, section 1.5.2).

There are at least three possible reasons for the reduction in the total number of individuals reporting dry eye symptoms at every follow-up assessment after LASIK. First, the cessation of lens wear may have alleviated the symptoms for some subjects. Contact lens wear is closely associated to dry eye symptoms. Second, the subjects in the present study were advised to use artificial teardrops the first month after surgery, and longer if the symptoms persisted. Artificial teardrops lessen symptoms of dry eyes after LASIK (Lenton and Albietz, 1999). Third, the LASIK subjects may be biased because choosing surgery over contact lens wear is a big decision for most individuals. The surgery is costly and the possibility that these subjects are biased towards a positive attitude of their surgery (their choice) cannot be totally ignored.

On the other hand, at each visit over a period of two years, the number of subjects reporting dry eye symptoms, and the severity of these, did not differ significantly from any of the contact lens wearing groups or the spectacle group. One interpretation of this result is that there may be other reasons for the reported dry eye symptoms than surgery or contact lens wear. During history taking at each visit, many subjects reported eye fatigue and dry eyes during the working hours. Most subjects reported computer related work several hours per day. Especially when specifically asked, dry eye symptoms are common in office workers (Doughty *et al.*, 2002a). Further studies should explore the impact of confounding factors such as the environment and vision demanding working tasks when assessing dry eye symptoms in contact lens wearers and after LASIK surgery.

4.2.2 Tear film characteristics does not change after LASIK

Dry eye symptoms are common after LASIK. Therefore, numerous studies have assessed tear film characteristics in subjects undergoing this kind of surgery (see section 1.5.4). Most of these are short time studies up to 6 months. However, since dry eye symptoms may persist longer at least for some patients, it was felt useful to assess the tear film characteristics over a prolonged period. Moreover, the present study chose to include less invasive tear film tests, which are less likely to initiate reflex tearing.

Using the phenol red thread (PRT) test to describe the tear volume, the present study found no significant changes in the PRT measures for soft contact lens wearers who underwent LASIK. Before surgery, group mean PRT value was 18.1 $\pm$ 8.3 mm. Although notably lower mean PRT values (up to -2.6 mm) were found up to 12 months after LASIK, these failed to reach statistical significance. This result is in close accordance with previous studies that have used the PRT test or a similar technique to describe the tear volume in post-LASIK subjects (Patel *et al.*, 2001; Albietz *et*

al., 2005; Stapleton *et al.*, 2006; Credie *et al.*, 2007). Although one previous study found similar small reductions in mean PRT values to actually be significant at one and three months post-LASIK, these were not significant 12 months after LASIK (Credie *et al.*, 2007).

While the present study chose to use the PRT test, most other studies have used the Schirmer test (with or without anaesthesia) reporting significant reductions in the test values several months after LASIK (see chapter 1, section 1.5.4). The discrepancy between the present study using the PRT test and previous studies that have used the Schirmer test may have arisen because the two tests probably measure different tear film characteristics. The Schirmer test without anaesthesia is believed to indicate the amount of residual tears plus reflex tear production from the foreign body stimulus of the test strip itself. The Schirmer test with anaesthesia was believed to measure the amount of residual tears plus the basal tear production without a foreign body stimulus. However, studies have shown that a reflex component was present even when anaesthesia was used (Jordan and Baum, 1980). It is currently not clear what the PRT test actually measures. However, a review of the literature (see chapter 1, section 1.5.4) indicates that the PRT test measure is probably related to the amount of tears situated in the eye. The present study also found a moderate correlation between PRT wetting lengths and TMH measures at baseline (Spearman's $r = 0.363$, $p = 0.001$, unpublished results), a result which has been reported previously as well (Mainstone *et al.*, 1996; Miller *et al.*, 2004). If it is accepted that the PRT predominantly is an estimate of the tear volume of the eye, it may be logical that no differences was seen post-LASIK. During the LASIK procedure, a majority of the corneal nerves are cut, and it takes several months for them to regenerate. Corneal nerves are essential in the process of reflex tearing. However, as the TMH values also did not change significantly after LASIK it may indicate that basal tear production itself is not reduced. Since the tear film stability (TBUT measures) also remained largely unchanged, increased tear evaporation was prevented. Therefore, it may not be surprising that tear film tests that primarily estimate tear volume, TMH values and PRT wetting lengths, remained largely unchanged 3 months or more after LASIK.

Another plausible reason for not detecting any statistically significant differences in the PRT wetting lengths was the large differences between individual measurements. Although inter-visit differences in the group mean PRT values differed by only 1-3 mm, individual values differed to a large extent at all visits with the inter quartile ranges being close to 90% of the median values (see Chapter 3, Figure 3.4-4). The 95% CI limits of agreement was -14 mm to +22 mm at baseline, and similar limits of agreement were found for the post-LASIK PRT assessments. This result is similar to the results from studies that have assessed repeatability of the PRT test by measuring PRT on different occasions. Group mean values have been reported to be "the same" on different occasions (Little and Bruce, 1994b) or reported to vary with as little as 0.9 (Nichols *et al.*, 2004b), 1-2 mm (Cho and Kwong, 1996) or 5 mm (Cho and Chan, 2003). However, individual values from the same studies have been reported differing to a large extent with 95% confidence intervals ranging from -10 mm to +10 mm (Little and Bruce, 1994b), -15.5 mm to +13.8 mm (Nichols *et al.*, 2004b), -14.8 mm to +8 mm (Cho and Chan, 2003).

Large inter-subject variability was also found for the other tear film tests that were undertaken. The inter-quartile ranges constituted 140%, 241% and 337% of the median values for TMH, NIBUT and f-TBUT tests, respectively. Similar large ranges were found for all four test groups. It is therefore not surprising that no statistically significant changes were detected. The observed TBUTs were generally longer than previously reported partly because any measurement was not truncated at 45 or 60 seconds and probably because the end-point used was when a clear interruption of the tear film was observed and not at the appearance of the first black spot (which often resolves without blinking, see chapter 1, section 1.4.4). For the LASIK group only, the large inter-subject variability for f-TBUT actually increased over the two years the present study lasted. This unexpected finding contradicts many previous studies and the reason for this is not clear. One possible reason could be failing to see peripheral tear break near the lid margin. A study on PRK subjects noted that the distributions of tear film break-up after surgery were more often localized in the upper and lower temporal regions than in normal eyes and the incidence of tear film break-up in the central cornea was decreased (Chen and Wang, 1999). Furthermore, subjects that have undergone LASIK surgery have a reduced blink-rate (Toda *et al.*, 2001). The observer's clinical impression from the present study was that small, peripheral tear film breaks often resolved before the patient blinked and thus extending the f-TBUT. The combination of a reduced blink rate and peripheral tear-break, which resolved or was overlooked, may have caused the greater frequency of longer f-TBUT values after LASIK.

In summary, all mean tear film measures were within normal limits and no clear indications of dry eye were found for any group at any visit. This result reflects that the subjects were all successful soft contact lens wearers when the study commenced, a finding which is also supported by the modest degrees of eye redness and fluorescein staining of the ocular surface. Furthermore, these results shows that after uneventful LASIK procedures in soft contact lens wearers otherwise suitable for such surgery, dry eye symptoms should not be expected to exceed pre-operative levels. The tear film test results in the present study support this conclusion.

4.3 Limitations of the study

Only one part of the mid-peripheral corneal endothelium was assessed

Endothelial analysis was only undertaken from photos taken of two regions of the cornea; one central picture and one mid-peripheral picture (approximately 3 mm superior-nasally) were taken at each subject at each occasion. A review of the literature indicates that differences between para-central corneal endothelium may exist. For example, the superior part of the corneal endothelial cell layer has been shown to have a higher cell density than inferior parts. However, the present study has shown that a detailed analysis of one mid-peripheral area gives a lot more information than only one central picture. Although a few other studies have analysed the endothelium from all four quadrants, the results have often been averaged which could conceal significant differences between central and mid-peripheral endothelial morphometry.

Environmental factors

The environment may significantly influence the results of clinical studies. There are variables that could not be controlled in the present study, such as temperature and humidity. However, an attempt was made to minimize such effects by assessing each subject group at the same time of the year, and each subject was assessed at approximately the same time of the day at each visit. All subjects were assessed at least two hours after wakening. The present study was initially a two-site study, but relocation of the LASIK clinic necessitated a third investigation room where also some of the instruments had been changed. Although great care was taken to keep the same set-up and sequence of tests that was originally initiated, environmental influence on the results cannot be totally excluded.

Bias of spectacle group

The spectacle group was not ideal as a control group. First, this group was small (N = 15) and second, some of the test results found for the spectacle wearers were rather unexpected. For example, the frequency and severity of ocular symptoms were very similar to lens wearers. The spectacle wearers proved to have a higher prevalence and more substantial complaints than expected with over 70% reporting dry eye symptoms to occur at least sometimes and an overall mean VAS severity of 35.9. In non-lens wearers, the prevalence of dry eye symptoms has been reported to be just 17.6% (emmetropes) (Nichols *et al.*, 2005) and 12.4% (not specifying whether or not these individuals wore spectacles) (Chalmers and Begley, 2006). However, up to 47% can be expected to report dryness symptoms in populations wearing spectacles (Vajdic *et al.*, 1999; Nichols *et al.*, 2005). The reported level of symptom severity was similar to the contact lens wearers and also in accordance with ocular comfort studies of spectacle wearers during VDU work or eye straining activities (Aaras *et al.*, 1998; Sheedy *et al.*, 2003; Horgen *et al.*, 2004). Since the questionnaire used in the present study was not specific as to when symptoms occurred the higher symptom frequencies and levels may be due to working tasks or environmental factors (Himebaugh *et al.*, 2009). Furthermore, spectacle wearers seek more frequent vision care for their refractive error, and may have more awareness of their ocular health and status than emmetropes.

Spectacle wearers may also have a previous diagnosis of dry eye disease; however the ocular surface and tear film test results of the subjects in the current study showed only minimal or no signs of dry eye. The way subjects were recruited could have mattered. All soft lens wearers (including pre-LASIK subjects) in the present study volunteered to participate through the response of a direct invitation via e-mail, telephone or 'by word of mouth'. The spectacle wearers, however, responded to a general invitation via an advertisement in the local newspaper. It is possible that people with some sort of problems will respond to such an advertisement rather than people who are completely symptom free. In spite of this, it may still be regarded as a strength of the study having a control group, or reference group, and for variables like endothelial morphometry characteristics, this group of non-lens wearers was not different from what has been reported by others.

Size of the test groups

The number of subjects who completed the study (for endothelial analyses) was 12, 18, 19 and 20 for the spectacle wearers, soft lens wearers, SiH lens wearers and LASIK subjects, respectively. While these groups are too small for any robust statistical between-groups comparisons, at least for variables like tear film measurements, it is legitimate to regard each group as reference groups because, for most variables, their test results were found to be very similar to what have been reported previously. However, the main aim of the present study was prospective analyses, and the power of the study of soft lens wearers, SiH lens wearers and LASIK subjects was ≥ 0.8 for endothelial analyses (not shown).

Admittedly, the study would have benefited from larger test groups. However, the group sizes were very similar compared to many previous studies. Moreover, it was considered more important to include reference groups of contact lens wearers and spectacle wearers than assessing only post-LASIK subjects, since a majority of the individuals seeking LASIK are contact lens wearers. The present study has shown that contact lens wear is an important factor that needs to be considered. Last, the total number of subjects who participated in the present study was 98. A larger study would have required other facilities and more than one (part-time) investigator.

4.4 Concluding remarks

In the literature and in the media, dry eye symptoms is often referred to as the most common complication after LASIK, especially if dry eyes are present pre-operatively. The present study suggest that if the soft contact lens wearers who plan to undergo LASIK are successful, full-time wearers, dry eye symptoms can be expected to reduce after surgery. Further studies should explore the impact of confounding factors such as the environment and vision demanding working tasks when assessing dry eye symptoms after LASIK surgery.

In light of the endothelial cells' capacity of migration and elongation in response to corneal wounding and stress, it is tempting to suggest that (minus-powered) contact lens wear initiate centrally directed migration of far-peripheral endothelial cells resulting in a higher mid-peripheral to central endothelial cell density ratio than observed for non-wearers. This theory would imply a reduced far-peripheral endothelial cell density in contact lens wearers compared to non-lens wearers, which the present study did not assess. Further studies should assess the far peripheral endothelium in addition to central and mid-peripheral parts.

By showing that higher MPECD:CECD ratio is only present in some individuals, the present study indicates that there is much more work that needs to be done. Further studies are needed to verify that the MPECD:CECD relationship in soft lens wearers is different from spectacle wearers. Several regions of the corneal endothelium should be evaluated, since some studies have reported that the cell density is higher superiorly. It would also be desirable to conduct a study where contact lens wearers ceased lens wear for at least three months to see if the same changes as seen in LASIK subjects would occur to eventually exclude any shape-change factor. A detailed analysis of the anterior and posterior corneal shape in relation to endothelial characteristics would also add to the current knowledge of how endothelial morphometry relate to corneal shape.

If it is accepted that the six-sided cell shape is optimal for a cell layer, which ensure barrier properties, then the proven reduction, and even reversible tendency, in pleomorphism observed for post-LASIK subjects must be interpreted as a highly desirable process. That this was not observed for subjects who were refitted with SiH contact lenses suggests that the continuous presence of a silicone-hydrogel contact lens, in spite of its high oxygen transmissibility, triggers mechanisms that maintain metabolic changes presumably due to inflammation processes. However, the continuous wear of a SiH lens is clearly superior to daily wear of soft hydrogel lenses with regard to corneal endothelial polymegethism, which undoubtedly reduced, or even reversed, during the study period for both post-LASIK subjects and continuous SiH contact lens wearers while it stayed unchanged in daily soft hydrogel lens wearers. Further studies should explore whether endothelial morphometry following refitting into daily wear of SiH lenses, which is associated with fewer and milder responses of inflammation, give different results than the present study. Likewise, it would be interesting to assess endothelial morphometry in wearers of SiH lenses of various modulus of rigidity, which would exert different mechanical pressure on the corneal tissue.

Last, the present study has shown that control groups in future studies on the influence of refractive surgery procedures on the cornea need to include contact lens wearers. The corneas in non-lens wearers have not been exposed to the same metabolic challenges as contact lens wearers and the present study has clearly expanded our knowledge on how different the endothelial cell morphometry is for contact lens wearers as opposed to spectacle wearers.

APPENDIX

5.0 Normality test results

Data on refractive error of a subject group was collected at 24 occasions. The Shapiro-Wilk test for normality gave p-values close to 0.3 in most instances. However, at six of 24 occasions, the probability of a normal distribution of the data was less than 5%, and at another six occasions, $p \leq 0.1$. The probability of a normal distribution was always less than 7% in the SiH group. Since the Shapiro-Wilk test cannot prove normality, but is rather a strong indicator of non-normality, non-parametric tests were chosen for all groups when refractive error was analysed.

Visual acuity tests were obtained from a test group at 80 different occasions over the two years the study lasted. In over 50% of the instances, the Shapiro-Wilk test for normality yielded probability values close to 0.2. At 31 occasions, Shapiro-Wilk test for normality gave p-values less than 0.05, and at further 10 occasions p was less or equal to 0.1. Therefore, the assumption of normality was rejected, and non-parametric tests were applied for the analyses of all visual acuity variables.

The Shapiro-Wilk test for normality showed that the grading of vision was clearly not normally distributed ($p \leq 0.05$ at all occasions). The severity of dryness symptoms was evaluated for a test group at 24 different occasions. In most cases, the Shapiro-Wilk test of normality gave p-values close to 0.3. However, at five occasions, p was less or equal to 0.05, and at another four instances p was less or equal to 0.1. Since the grading of vision was clearly a non-parametric variable and since both parametric and non-parametric tests gave similar results, non-parametric tests were chosen for the variable "symptom severity".

For non-invasive and fluorescein-tear break up times (NIBUT and f-TBUT) the Shapiro-Wilk test for normality showed that these sets of data were clearly not normally distributed ($p \leq 0.05$ at all occasions). Similarly, the distribution of tear meniscus heights (TMH), within each group at each occasion, was not normally distributed ($p<0.05$, Shapiro-Wilk test). Thus, these variables were treated in a non-parametric manner. Phenol red thread (PRT) test results were obtained for a group of subjects at 24 instances. In the majority cases, the Shapiro Wilk test probability value was between 0.2 and 0.4 and only at 4 occasions was it found to be < 0.05. However, since the other tear film variables were non-parametric and since parametric and non-parametric tests gave similar results, PRT was treated as a non-parametric variable for the reason of consistency.

The ocular surface was assessed using a slit-lamp and each characteristic was graded with Efron's grading scale in 0.1 units. None of the ocular surface characteristics were found to be evenly distributed ($p < 0.05$, Shapiro-Wilk test). Consequently, non-parametric tests were chosen for the analyses of these.

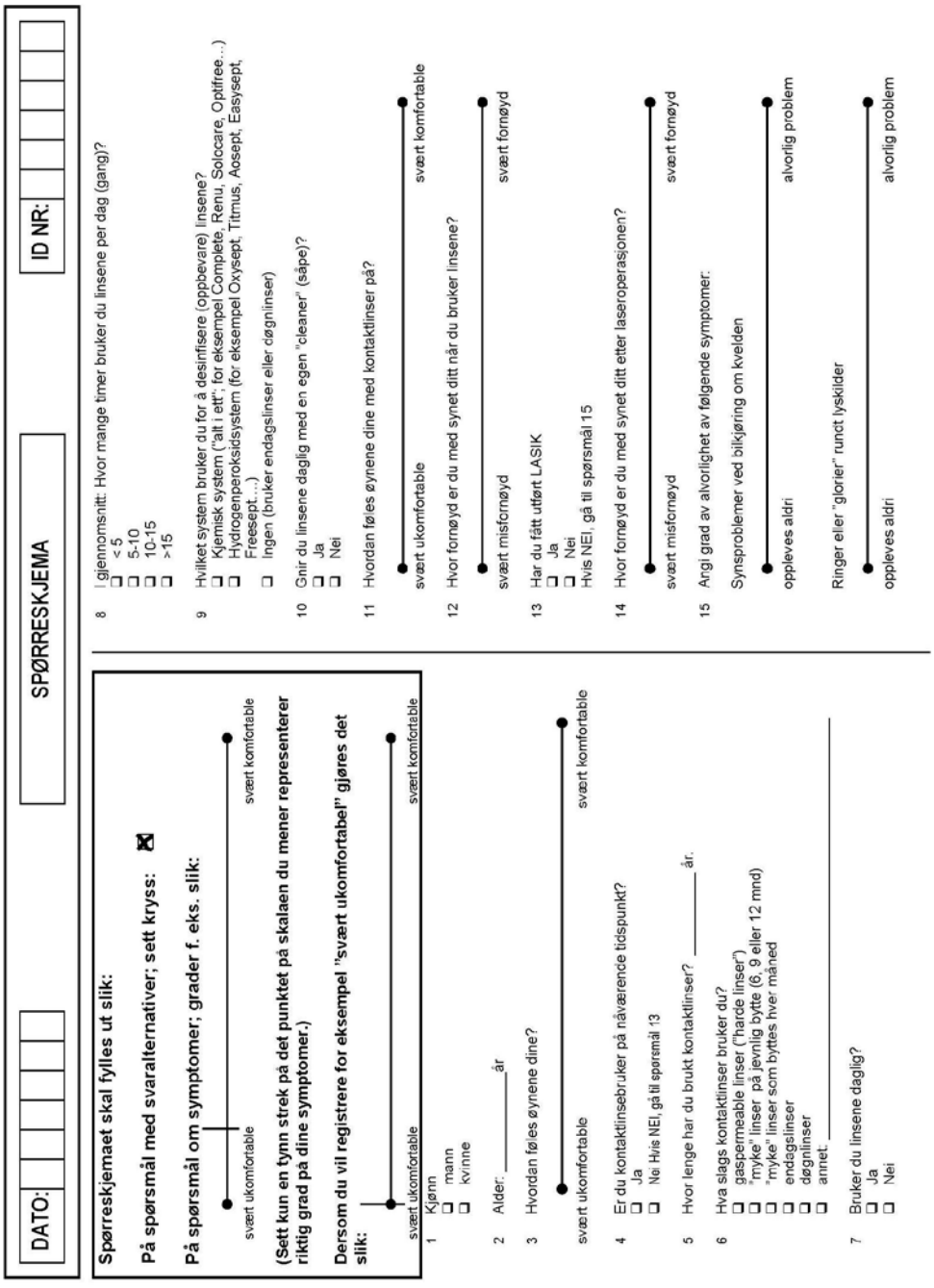
For central and mid-peripheral corneal thickness data, the Shapiro-Wilk test for normality gave probability values close to 0.5 in most cases. In two out of 48 instances, p was less or equal to

0.05. In a series of experiments, one would expect one out of 20 to reach significance by chance. Thus, it was considered reasonable to make the assumption of normality and parametric tests were chosen for the following analyses. For anterior radius of curvature, the Shapiro-Wilk test for normality yielded p-values that were ≤ 0.05 at 12 occasions out of 24, which means that there is less than 5% probability that these distributions were normal. Therefore, non-parametric tests were chosen for the analyses where the variable K (central anterior radius of curvature) was involved.

For the morphometric variables, the Shapiro-Wilk test for normality gave p-values close to 0.4 in most instances. On a few occasions (11 out of 144), p was less or equal to 0.05, which can be a result by chance since in a series of experiments, one would expect one out of 20 to reach significance by chance. Moreover, three of the groups had sample sizes close to 20 and the probability values from non-parametric tests were close to parametric test results. Therefore, it was considered reasonable to make the assumption of normality for the morphometric data. Consequently, parametric tests were chosen.

5.1 Clinical evaluations

5.1.1 Questionnaire



DATO: | | | | | | SPØRRESKJEMA ID NR: | | | | | |

Spørreskjemaet skal fylles ut slik:

På spørsmål med svaralternativer; sett kryss: ☒

På spørsmål om symptomer; grader f. eks. slik:

svært ukomfortable — svært komfortable

(Sett kun en tynn strek på det punktet på skalaen du mener representerer riktig grad på dine symptomer.)

Dersom du vil registrere for eksempel "svært ukomfortabel" gjøres det slik:

svært ukomfortable — svært komfortable

1 Kjønn
- ☐ mann
- ☐ kvinne

2 Alder: _____ år

3 Hvordan føles øynene dine?

svært ukomfortable — svært komfortable

4 Er du kontaktlinsebruker på nåværende tidspunkt?
- ☐ Ja
- ☐ Nei Hvis NEI, gå til spørsmål 13

5 Hvor lenge har du brukt kontaktlinser? _____ år.

6 Hva slags kontaktlinser bruker du?
- ☐ gasspermeable linser ("harde linser")
- ☐ "myke" linser på jevnlig bytte (6, 9 eller 12 mnd)
- ☐ "myke" linser som byttes hver måned
- ☐ endagslinser
- ☐ døgnlinser
- ☐ annet: _____

7 Bruker du linsene daglig?
- ☐ Ja
- ☐ Nei

8 I gjennomsnitt: Hvor mange timer bruker du linsene per dag (gang)?
- ☐ < 5
- ☐ 5-10
- ☐ 10-15
- ☐ >15

9 Hvilket system bruker du for å desinfisere (oppbevare) linsene?
- ☐ Kjemisk system ("alt i ett"; for eksempel Complete, Renu, Solocare, Optifree...)
- ☐ Hydrogenperoksidsystem (for eksempel Oxysept, Titmus, Aosept, Easysept, Freesept...)
- ☐ Ingen (bruker endagslinser eller døgnlinser)

10 Gnir du linsene daglig med en egen "cleaner" (såpe)?
- ☐ Ja
- ☐ Nei

11 Hvordan føles øynene dine med kontaktlinser på?

svært ukomfortable — svært komfortable

12 Hvor fornøyd er du med synet ditt når du bruker linsene?

svært misfornøyd — svært fornøyd

13 Har du fått utført LASIK
- ☐ Ja
- ☐ Nei

Hvis NEI, gå til spørsmål 15

14 Hvor fornøyd er du med synet ditt etter laseroperasjonen?

svært misfornøyd — svært fornøyd

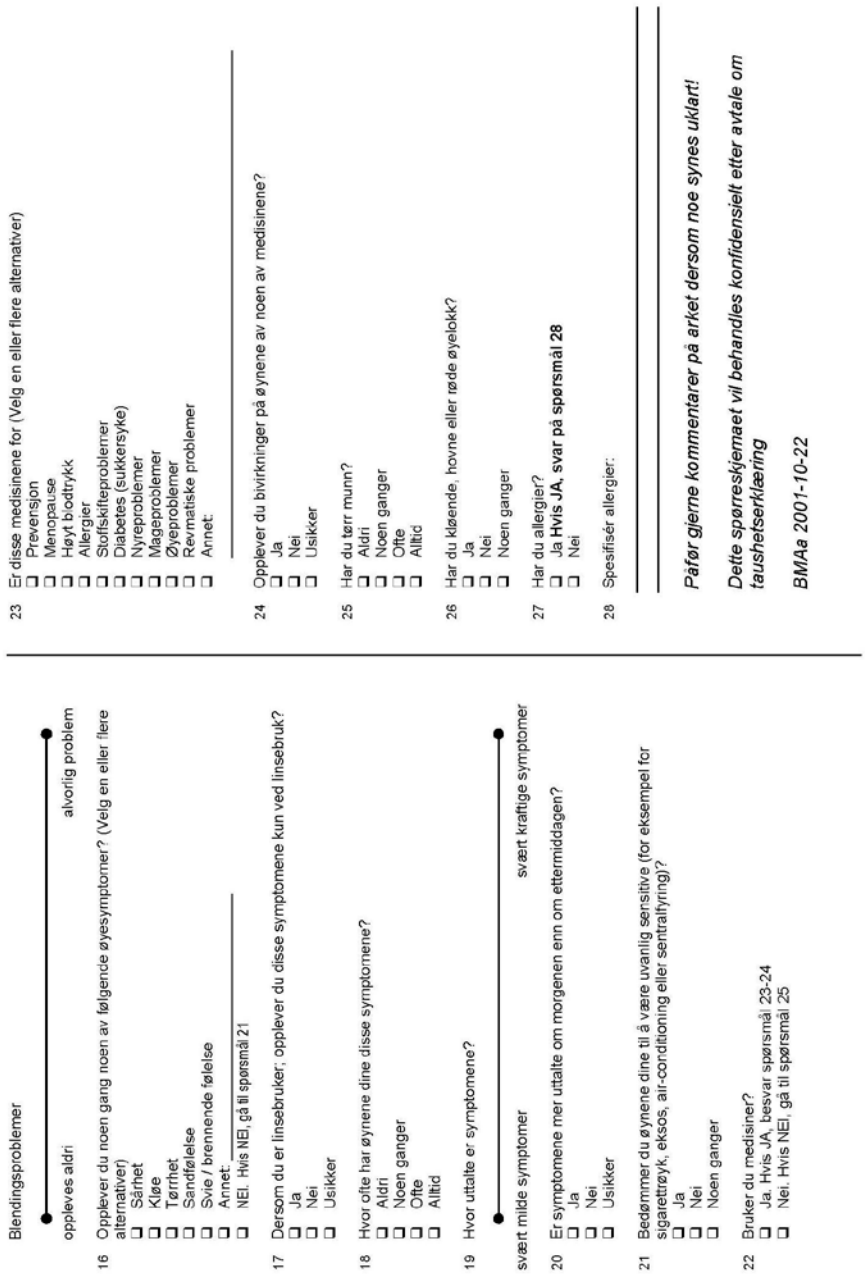
15 Angi grad av alvorlighet av følgende symptomer:

Synsproblemer ved bilkjøring om kvelden

oppleves aldri — alvorlig problem

Ringer eller "glorier" rundt lyskilder

oppleves aldri — alvorlig problem


Blendingsproblemer

opplevdes aldri — alvorlig problem

16 Opplever du noen gang noen av følgende øyesymptomer? (Velg en eller flere alternativer)
- ❑ Sårhet
- ❑ Kløe
- ❑ Tørrhet
- ❑ Sandfølelse
- ❑ Svie / brennende følelse
- ❑ Annet: _____
- ❑ NEI. Hvis NEI, gå til spørsmål 21

17 Dersom du er linsebruker; opplever du disse symptomene kun ved linsebruk?
- ❑ Ja
- ❑ Nei
- ❑ Usikker

18 Hvor ofte har øynene dine disse symptomene?
- ❑ Aldri
- ❑ Noen ganger
- ❑ Ofte
- ❑ Alltid

19 Hvor uttalte er symptomene?

svært milde symptomer — svært kraftige symptomer

20 Er symptomene mer uttalte om morgenen enn om ettermiddagen?
- ❑ Ja
- ❑ Nei
- ❑ Usikker

21 Bedømmer du øynene dine til å være uvanlig sensitive (for eksempel for sigarettrøyk, eksos, air-conditioning eller sentralfyring)?
- ❑ Ja
- ❑ Nei
- ❑ Noen ganger

22 Bruker du medisiner?
- ❑ Ja. Hvis JA, besvar spørsmål 23-24
- ❑ Nei. Hvis NEI, gå til spørsmål 25

23 Er disse medisinene for (Velg en eller flere alternativer)
- ❑ Prevensjon
- ❑ Menopause
- ❑ Høyt blodtrykk
- ❑ Allergier
- ❑ Stoffskifteproblemer
- ❑ Diabetes (sukkersyke)
- ❑ Nyreproblemer
- ❑ Mageproblemer
- ❑ Øyeproblemer
- ❑ Revmatiske problemer
- ❑ Annet: _____

24 Opplever du bivirkninger på øynene av noen av medisinene?
- ❑ Ja
- ❑ Nei
- ❑ Usikker

25 Har du tørr munn?
- ❑ Aldri
- ❑ Noen ganger
- ❑ Ofte
- ❑ Alltid

26 Har du kløende, hovne eller røde øyelokk?
- ❑ Ja
- ❑ Nei
- ❑ Noen ganger

27 Har du allergier?
- ❑ Ja **Hvis JA, svar på spørsmål 28**
- ❑ Nei

28 Spesifiser allergier: _____

Påfør gjerne kommentarer på arket dersom noe synes uklart!

Dette spørreskjemaet vil behandles konfidensielt etter avtale om taushetserklæring

BMAø 2001-10-22

Table 5.1-1
Variable list, obtained from questionnaire (page 1 of 3)

Variable	Definition	Type	Unit / definition
Subject demographics			
Gender	Male or female	Nominal	1=male 2=female
Age		Interval	Years
Contact lens wear			

Variable	Definition	Type	Unit / definition
Length of contact lens wear		Interval	Years
Contact lens modality		Nominal	1 = Rigid 2 = Soft 3 = Soft, replaced monthly 4 = Soft, disposed daily 5 = Silicone-hydrogel 6 = Other
Daily wear	"Do you wear your lenses every day?"	Nominal	1=Yes 2=No
Length of wear each day		Ordinal	1 = < 5 h 2 = 5 to 10 h 3 = 10 to 15 h 4 = > 10 h
Disinfection system		Nominal	1 = Chemical (all-in-one) 2 = H_2O_2 – based 3 = None
Daily cleaner	"Do you clean your lenses with a special cleaner?"	Nominal	1=Yes 2=No
Days without SiH lenses	Number of days without SiH lenses in-situ last 6 months	Interval	Number
Nights without SiH lenses	Number of nights without SiH lenses in-situ last 6 months	Interval	Number
Satisfaction of vision			
Satisfaction with corrected vision	Grading of satisfaction of corrected (i.e. with contact lenses, spectacles or LASIK) on a 100 mm long horizontal line: Visual Analogue Scale (VAS); endpoints representing "very comfortable" and "very uncomfortable"	Continuous	0.5 mm
Vision problems when driving at night	Grading of severity of vision problems (VAS); endpoints representing "Never experiencing it" to "severe problem".	Continuous	0.5 mm
Experience of halos	Grading of the severity of experiencing halos (VAS); endpoints representing "Never experiencing it" to "severe problem".	Continuous	0.5 mm
Problems with glare	Grading of the severity of glare problems (VAS); endpoints representing "Never experiencing it" to "severe problem".	Continuous	0.5 mm
Ocular symptoms			
Ocular symptom(s)	Type and presence of ocular symptom(s)	Nominal	1 = Soreness 2 = Itchiness 3 = Dryness 4 = Grittiness 5 = Burning 6 = Other 7 = No
Contact lens related symptoms	"Do you experience these symptoms only when wearing contact lenses?"	Nominal	1 = Yes 2 = No

Variable	Definition	Type	Unit / definition
Frequency of ocular symptoms		Ordinal	1 = Never 2 = Sometimes 3 = Often 4 = Always
Severity of ocular symptoms	Grading of the severity of ocular symptoms (VAS); endpoints representing “very mild symptoms” and “Very severe symptoms"	Continuous	0.5 mm
Symptoms in the morning	Symptoms in the morning rather than in the afternoon.	Nominal	1 = Yes 2 = No 3 = Uncertain
Sensitive eyes	Extraordinary sensitivity to smoke, air-condition etc.	Nominal	1 = Yes 2 = No 3 = Sometimes
Dry mouth		Nominal	1 = Yes 2 = No 3 = Uncertain
Itchy, swollen or red eyelids		Nominal	1 = Yes 2 = No 3 = Sometimes
Medication and allergy			
Medication use		Nominal	1 = Yes 2 = No
Condition(s)	One or several conditions that the medications are used for	Nominal	1 = Birth control 2 = Menopause 3 = High Blood Pressure 4 = Allergies 5 = Thyroid problems 6 = Diabetes 7 = Kidney problems 8 = Stomach problems 9 = Eye problems 10 = Rheumatoid disease 11 = Other
Side effects of medication		Nominal	1 = Yes 2 = No 3 = Uncertain
Presence of allergy		Nominal	1 = Yes 2 = No
Specification of allergy		Nominal	Open answer

Table 5.1-2
Conversion Table for presentation of visual acuity

6 m	Decimal	Log MAR
6 / 12	0.5	+0.3
6 / 9.5	0.63	+0.2
6 / 7.5	0.8	+0.1
6 / 6	1.0	0
6 / 4.8	1.25	-0.1
6 / 3.8	1.6	-0.2
6 / 3	2.0	-0.3

5.2 Spectacle wearers

Table 5.2-1
Linear regression analysis of time dependent changes in endothelial morphometric parameters in spectacle wearers (n=12).

Slope (p.y.)[1]	ECD (cells/mm^2)		COV (%)		%6-sided cells	
	central	mid-peripheral	central	mid-peripheral	central	mid peripheral
Mean ± SD (min -max)	-3.1 ± 50.4 (-82.3 to 96.0)	-29.2 ± 63.7 (-111.4 to 94.2)	-0.1 ± 1.3 (-2.8 to 1.7)	0.3 ± 1.9 (-2.5 to 3.6)	-0.9 ± 3.0 (-5.6 to 6.4)	-1.0 ± 4.0 (-6.0 to 5.6)
Pearson's R	-0.015	-0.099	-0.015	0.045	-0.064	-0.078
p	0.900	0.410	0.900	0.710	0.595	0.514

Table 5.2-2
Subject details of all spectacle wearers at a single occasion ('baseline').

	Initial assignment	Extra subjects	p (t-test)	Total
Number of subjects	15	6		21
Age (years)	32.3 ± 5.8	28.2 ± 6.1	0.192	31.1 ± 6.0
Age range (years)	23 to 41	20 to 36		20 to 41
Gender (F:M)	6:9	3:3		9:12
Mean RE[a]	-2.71 ± 1.69	-0.63 ± 0.31*	<0.001	-2.11 ± 1.72

[a] Mean refractive error (MSE) in spherical equivalent power (DS). All other data are mean ± S.D.
* Significantly different from initially assigned group ($p < 0.001$)

Table 5.2-3
Inter-relationships (bivariate correlation analyses; Pearson's correlation coefficient) of mean morphometric parameters (at six occasions over two years) in spectacle wearers (N=15).

SPX		CECD	MPECD	CCOV	MPCOV	C%SIX	MP%SIX
CECD	r	1	0.563*	0.196	0.185	0.249	-0.016
	p		0.029	0.484	0.509	0.370	0.954
MPECD	r	0.563*	1	0.390	0.189	-0.316	-0.166
	p	0.029		0.150	0.500	0.251	0.555
CCOV	r	0.196	0.390	1	0.904**	-0.535*	-0.755**
	p	0.484	0.150		0.000	0.040	0.001
MPCOV	r	0.185	0.189	.904**	1	-0.414	-0.807**
	p	0.509	0.500	0.000		0.125	0.000
C%SIX	r	0.249	-0.316	-0.535*	-0.414	1	0.674**
	p	0.370	0.251	0.040	0.125		0.006
MP%SIX	r	-0.016	-0.166	-0.755**	-0.807**	.674**	1
	p	0.954	0.555	0.001	0.000	0.006	

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

5.3 Soft contact lens wearers

Table 5.3-1
Linear regression analysis of time dependent changes in endothelial morphometric parameters in soft lens wearers (n = 19).

Slope (p.y.) [1]	ECD (cells/mm^2)		COV (%)		% six-sided cells	
	central	mid-peripheral	central	mid-peripheral	central	mid peripheral
Mean ± SD (min -max)	4.0 ± 51.2 (-85.3 to 103.6)	-47.5 ± 106.9 (-375.1 to 159.5)	-0.2 ± 2.0 (-4.0 to 2.7)	0.8 ± 2.3 (-4.0 to 3.7)	0.9 ± 3.7 (-5.1 to 8.4)	-2.7 ± 3.1 (-7.9 to 2.7)
Pearson's R	0.008	-0.110	-0.030	0.049	0.067	-0.179
p	0.930	0.244	0.750	0.603	0.479	0.057

Table 5.3-2
Inter-relationships (bivariate correlation analyses; Pearson's correlation coefficient) of mean morphometric parameters (at six occasions over two years) in soft lens wearers (N=21).

SCL		CECD	MPECD	CCOV	MPCOV	C%SIX	MP%SIX
CECD	r	1.000	0.639**	-0.056	-0.264	-0.194	0.155
	p		0.002	0.810	0.248	0.400	0.503
MPECD	r	0.639**	1.000	-0.251	-.471*	0.108	0.153
	p	0.002		0.272	0.031	0.640	0.508
CCOV	r	-0.056	-0.251	1.000	0.648**	-0.826**	-0.533*

	p	0.810	0.272		0.001	0.000	0.013
MPCOV	r	-0.264	-0.471*	0.648**	1.000	-0.365	-.759**
	p	0.248	0.031	0.001		0.104	0.000
C%SIX	r	-0.194	0.108	-0.826**	-0.365	1.000	0.490*
	p	0.400	0.640	0.000	0.104		0.024
MP%SIX	r	0.155	0.153	-0.533*	-.759**	0.490*	1.000
	p	0.503	0.508	0.013	0.000	0.024	

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

5.4 SiH contact lens wearers

Table 5.4-1
Linear regression analysis of time dependent changes in endothelial morphometric parameters in *SiH lens wearers* (n = 18).

Slope (p.y.) [1]	ECD (cells/mm^2)		COV (%)		%6-sided cells	
	central	mid-peripheral	central	mid-peripheral	central	mid peripheral
Mean ± SD (min -max)	-24.2 ± 53.0 (-101.3 to 71.3)	-57.5 ± 84.7 (-277.5 to 144.7)	-2.2 ± 2.1 (-7.0 to 1.1)	-1.9 ± 3.1 (-10.0 to 3.7)	0.3 ± 5.3 (-6.6 to 13.5)	-0.3 ± 4.5 (-6.0 to 10.1)
Pearson's R	-0.066	-0.136	-0.296	-0.219	0.023	-0.023
p	0.500	0.161	0.002	0.023	0.810	0.810

Table 5.4-2
Inter-relationships (bivariate correlation analyses; Pearson's correlation coefficient) of morphometric parameters in soft lens wearers before refitting with SiH lenses (N=22).

pre-refitting		CECD	MPECD	CCOV	MPCOV	C%SIX	MP%SIX
CECD	r	1	0.634**	-0.034	-0.226	0.054	0.029
	p		0.002	0.882	0.311	0.813	0.898
MPECD	r	0.634**	1	0.371	-0.076	-0.053	-0.078
	p	0.002		0.089	0.736	0.816	0.729
CCOV	r	-0.034	0.371	1	0.636**	-0.586**	-0.388
	p	0.882	0.089		0.001	0.004	0.074
MPCOV	r	-0.226	-0.076	0.636**	1	-0.294	-0.783**
	p	0.311	0.736	0.001		0.185	0.000
C%SIX	r	0.054	-0.053	-0.586**	-0.294	1	0.086
	p	0.813	0.816	0.004	0.185		0.702
MP%SIX	r	0.029	-0.078	-0.388	-0.783**	0.086	1
	p	0.898	0.729	0.074	0.000	0.702	

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Table 5.4-3
Inter-relationships (bivariate correlation analyses; Pearson's correlation coefficient) of mean morphometric parameters (at five occasions over two years) after refitting with SiH lenses (N=22).

post-refitting		CECD	MPECD	CCOV	MPCOV	C%SIX	MP%SIX
CECD	r	1	0.781**	-0.008	-0.126	0.134	0.019
	p		0.000	0.973	0.575	0.552	0.932
MPECD	r	0.781**	1	-0.035	-0.214	0.190	-0.022
	p	0.000		0.876	0.340	0.397	0.922
CCOV	r	-0.008	-0.035	1	0.791**	-0.753**	-0.647**
	p	0.973	0.876		0.000	0.000	0.001
MPCOV	r	-0.126	-0.214	0.791**	1	-0.662**	-0.745**
	p	0.575	0.340	0.000		0.001	0.000
C%SIX	r	0.134	0.190	-0.753**	-0.662**	1	0.627**
	p	0.552	0.397	0.000	0.001		0.002
MP%SIX	r	0.019	-0.022	-0.647**	-0.745**	0.627**	1
	p	0.932	0.922	0.001	0.000	0.002	

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

5.5 LASIK subjects

Table 5.5-1
Linear regression analysis of time dependent changes in endothelial morphometric parameters in *post-LASIK subjects* (n = 20).

Slope (p.y.) [1]	ECD (cells/mm^2)		COV (%)		%6-sided cells	
	central	mid-peripheral	central	mid-peripheral	central	mid peripheral
Mean ± SD (min -max)	-13.9 ± 47.2 (-135.9 to 48.2)	-77.0 ± 60.3 (-208.1 to 27.7)	-2.2 ± 1.7 (-6.7 to 1.3)	-1.8 ± 2.5 (-7.5 to 4.7)	2.4 ± 4.5 (-8.3 to 11.2)	2.6 ±3.9 (-2.5 to 13.6)
Pearson's R	-0.031	-0.223	-0.278	-0.199	0.171	0.182
p	0.737	0.014	0.002	0.029	0.061	0.047

Table 5.5-2
Inter-relationships (bivariate correlation analyses; Pearson's correlation coefficient) of morphometric parameters in soft lens wearers before LASIK (N=25).

pre-LASIK		CECD	MPECD	CCOV	MPCOV	C%SIX	MP%SIX
CECD	r	1	0.479*	-0.100	0.342	-0.164	-0.005
	p		0.015	0.635	0.094	0.432	0.980
MPECD	r	0.479*	1	-0.237	-0.160	0.049	0.021
	p	0.015		0.253	0.444	0.817	0.921
CCOV	r	-0.100	-0.237	1	0.683**	-0.660**	-0.701**
	p	0.635	0.253		0.000	0.000	0.000
MPCOV	r	0.342	-0.160	0.683**	1	-0.607**	-0.690**
	p	0.094	0.444	0.000		0.001	0.000
C%SIX	r	-0.164	0.049	-0.660**	-0.607**	1	0.631**
	p	0.432	0.817	0.000	0.001		0.001
MP%SIX	r	-0.005	0.021	-0.701**	-0.690**	0.631**	1
	p	0.980	0.921	0.000	0.000	0.001	

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Table 5.5-3
Inter-relationships (bivariate correlation analyses; Pearson's correlation coefficient) of mean morphometric parameters (at five occasions over two years) after LASIK (N=25).

post-LASIK		CECD	MPECD	CCOV	MPCOV	C%SIX	MP%SIX
CECD	r	1	0.652**	-0.260	-0.046	0.034	-0.003
	p		0.000	0.209	0.828	0.872	0.990
MPECD	r	0.652**	1	-0.261	-0.139	-0.036	0.045
	p	0.000		0.208	0.507	0.863	0.832
CCOV	r	-0.260	-0.261	1	0.847**	-0.734**	-0.788**
	p	0.209	0.208		0.000	0.000	0.000
MPCOV	r	-0.046	-0.139	0.847**	1	-0.712**	-0.872**
	p	0.828	0.507	0.000		0.000	0.000
C%SIX	r	0.034	-0.036	-0.734**	-0.712**	1	0.816**
	p	0.872	0.863	0.000	0.000		0.000
MP%SIX	r	-0.003	0.045	-0.788**	-0.872**	0.816**	1
	p	0.990	0.832	0.000	0.000	0.000	

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

5.5.1 Consideration of magnification error

For the post-LASIK group, endothelial cell densities were also assessed after correcting for the possible effects of image magnification changes that arise since the central and mid-peripheral corneal thickness is significantly altered by the surgery (Table 3.4-11). It has been concluded, partly based on theoretical considerations, the estimates of ECD in a thin cornea will be over-estimated by the IMAGEnet software (Isager *et al.*, 1996). As suggested by Isager and colleagues (Isager *et al.*, 1999), each individual ECD value at all 6 visits was adjusted by multiplying the cell density with the normalized magnification (M^2/M_o^2). The Topcon SP-200P is calibrated by the company assuming a standard central corneal thickness of 500nm and anterior corneal radius of 8.00 mm (M_o) (Personal communication with Essilor Norge). From equations of geometric optics, magnification can be expressed as: $M = (-n/d)^2 \big/ (-n/d + (n - n_{air})/r)^2$, where *n* is the refractive index of the cornea (1,376), *d* is the corneal thickness and *r* is corneal curvature. Since both central and mid-peripheral corneal thicknesses were available, M^2/M_o^2 was calculated for each occasion, using central radius of curvature values in both calculations. For those subjects where corneal radius was not available, r = 8.00 mm was used.

These additional analyses indicated that, even with correction for possible magnification errors, the overall result was still the same as that outlined in section 3.4.6. The data from these analyses are included in Table 5.5-4 and Table 5.5-5. All mean cell densities adjusted for magnification were all significantly different from the initial cell density estimated by IMAGEnet cell analyzer (paired t-test). However, when judged clinically the differences were small, ranging from only 6 to 25 cells / mm^2. Moreover, when looking at time-dependent changes, the results remained essentially unchanged: Mean central cell density did not change from visit to visit whereas mid-peripheral cell density decreased post-operatively (Table 3.4-11). The mid-peripheral normalized cell densities at 18 and 24 months were on average 163 cells / mm^2 (p = 0.042) and 185 cells / mm^2 (p = 0.004) lower than the normalized cell density of 2641 $\pm$ 371 cells / mm^2 at baseline (repeated ANOVA). Linear regression analysis of time-dependent changes in cell densities showed the same trends for normalized cell densities as for the original (IMAGEnet) cell densities. No time dependent trends could be detected for central cell densities (Pearson's r = 0.044, p = 0.635) whereas for mid-peripheral cell densities a tendency of a reduction in cell numbers with time could be seen (Pearson's r = -0.23, p = 0.012).

Table 5.5-4
Mean ± SD endothelial cell density estimations (minimum to maximum values in brackets) before and after normalization. Each individual's estimated cell density (available from IMAGEnet cell analyzer) was multiplied with the normalized magnification (M^2/M_o^2). See text for details.

		Cell density (cells / mm^2)		
	Time	IMAGEnet	normalized	Sig.*
Central region	Baseline (pre-LASIK)	2635 ± 370 (2041 to 3559)	2641 ± 371 (2050 to 3578)	0.000132
	3 months	2642 ± 328 (2102 to 3447)	2627 ± 323 2098 to 3428	$5.251\ 10^{-7}$
	6 months	2633 ± 303 (2106 to 3350)	2617 ± 299 (2094 to 3328)	$7.23\ 10^{-8}$
	12 months	2653 ± 340 (2146 to 3614)	2637 ± 334 (2133 to 3592)	$2.39\ 10^{-8}$
	18 months	2607 ± 301 (2063 to 3425)	2593 ± 298 (2052 to 3404)	$1.38\ 10^{-7}$
	24 months	2615 ± 299 (2200 to 3324)	2601 ± 295 (2188 to 3310)	$2.79\ 10^{-7}$
Mid-peripheral region	Baseline (pre-LASIK)	2913 ± 274 (2519 to 3673)	2939 ± 281 (2532 to 3724)	$8.67\ 10^{-10}$
	3 months	2850 ± 215 (2538 to 3302)	2862 ± 211 (2554 to 3306)	$1.64\ 10^{-5}$
	6 months	2820 ± 286 (2363 to 3494)	2832 ± 289 (2360 to 3511)	$2.22\ 10^{-5}$
	12 months	2799 ± 198 (2413 to 3259)	2811 ± 197 (2421 to 3261)	$9.13\ 10^{-7}$
	18 months	2765 ± 249 (2394 to 3521)	2776 ± 245 (2411 to 3514)	$2.77\ 10^{-5}$
	24 months	2739 ± 221 (2417 to 3219)	2754 ± 224 (2427 to 3242)	$4.02\ 10^{-7}$

* paired, two-sided t-test

Table 5.5-5
Mean percentage difference between endothelial cell densities before and after LASIK (pre-operative minus post-operative) as estimated by IMAGEnet and the normalized cell densities (see text for details). Comparisons were made using repeated ANOVA test with Sidak adjustment for multiple comparisons.

		Change in cell density (%) from pre-LASIK values					
		IMAGEnet			Normalized		
	Time	Mean % df. from baseline	95% CI of mean df.	Sig.*	Mean % df. from baseline	95% CI of mean df.	Sig.*
Central region	3 months	-0.3	-4.3 to 3.7	1.000	0.5	-3.5 to 4.6	1.000
	6 months	0.1	-5.6 to 5.7	1.000	0.9	-4.8 to 6.6	1.000
	12 months	-0.7	-3.9 to 2.6	1.000	0.2	-3.1 to 3.4	1.000
	18 months	1.0	-3.4 to 5.5	1.000	1.8	-2.8 to 6.4	0.964
	24 months	0.8	-3.8 to 5.4	1.000	1.5	-3.1 to 6.2	0.994
Mid-periph. region	3 months	2.2	-3.5 to 7.9	0.973	2.6	-3.1 to 8.4	0.904
	6 months	3.2	-2.2 to 8.7	0.622	3.6	-1.8 to 9.1	0.435
	12 months	3.9	-0.9 to 8.7	0.182	4.4	-0.5 to 9.2	0.105
	18 months	5.1	-0.4 to 10.6	0.087	5.5	0.1 to 10.9	0.042
	24 months	6.0	1.3 to 10.7	0.006	6.3	1.5 to 11.1	0.004

* Adjustment for multiple comparisons: Sidak.

5.6 Between-group comparisons

Table 5.6-1
Correlations of MPECD:CECD ratio and age, central K and MPCT:CCT ratio for all spectacle wearers at baseline N = 21

		MPECD:CECD ratio	Age at the first visit (years)	Central K (mm)	MPCT:CCT ratio
MPECD:CECD ratio	r	1	-0.044	-0.433	0.004
	p		0.851	0.107	0.986
Age at the first visit (years)	r	-0.044	1	-0.205	0.179
	p	0.851		0.464	0.438
Central K (mm)	r	-0.433	-0.205	1	0.364
	p	0.107	0.464		0.183
MPCT:CCT ratio	r	0.004	0.179	0.364	1
	p	0.986	0.438	0.183	

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Table 5.6-2
Correlation of MPECD:CECD ratio and age, refractive error, length of lens wear, central K and MPCT:CCT ratio for all soft lens wearers at baseline N = 77

		MPECD:CECD ratio	Refractive error (MSE)[a]	Age at the first visit (years)	Duration of lens wear at the first visit (years)	Central K (mm)	MPCT:CCT ratio
MPECD:CECD ratio	r	1	-0.088	0.119	0.200	-0.023	-0.194
	p		0.445	0.303	0.080	0.848	0.091
Refractive error (MSE)[a]	r	-0.088	1	0.108	-0.316**	0.283*	.286*
	p	0.445		0.349	0.005	0.017	0.012
Age at the first visit (years)	r	0.119	0.108	1	0.620**	0.325**	-0.008
	p	0.303	0.349		0.000	0.006	0.947
Duration of lens wear at the first visit (years)	r	0.200	-0.316**	0.620**	1	.248*	-0.027
	p	0.080	0.005	0.000		0.037	0.815
Central K (mm)	r	-0.023	0.283*	0.325**	0.248*	1	0.027
	p	0.848	0.017	0.006	0.037		0.825
MPCT:CCT ratio	r	-0.194	0.286*	-0.008	-0.027	0.027	1.000
	p	0.091	0.012	0.947	0.815	0.825	

[a] Mean refractive error (MSE) in spherical equivalent power (DS)
*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

End notes

[1] The group mean slope of the individual linear regression lines' slopes (α), was derived from the formula

$$a = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{\sum (x_i - \bar{x})^2}$$

where x_i is the time of a visit (i.e. 0, 3, 6, 12, 18 and 24) and $\bar{x}$ is the average time of visits (i.e. (0+3+6+12+18+24)/6). y_i is cell density at a given visit, whereas $\bar{y}$ is the individual mean ECD from 6 visits. To obtain change per year, the calculated slope a (cells/mm^2 per month) was multiplied by 12.

GLOSSARY

%SIX	Percentage of six-sided corneal endothelial cells, i.e.the magnitude of corneal endothelial *pleomorphism*.
%SIX-ratio	MP%SIX divided by C%SIX
ANOVA	Analysis of variance
BCHCVA	Best Corrected High Contrast Visual Acuity
BCLCVA	Best Corrected Low Contrast Visual Acuity
Bonferroni correction	A multiple comparison test where the probability is multiplied by the number of comparisons.
C%SIX	Central %SIX
CCOV	Central COV. See also COV.
CCT	Central Corneal Thickness
CECD	Central ECD
COV	Coefficient of Variation (standard deviation of cell areas divided by the mean cell area), i.e. the magnitude of endothelial *polymegethism.*
COV-ratio	MPCOV divided by CCOV
D	Dioptres
ECD	Endothelial Cell Density (cells per sq.mm)
ECD-ratio	MPECDdivided by CECD
Friedman	Non-parametric comparison test of more than two related samples (non-parametric analog to repeated ANOVA)
f-TBUT	Fluorescein Tear Break Up Time
K	Keratometry reading (Central Corneal Radius of Curvature)
Kruskal-Wallis	Non-parametric comparison test of more than two independent samples (non-parametric analog of one-way ANOVA)
Laser	Light amplification by stimulated emission of radiation
LASIK	Laser-Assisted In-Situ Keratomileusis
LogMAR	Logarithm (to base 10) of the Minimum Angle of Resolution
Mann-Whitney U	Non-parametric comparison test of two independent samples
MP%SIX	Mid-Peripheral %SIX
MPCOV	Mid-Peripheral COV. See also COV.
MPCT	Mid-Peripheral Corneal Thickness
MPECD	Mid-Peripheral ECD
MSE	Mean Spherical Equivalent
NIBUT	Non Invasive Tear Break Up Time
One-way ANOVA	Parametric comparison test of more than two independent samples.
paired t-test	Parametric comparison test of two related samples (e.g. repeated measurements).
Pleomorphism	Variation in corneal endothelial cell shapes. See also %SIX
Polymegethism	Variation in corneal endothelial cell areas. See also COV
post-hoc test	Multiple comparison tests used at the second stage of the analysis of variance (ANOVA) if the null hypothesis is rejected. E.g. Bonferroni correction.
PRK	Photorefractive keratectomy.

PRT	Phenol Red Thread
Repeated ANOVA	Parametric comparison test of more than two related samples (e.g. repeated measurements).
SCL	Soft Contact Lens
Shapiro-Wilk	Test of the hypothesis that the sample comes from a normally distributed population.
SiH	Silicone-Hydrogel
SPX	Spectacles
TMH	Tear Meniscus Height
t-test	parametric comparison test of two indenpendent samples
VAS	Visual Analogue Scale
Wilcoxon Signed-Rank Test	Non-parametric comparison test of two or more related samples (e.g. repeated measurements)

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