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On the structural origin of refractive instability and corneal haze after excimer laser keratectomy for myopia

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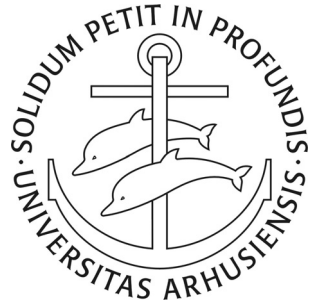

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This review together with the following original publications have been accepted by the Faculty of Health Sciences, University of Aarhus to be defended for the degree Doctor Medicinae. The public defence will take place, Friday, April 4, 2003 at 14.00 in Auditorium 424, Institute of Anatomy, University of Aarhus, Denmark.

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I. Li HF, Petroll WM, Møller-Pedersen T, Maurer JK, Cavanagh HD & Jester JV (1997): Epithelial and corneal thickness measurements by in vivo confocal microscopy through focusing (CMTF). Curr Eye Res 16: 214–221.

II. Møller-Pedersen T, Vogel M, Li HF, Petroll WM, Cavanagh HD & Jester JV (1997): Quantification of stromal thinning, epithelial thickness, and corneal haze following photorefractive keratectomy using in vivo confocal microscopy. Ophthalmology 104: 360–368.

III. Møller-Pedersen T, Li HF, Petroll WM, Cavanagh HD & Jester JV (1998): Confocal microscopic characterization of wound repair following photorefractive keratectomy. Invest Ophthalmol Vis Sci 39: 487–501.

IV. Møller-Pedersen T, Cavanagh HD, Petroll WM & Jester JV (1998): Corneal haze development after PRK is regulated by volume of stromal tissue removal. Cornea 17(6): 627–639.

V. Møller-Pedersen T, Cavanagh HD, Petroll WM & Jester JV (1998): Neutralizing antibody to TGF_{β} modulates stromal fibrosis but not regression of photoablative effect following PRK. Curr Eye Res 17: 736–747.

VI. Jester JV, Møller-Pedersen T, Huang J, Sax CM, Kays WmT, Cavanagh HD, Petroll WM & Piatigorski J (1999): The cellular basis of corneal transparency: evidence for corneal crystallins. J Cell Sci 12: 613–622.

VII. Møller-Pedersen T, Cavanagh HD, Petroll WM & Jester JV (2000): Stromal wound healing explains refractive instability and haze development after photorefractive keratectomy – a one year confocal microscopic study. Ophthalmology 107, 1235–1245.

These papers have not previously been evaluated in order to obtain an academic degree.

Preface

This doctor of medical science thesis is based on work conducted at the Department of Ophthalmology, University of Texas Southwestern Medical Center, Dallas, USA and at the Department of Ophthalmology, Aarhus University Hospital, Aarhus, Denmark.

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To Mette, Emilie & Line

Contents

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Introduction

Myopia in surgical perspective

Refractive errors are due to an improper relationship between the refractive power of the cornea and lens, the depth of the anterior chamber, and the length of the eye globe. Myopia represents the most common refractive disorder with a prevalence of approximately 20–25% for Europeans, 50–70% for Chinese, and less than 10% for Eskimos. Despite intense research efforts, the etiology and pathogenetic mechanism(s) responsible for development of myopia, and for the exceedingly wide variation among ethnic groups, remain elusive. Traditionally, refractive errors have been corrected with spectacles or corneal contact lenses. However, the idea of obtaining maximal visual acuity without corrective eyewear has motivated a search for surgical alternatives to manipulate the ocular refraction permanently and predictably. The prime focus has been the cornea that represents the main refractive element of the eye. The anterior corneal surface has a refractive power of approximately 49 diopters (D) as compared to −6 D of the posterior corneal surface. Thus, of the eye's total refractive power of about 60 D, approximately 43 D or 72% is located in the cornea. Alteration of the anterior corneal curvature therefore offers a good opportunity for surgical correction of visual refractive errors. For the treatment of myopia, the overall intention has been to induce a controlled flattening of the central cornea (and a steepening of the periphery), and still maintain a smooth and optically transparent surface. During the last 25 years, many keratorefractive surgical procedures have been designed and tested based on various incisional, lamellar, and thermal principles. However, since the introduction of non-thermal, ultraviolet excimer laser photoablation, the field of refractive corneal surgery has widely expanded.

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Excimer laser photoablation

In biophysical terms, excimer laser photoablation is based on ultraviolet radiation from an 'excited dimer' of argon and fluorine gas molecules, that exists transiently before dissociating with intense emission of energetic photons with a wavelength of 193 nm. The emitted photons are absorbed within a thin layer of the treated surface and have sufficient energy to break intermolecular bonds leading to ablative decomposition of the tissue into minor fragments that are ejected. Currently, there are two main treatment approaches: (1) photorefractive keratectomy (PRK), where the apical corneal epithelium initially is removed (by manual debridement or by transepithelial photoablation) followed by ablation of the denuded stromal surface (anterior stromectomy); (2) laser *in situ* keratomileusis (LASIK), where the photoablation is performed in the mid-stroma following temporary displacement of a 130–160 μm hinged (epithelial and stromal) tissue flap, formed with a microkeratome (intracorneal stromectomy). To reduce the anterior corneal convexity and correct 1·00 D of myopia, both procedures require removal of a stromal lenticule shaped as a biological contact lens with a central thickness of approximately 13 μm for a 6-mm diameter optical zone and 9 μm/D for a 5-mm diameter ablation zone (Munnerlyn et al. 1988; Colliac et al. 1994).

Study rationale and hypotheses

Predictability of refractive correction

The predictability of excimer laser surgery for myopia has so far never been comparable to that of corrective eyewear including spectacles and contact lenses. Today as well as in the past, the refractive outcomes of both PRK and LASIK have shown an exceedingly wide variation, with 70–100% of low myopes, 40–85% of moderate myopes, and 20–67% of high myopes being within ±1·00 D of intended refraction by 1 year post-surgery (Dutt et al. 1994; Epstein et al. 1994; Maguen et al. 1994; Talley et al. 1994; Seiler et al. 1994; Sher et al. 1994; Piebenga et al. 1995; McCarty et al. 1996; Schallhorn et al. 1996; Hersh et al. 1998; Tuunanen & Tervo 1998; Shah et al. 1998; Han et al. 2000). There appears to be two main sources of variation to account for this low predictability. Firstly, there is no precise control of the actual photoablation depth during surgery in each individual patient (i.e. the exact amount of stromal tissue removed). Secondly, there is no precise control of the postoperative wound-healing response that tends to distort the induced refractive correction by addition of variable amounts of new repair tissue.

Variations in ablation depth

The threshold for achieving photoablation of the corneal stroma is about 30 mJ/cm^2 (beam energy density) for collagen and 40 mJ/cm^2 for keratocytes (Berns et al. 1999). Below these levels, no ablation will occur. Above these thresholds, a linear relationship exists between ablation rate and beam energy density with 0·4–0·5 μm stroma removed per pulse at the energy levels (160–180 mJ/cm^2) currently used in most excimer lasers (Dougherty et al. 1994; Huebscher et al. 1996). However, the laser–cornea interaction is depending of tissue hydration at a given energy level. Dehydrated corneas generally have more dry tissue mass removed per laser pulse (leading to over-correction), whereas edematous corneas show less dry tissue mass removed per laser pulse (leading to under-correction) (Dougherty et al. 1994). Many factors may influence corneal tissue hydration prior to treatment (including time and technique for epithelial denudation, temperature and humidity of environment, use of irrigation solutions, etc.) leading to inter-session variation in the photoablation process in the order of ±10% (Dougherty et al. 1994). Besides, there may exist unique inter-individual variation in the physiological (anterior–posterior) tissue hydration profile (and dry tissue mass components) of the preoperative stroma, leading to differences in the actual photoablation rate. These potential variations are currently not accounted for. It is also important to note that the actual laser beam intensity (in most lasers) is manually adjusted prior to surgery, based on the surgeons' subjective judgement of the ablative performance (e.g. on a piece of metal foil). This calibration procedure may be an important source of inter-session variation. Thus, the nominal photoablation depth displayed on the excimer laser prior to treatment (i.e. the nomogram) is empirical and based on mean population responses. Currently, no valid method exists for the assessment of individual photoablation depths in single patients. *There is therefore a fundamental need to develop an accurate corneal biometry method to see if the laser treatment actually has induced the desired thinning immediately post-ablation (I, II).*

Refractive instability

The refractive changes after excimer laser photoablation usually follow a characteristic temporal pattern. Immediately following treatment for myopia, the patient is left with an intentional (hyperopic) over-correction to account for the subsequent regression of treatment effect. During the first year after both PRK and LASIK, the refraction typically undergo a 0·8–3·0 D myopic regression (gradually becoming less hyperopic and more myopic), especially following higher corrections (Dutt et al. 1994; Epstein et al. 1994; Maguen et al. 1994; Seiler et al. 1994; Sher et al. 1994; Talley et al. 1994; Piebenga et al. 1995; McCarty et al. 1996; Kim et al. 1997; Williams 1997; Chayet et al. 1998; Gartry et al. 1998; Hersh et al. 1998; Tuunanen & Tervo 1998; Han et al. 2000). For this reason, all excimer laser algorithms work with built-in (over-) correction factors to account for the loss of treatment effect (Huebscher et al. 1996). However, these adjustment factors are not based on direct measurements of corneal sublayer wound healing but are empirically modified from the average refractive outcome (mean population response) of clinical trials. Since the magnitude of the initial over-correction is empirical and since the subsequent refractive regression varies considerably among individuals, the procedure may leave the patient emmetropic, hyperopic, or myopic. There is currently no way of predicting those patients who will develop profound refractive shifts after photoablation. Although the refractive changes appear to level off after 1 year, longer term studies of both PRK (2–5-year follow-up) and LASIK (2-year follow-up) have revealed a continued potential for ongoing myopic regression with time (Epstein et al. 1994; Haviv et al. 1997; Kim et al. 1997; Williams 1997; Tuunanen & Tervo 1998; Han et al. 2000), suggesting that corneal wound healing is considerably delayed in humans and may require decades to be completed. Still the permanency and long-term consequences of excimer laser procedures are unknown.

Structural origin of regression

Despite the high frequency and clinical importance of postoperative refractive regression following PRK and LASIK, there has been a major lack of clinical studies of the underlying pathogenetic mechanism(s). The limited knowledge available originate predominantly from *ex vivo* histology of fixed and processed corneal specimens from experimental animals. Although the validity of such observations clearly is questionable, it is generally suspected that both epithelial and stromal wound healing may distort the induced refractive correction by addition (regrowth) of new tissue filling in the photoablated region. Thus, many investigators of PRK-procedures have noted an apparent and most often transient compensatory hyperplasia of the corneal epithelium (up to 60–70 μm in primates and rabbits as compared to 40–50 μm in normals) (Hanna et al. 1989, 1990, 1992; Tuft et al. 1989; Del Pero et al. 1990; Fantes et al. 1990; Beuerman et al. 1994; Amm et al. 1996; Lohmann et al. 1999). Subepithelial deposition of various new and abnormal extracellular matrix components (i.e. stromal fibrosis) has also been noted post-PRK (Tuft et al. 1989; Del Pero et al. 1990; Hanna et al. 1990, 1992; Malley et al. 1990; Fitzsimmons et al. 1992; Latvala et al. 1995; Amm et al. 1996; Weber et al. 1997). However, none of these static *ex vivo* studies have provided true quantitative or prospective data on the stromal regrowth, why important characteristics such as magnitude, time course, and regulatory stimuli have remained unidentified. As for human *in vivo* data, a few clinical studies have indicated central corneal re-thickening after both PRK (Ehlers & Hjortdal 1992; Krueger et al. 1995) and LASIK (Chayet et al. 1998; Perez Santonja et al. 1999), symmetric in both time course and magnitude with the induced myopic regression and leading to central corneal re-steepening. However, the magnitude and relative contribution of epithelial and stromal wound healing has largely remained unidentified due to a lack of sensitive corneal biometry methods. It should also be noted that biomechanical distortion of the remaining photoablated tissue (progressive stromal ectasia or 'bulging') has been proposed as a third potential mechanism of regression following both PRK (Ramirez-Florez & Maurice 1996) and LASIK (Seiler et al. 1998; Seiler 1999). Due to the linear relationship between attempted correction and keratectomy depth, the biomechanical stability of the residual stromal lamellae may obviously be of concern following high corrections. Currently, the accepted limit for the minimum thickness of the stress-bearing residual stroma is 250 μm (Chayet et al.

1998; Seiler et al. 1998; Seiler 1999), which relates directly to the possible upper limit of myopic laser correction without inducing stromal weakening and refractive destabilisation. Clearly, identification of the specific mechanism(s) involved in the development of regression following both PRK and LASIK will be essential to our ability to formulate therapeutic strategies to effectively control and improve the refractive surgical outcomes of these procedures. *It is therefore essential to develop an accurate in vivo technique for direct and simultaneous in vivo measurements of epithelial and stromal thickness to specifically assess the role of tissue addition (re-thickening) in explaining refractive regression after excimer laser refractive surgery (I, II, VII).*

Loss of corneal transparency

Excimer laser PRK usually causes the very transparent cornea to lose some of its optical clarity and develop diffuse and reticular haze, within the photoablated subepithelial region (Epstein et al. 1994; Maguen et al. 1994; Seiler et al. 1994; Sher et al. 1994; Talley et al. 1994; McCarty et al. 1996; O'Brart et al. 1996; Schallhorn et al. 1996; Hersh et al. 1997; Williams 1997; Kremer et al. 1999). By contrast, LASIK patients show a lower incidence of opacities at the photoablated interface, but the optical quality of LASIK is often variable due to flap-related complications including wrinkles or striae (Hersh et al. 1998; Perez Santonja et al. 1999; Stulting et al. 1999). Clinically on the slit-lamp, the subepithelial haze is apparent several weeks following PRK, peaks at about 3–6 months, and progressively clears up during the following 9–12 months. During this healing period, haze often reduces visual function and limits low contrast visual acuity performance (Lohmann et al. 1991; McCarty et al. 1996; Shah et al. 1998). Most important, however, visually significant haze persists in the optical axis of 3–5% of all PRK-patients, especially following higher corrections. However, there is currently no method available for predicting those patients who might have an exaggerated wound-healing response and develop severe haze. Although multiple factors may be associated with the occurrence of haze, only a few have been identified. It appears that the likelihood and severity of haze is principally related to the maximal photoablation depth, with higher corrections (i.e. deeper ablations) generally being associated with greater corneal opacification (Ehlers & Hjortdal 1992; Carson & Taylor 1995; McCarty et al. 1996; Williams 1997; Shah et al. 1998; Kremer et al. 1999). By contrast, there appears to be no consistent association with the presence of refractive regression (Kim et al. 1997; Gartry et al. 1998). Currently, there is no good hypothesis to explain why haze occurs and specifically why some eyes have persistent haze. *Clearly, the biological causes of corneal haze development need to be fully elucidated before rational strategies to eliminate them can be initiated.*

Structural origin of haze

Although previous studies have attempted to relate the development of postoperative corneal haze to stromal wound repair, the true origin and location of haze remains unknown. Based on histologic *ex vivo* studies of photoablated human, primate, and rabbit corneas, a number of potential causes of increased light scattering have been suggested. These include: (1) subepithelial deposition of new stromal extracellular matrix components (collagen, proteoglycans, and other macromolecules) with abnormal composition and organization (i.e. stromal fibrosis); (2) appearance of vacuoles or membranous inclusions in the superficial stroma; (3) altered tissue hydration with focal areas of intercellular edema; (4) irregularities at the photoablated stromal surface with disruption of the regular arrangement of stromal lamellae; and (5) increased cellularity of activated wound-healing keratocytes in the anterior stroma (Hanna et al. 1989, 1992; Tuft et al. 1989; Del Pero et al. 1990; Fantes et al. 1990; Malley et al. 1990; Lohmann et al. 1991; Campos et al. 1992; Fitzsimmons et al. 1992; Rawe et al. 1992; Beuerman et al. 1994; Latvala et al. 1995; Amm et al. 1996; Ramirez-Florez & Maurice 1996; Weber et al. 1997). Although these *ex vivo* studies have provided valuable insight into corneal wound healing, the exact structural and cellular basis for haze after photoablation is disputed and has remained unidentified. Specifically, it is uncertain whether haze predominantly origins form cellular or extracellular structures. This is largely a result of an inability in previous studies to assess the light scattering properties of the observed abnormal histopathologic structures. *It is therefore important to identify the exact structural origin of corneal haze after photorefractive surgery by a direct correlation of in vivo histopathology with the amount and z-axis localization of corneal light reflectivity over time (III).*

Wound-healing intensity

In the search for specific factors controlling the magnitude of corneal wound healing following excimer laser photoablation, authors have focussed on the initial keratocyte loss seen after manual epithelial debridement. This interesting observation was first made by Dohlman (Dohlman et al. 1968) and later confirmed by others (Nakayasu 1988; Crosson 1989; Hanna et al. 1989; Fantes et al. 1990; Campos et al. 1994a, b; Szerenyi et al. 1994). Thus, it appears that the anterior keratocytes promptly die after simple epithelial abrasion (scraping) and leave a continuous 50–150 μm acellular stromal zone that subsequently becomes repopulated over the next 4 weeks. Investigators have focussed on possible epithelial–stromal interactions underlying this phenomenon, and it has been proposed that specific mediators (cytokines) may be released from the injured epithelium and may induce apoptosis (i.e. programmed cell death) in the underlying keratocytes (Wilson et al. 1996a, b). These authors also identified slightly lower levels of keratocyte apoptosis following excimer laser transepithelial photoablation (Helena et al. 1998; Kim et al. 1998). Taken together, these *ex vivo* findings have prompted the idea that a decreased keratocyte loss may lead to a diminished corneal wound-healing response, providing a fundamental rationale for performing transepithelial photoablation, especially for treatment of higher levels of myopia and in retreatment of regression (Wilson 1997; Helena et al. 1998; Kim et al. 1998; Wilson & Kim 1998). However, this hypothesis has never been confirmed by actual comparative measurements of the initial keratocyte loss and subsequent wound-healing parameters. *It is therefore important to conduct an in vivo study to establish definitively the relationship between initial depth of keratocyte killing and stromal loss, and subsequent repopulation and development of corneal haze (IV).*

Therapeutic modulation of wound repair

It is well recognized that many growth factors and cytokines provide important regulatory signals in the complex cascade of wound-healing events after

corneal injury. The most potent and direct mediator of new stromal tissue formation appears to be transforming growth factor beta (TGF_β) that promotes the formation of fibrosis in both corneal and dermal tissue. As a multifunctional and auto-inductive cytokine, TGF_β stimulates corneal keratocyte proliferation, migration, and myofibroblast transformation and enhances the synthesis of stromal extracellular matrix components such as fibronectin and collagen, while reducing stromal degradation by inhibiting the synthesis of matrix metalloproteinases (Girard et al. 1991; Grant et al. 1992; Ohji et al. 1993; Jester et al. 1996; Andresen & Ehlers 1998). The critical role of the TGF_β-mediated cytocrine pathway has further been supported by the finding in experimental animal models that topical application of neutralizing antibodies to TGF_β significantly reduces both dermal scar tissue formation and corneal fibrosis following lamellar keratectomy (Shah et al. 1994, 1995; Jester et al. 1997). By analogy, early inhibition of the concentration of TGF_β may prove to be a clinically useful strategy to directly control and modulate corneal wound healing following excimer laser procedures. *It is therefore of interest to perform a detailed analysis of the therapeutic potential of anti-TGF_β treatment in preventing haze development and regression of photoablative effect following excimer laser PRK (V).*

Potential mechanisms for light scattering

According to the well-established lattice theory of Maurice, the optical transparency of the normal cornea depends on the uniform diameter and spacing of collagen fibrils in the stromal extracellular matrix, leading to destructive interference of scattered incident light, but allowing light transmission in the forward direction (Maurice 1957). Attempts to explain loss of transparency, including haze development after excimer laser procedures, have therefore understandably focussed on the disruption of the collagen fiber array (i.e. stromal fibrosis) as the principal source of corneal light scattering. However, it appears from *in vivo* confocal microscopic studies (II–V, VII) that the major intracorneal structures showing enhanced light scattering after photoablation are the cell body and cell processes of the stromal keratocytes, challenging the current explanations of corneal tissue transparency. There appears to be a close relationship between the loss of corneal transparency (haze) and the presence of highly reflective wound-healing keratocytes which exhibit enhanced cellular-based reflections from their normally invisible cytoplasm. Thus, it seems that the reflectivity of keratocytes can be environmentally altered, suggesting a dynamic regulation of the relative refractive index between the cells and their extracellular matrix. This cell-related loss of corneal clarity is not taken into account by the conventional models of corneal transparency based on the regular ultrastructural array of tightly packed, orthogonally arranged collagen fibers and suggests a novel and previously unrecognized *cellular* contribution to the maintenance of normal corneal transparency. Interestingly, a similar enhancement of keratocyte reflections can be induced by simple formaldehyde fixation of *ex vivo* eyes, revealing fine details of the broad cell processes (Jester et al. 1992). This finding suggests that fixation may alter the relative refractive index of either the matrix or the keratocyte contents, which may be explained by the precipitation of intracellular soluble proteins, resulting in a change in the cellular refractive index relative to that of the surrounding matrix. It should be noted that the transparent intraocular lens of the normal eye is known to contain major water-soluble proteins, termed crystallins, responsible for maintaining its optical properties (Benedek 1997). By analogy, the clear lens loses its normal transparency when exposed to temperatures below 15°C due to precipitation of these intracellular lens proteins (i.e. 'cold cataract') (Benedek 1997). *In an attempt to further explore the 'stealth-like' invisibility of normal corneal keratocytes and the cellular-based light scattering of wound-healing fibroblasts, it is therefore relevant to search for unrecognized, water-soluble crystallin-like proteins in the cytoplasm of keratocytes from clear and opaque corneal tissue (VI).*

Specific aims

Based on the considerations stated above, a parallel series of clinical and experimental animal studies (I–VII) were designed and initiated. The overall intention was to assist in elucidating the biological causes and structural origin of: (1) instability of the intended refractive changes (regression); and (2) loss of corneal optical clarity (haze) following excimer laser PRK. The specific aims of these investigations were:

(1) To develop a new biometry method based on *in vivo* confocal microscopy that allows for quantitative evaluation of corneal sublayer wound healing by providing precise epithelial and stromal thickness measurements and unbiased assessment of corneal light backscattering (haze).
(2) To measure the actual photoablation depth in PRK-patients and to assess the role of epithelial and stromal re-thickening in explaining regression of refractive effect.
(3) To identify the true location and structural basis for corneal haze development following PRK by correlating *in vivo* histopathology with the amount and *z*-axis localization of corneal light scattering at differing temporal intervals in humans and rabbits.
(4) To determine how temporal changes in rabbit corneal thickness after PRK relate to stromal acellularity, fibroblast activation and migration, epithelial regeneration, and stromal regrowth.
(5) To characterize corneal wound repair following epithelial abrasio in rabbits and to determine whether excimer laser transepithelial photoablation can reduce the initial keratocyte killing.
(6) To establish the relationship between initial keratocyte loss, volume of stromal tissue removal, and the magnitude of wound healing and haze development in photoablated rabbit corneas.
(7) To evaluate the role of TGF_β in post-PRK stromal wound repair by neutralizing the bioactivity of TGF_β in a rabbit eye model using specific blocking antibodies.
(8) To investigate the basis for cellular transparency by analyzing the expression of specific water-soluble crystallin-like proteins in the cytoplasm of keratocytes from clear and hazy rabbit corneas.

Methodological aspects

Confocal microscopic imaging

Due to their non-invasive optical sectioning ability, *in vivo* confocal

microscopes are ideally suited for dynamic evaluation of corneal wound healing in three or four dimensions (x, y, z, time). Using this form of vital microscopy, high-resolution en face images can be obtained in real time from different depths within the intact living cornea without the need for staining or processing. The technique enables direct visualization of temporal changes in corneal wound-healing morphology in the same living eye at the cellular level of magnification. Cellular responses and structural changes can thereby be correlated directly and sequentially over time to clinical observations.

During the past few years, several such *in vivo* confocal microscopes have been developed and applied clinically (Cavanagh et al. 1993; Auran et al. 1995; Wiegand et al. 1995). However, the tandem scanning confocal microscope (TSCM) used in present studies has one major advantage compared to other systems. The TSCM has a specially designed objective in which internal lenses can be accurately moved to vary the distance from the objective tip to the position of the focal plane (optical section) without changing the position of the objective surface (Petroll et al. 1993). The exact z-axis depth from which the image originates can therefore be precisely defined and controlled (in microns) and concurrently displayed during *in vivo* imaging using a depth encoding system (Petroll et al. 1996). Due to a tissue penetration of 0–1·5 mm (working distance), the TSCM system enables a direct quantitative evaluation of the three-dimensional location (z-axis position) of cellular structures throughout the cornea.

Besides the objective lens, the TSCM has the following main characteristics. It is mounted horizontally to operate 12 similar to a standard slit-lamp (Cavanagh et al. 1993). The light source is a 100-W mercury arc lamp (broadband white light) that is filtered using ultraviolet and heat filters to prevent light toxicity to the eye. The optical path contains a 0·25% transmittance, glass Nipkow disc 13 with 35 000 pinholes (20 μm in diameter) arranged densely along multiple Archimedean spirals (Cavanagh et al. 1993; Petroll et al. 1996). This disc pattern has a bilateral symmetry so that light beams passing through (illuminated) pinholes on one side of the disc are focussed into a thin slice of the specimen. Reflected light from the illuminated volume are then brought into focus at the exact conjugated (detector) pinholes on the opposite side of the disc (dual light path). When the disc is rotated at a speed of about 1000 rev/min, the pinhole arrangement covers and rebuilds the whole field of view, although each single pinhole only scans a single line. Most importantly, all light beams that do not originate from the focal plane are intercepted by the opaque areas on the disc and are prevented from reaching the video camera (rejection of out-of-focus signals). Overall, this TSCM design produces serial, two-dimensional images with an approximate field-of-view of 450×360 µm, an effective lateral (x, y) resolution of about 1 μm, and an axial (z) resolution of approximately 9 μm (optical slice thickness). Moreover, the technique has a remarkably enhanced sharpness and contrast (high signal-to-noise ratio) that enables visualization of low reflecting structures such as keratocyte nuclei (Petroll et al. 1993, 1996) (I, II).

Although TSCM enables direct imaging of living cells and undisturbed physiological processes within the cornea, it is important to validate the *in vivo* morphological findings by comparison to well-known *ex vivo* histology. Such comparisons may be compromised by the uncontrollable processing artifacts inherently associated with *ex vivo* techniques including conventional light and electron microscopy. However, there generally appears to be a good correlation between parallel *in vivo* and *ex vivo* observations of all layers in the normal cornea including: various layers of the epithelium, epithelial basal lamina, subepithelial nerve plexus, intrastromal nerves, different subpopulations of keratocytes, and endothelium (Ichijima et al. 1992; Jester et al. 1992; Somodi & Guthoff 1995; Böhnke & Masters 1999). During corneal wound repair, it is similarly important to verify *in vivo* histopathological observations by coupling to *ex vivo* techniques. Such documentation has included identification of specific keratocyte repair phenotypes (by immunofluorescently labelled stress fibers) and verification of extracellular changes such as deposition of new matrix components (by specialized stains) (III, IV) (Ichijima et al. 1994; Jester et al. 1995). Still, morphological changes detected by TSCM should be interpreted with caution.

Corneal biometry

To evaluate the biological mechanisms causing refractive instability following PRK, it is essential to obtain accurate information on total corneal and sublayer (epithelial and stromal) thickness. The traditional and most widely used corneal biometry methods include (non-contact) optical pachymetry (Mishima & Hedbys 1968; Olsen et al. 1980a), (non-contact) specular microscopy (Olsen & Ehlers 1984), and (contact) ultrasonic pachymetry (Salz et al. 1983). Over the past few years, new and more sophisticated systems have been introduced including (non-contact) automated video slit-lamp optical pachymetry (McLaren & Bourne 1999), (non-contact) Orbscan optical scanning slit pachymetry (for mapping the thickness of the entire cornea) (Yaylali et al. 1997), and (contact) high-frequency ultrasound pachymetry (Reinstein et al. 1994). Although these methods markedly have improved our ability to detect changes in corneal thickness, only few techniques have provided simultaneous information on epithelial thickness. At high magnifications, modified Haag-Streit optical pachymeters allow for measurements of epithelial thickness; however, the method has a relatively low reproducibility (Wilson et al. 1980; Gauthier et al. 1997). The more recent high-frequency ultrasound pachymeter provides more precise epithelial thickness measurements; however, the acoustic reflection assumed to be the epithelial–stromal interface may not originate from the exact epithelial basal lamina (Reinstein et al. 1994).

As a novel approach, the ability of *in vivo* confocal microscopy to optically section through the cornea and provide z-axis positional depth information was used to perform corneal biometry (I, II). This (contact) methodology, which was named confocal microscopy through focusing (CMTF), takes advantage of the observation that different corneal sublayers have different reflective intensities when imaged with confocal microscopes. Following video-recording of a continuos confocal scan from the epithelium to the endothelium, a CMTF light intensity profile was generated by calculating the average grayscale value (pixel intensity) in the central 180×180 pixel region (approximately 285×285 µm) of each image in the scan. Distinct peaks on this light intensity curve were then correlated directly to corresponding (and concurrently displayed) high-resolution images of well-known anatomical landmarks including superficial epithelium, subepithelial nerve plexus, first layer of keratocytes,

and endothelium. Subsequently, corneal sublayer thickness values (for the epithelium, Bowman's layer, and stroma) were calculated from the exact *z*-axis positions of the relevant peaks on the pixel intensity profile (I, II). At the maximum (average) focal plane speed of 64 μm/s, the average sampling resolution (i.e. the *z*-axis distance between consecutive video frame images) was 2·1–2·6 μm using standard video rates of, respectively, 30 (NTSC) or 25 (PAL) frames/s (I, II). However, the actual sampling rate has recently been doubled by calculating the CMTF profile based on the individual (odd and even) video-fields instead of using the combined (interlaced) video frames (Li et al. 2000; Ivarsen et al. 2002), leading to an improved resolution of CMTF biometry by a factor 2.

For validation of the method, CMTF pachymetry was compared to ultrasound pachymetry, although ultrasound readings may not represent the 'true' (accurate) measure of corneal thickness. The mean difference in central corneal thickness between CMTF and ultrasound pachymetry was found to be only $-2{\cdot}8 \pm 7{\cdot}1$ μm (not significantly different), indicating that the two methods overall were comparable for normally hydrated corneas (I). Also the average epithelial thickness detected by CMTF (50·6 μm for humans and 47·7 μm for rabbits) (I) was consistent with previously reported data using high-frequency ultrasound (50·5 μm for humans and 47 μm for rabbits) (Allemann et al. 1993; Reinstein et al. 1994) but thinner than the data recorded by optical pachymetry (57–62 μm for humans) (Wilson et al. 1980; Gauthier et al. 1997). The average coefficient of variation (CV) for repeated CMTF corneal thickness measurements was 0·7% for rabbits and 1·6% for humans (I, II) as compared to 1·3–3·1% for optical pachymetry (Olsen et al. 1980b; Salz et al. 1983; McLaren & Bourne 1999), 1·5% for the Orbscan system, and 0·6–1·1% for ultrasound pachymetry (Salz et al. 1983; Yaylali et al. 1997). As for repeated epithelial thickness measurements by CMTF, the average CV was 2·5% for rabbits and 6·6% for humans (I, II), as compared to 3·7% (humans) using high-frequency ultrasound (Reinstein et al. 1994). Overall, CMTF appeared to be a new, reproducible, and observer-independent method for obtaining reliable estimates of total corneal and sublayer thickness in both rabbits and humans. More elegantly, the CMTF methodology was recently validated using PMMA calibration contact lenses, demonstrating a high accuracy of ± 1 μm for (repeated) corneal thickness measurements, regardless of changes in target thickness or radius of curvature (Ivarsen et al. 2002).

In comparison to other more widely used methods such as ultrasound and optical pachymetry, the CMTF technique has one major advantage. CMTF concurrently provides a series of high lateral resolution histological images corresponding to all points and peaks on the light intensity curve. This feature enables the identification of specific intracorneal structures and answers questions regarding the cellular or extracellular origin of specific points on the CMTF profile. Over time, such additional imaging information can be used to define and characterize how changes in corneal sublayer depth and thickness relate to specific changes in cellular structures and extracellular matrix components.

However, the CMTF method has four main limitations. First, like other optical techniques, CMTF is limited to optically clear or semi-transparent media and may not be able to image through and detect an endothelial peak in severely fibrotic or edematous corneas. Second, changes in corneal refractive index and anterior radius of curvature may induce errors in the CMTF depth estimation, similar to optical and specular pachymetry (Olsen et al. 1980a; Olsen & Ehlers 1984). However, corneal refractive index does not vary substantially between normals (Patel 1987), and changes in anterior radius of curvature seem negligible for CMTF accuracy under most conditions (Ivarsen et al. 2002). Third, the algorithm calculating the exact focal plane position does not correct for potential differences in refractive index between corneal sublayers. However, these differences should be small and would affect other methods of measurement as well (Patel 1987; Patel et al. 1995). Fourth, the relatively high sampling time of about 8 s per CMTF-scan (at a speed of 64 μm/s) may allow for axial distortion and displacement due to involuntary eye movement which may degrade the reproducibility of the measurement. Thus, up to 15% of all human scans had significant eye movement such as blinking or loss of fixation and were excluded from further evaluation (I, II); only about 85% of all scans were considered complete. The use of an improved eye-fixation target (with a built-in refractive correction mechanism) did not increase the frequency of complete scans (II). Moreover, even the complete CMTF-scans may still be influenced by fine saccadic eye movements, respiration, and ocular pulsation that can only be minimized by increasing the speed of the scan. However, if the maximum focal plane speed is further increased, a decrease in *z*-axis sampling resolution will result using the current standard video rates. Clearly, this is an important issue for further development.

Assessment of corneal haze

Although disturbances in corneal transparency may scatter light in all directions, the clinical term 'haze' refers specifically to the backscattered (and reflected) light that is visible for the observer. By contrast, only forward-light scatter may compromise visual function by optical degradation of the retinal image and thereby real bothersome 'glare' as experienced by the patient (Lohmann et al. 1993; Harrison et al. 1995; Schallhorn et al. 1996). For the assessment of haze (i.e. backscatter and reflected light), the traditional and most widespread method has been semi-quantification on an arbitrary scale from 0 (clear cornea) to 4 (dense haze that obscures anterior chamber details) using slit-lamp biomicroscopy. Unfortunately, this simple grading method is imprecise and subjective and has a high inter-observer variation, despite the use of standardized reference photographs as clinical classification guidelines. Over the past few years, more sophisticated and objective methods have therefore been introduced.

One approach has been to mount a digital video camera on a slit-lamp and capture 8-bit grayscale images using fixed angles for both incident light and light collection (Lohmann et al. 1992; Chang et al. 1996; Maldonado et al. 1997). Subsequently, computerized digital image analysis has been used to measure grayscale disturbances (densitometry) either across the overall image (Lohmann et al. 1992; Chang et al. 1996)

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or in various minor topographic regions within the ablation zone (Maldonado et al. 1997). Furthermore, incorporation of polarizing filters (in front of both light source and camera) has been used to discriminate between reflected and backscattered light in the haze signal (Lohmann et al. 1992). Another strategy has been the use of a photomultiplier tube (instead of a video camera) to detect backscattered light from the axial cornea (scatterometry) (Olsen 1982; Braunstein et al. 1996). Objective haze assessment has also been attempted using high-frequency ultrasound (Allemann et al. 1993), although acoustic backscatter may not mirror the optical backscatter. Finally, devices designed to measure lens opacification have been evaluated (Andrade et al. 1990; Binder et al. 1996); however, none of these techniques have shown sufficient sensitivity to monitor fine disturbances in corneal clarity.

As a novel approach, corneal haze was evaluated using the CMTF method (II, III). To enable confocal microscopic quantification of corneal backscatter, the video camera was set to a fixed detector sensitivity with a constant gain, voltage, and black level throughout CMTF image acquisition. Under this circumstance, the increase
15 of incident photons detected by the video camera is principally related to increased light scattering of the hazy tissue (II, III). Therefore, corneal haze was quantified directly from the CMTF profile by integrating the area under the haze peak (relative to that of the surrounding stroma). This objective CMTF haze estimate combines information on both haze thickness (peak width) and haze intensity (peak height) and is expressed in arbitrary unit (U) defined as $\mu m \times$ pixel intensity. When compared to the routine clinical examination, a significant correlation
16 ($r = 0{\cdot}76$) was found between subjective haze grading by slit-lamp biomicroscopy and the objective CMTF haze estimate (II, VII), thereby verifying the methodology. However, it is recognized
17 that other factors than corneal reflectivity (and backscatter) may influence the CMTF haze estimate including: illumination (stability of light source), transparency of lens surfaces, detector sensitivity, and microscope alignment. However, care was taken to keep these factors constant (II, III). Moreover, the CV (SD/mean) for a daily reference scan (internal standard) was only 6% with no significant increase or decrease with time. Thus, when extraneous factors are controlled, it appears that the detected variation in the CMTF haze estimate is related primarily to changes in corneal light scattering (II, III).

When compared to other objective haze assessment methods, the CMTF technique has one major advantage. It combines the ability to measure haze with simultaneous display of high-resolution confocal images of the abnormal light scattering structures within the haze region. The CMTF method thereby provides a direct correlation between the amount and z-axis depth localization of increased backscatter and the underlying *in vivo* histopathologic changes, which is mandatory for obtaining a precise description of the structural origin of haze. In essence, CMTF is analogous to a high-power, magnified, slit-lamp examination of an optical 'slice' of the cornea. However, the CMTF method has two main limitations. First, each CMTF-scan samples light from a very small area (about $285 \times 285\,\mu m$) within the photoablated region. This 'field-of-view' limitation may disturb the accuracy of the haze measurement because haze often appears inhomogeneous with focal areas of intense and intermediate scatter, interspersed with other more clear regions. However, it should be noted that the reticular nature of haze obviously will affect other methods of measurement as well. One way to overcome this problem is to integrate over a larger haze area by performing multiple CMTF-scans within the ablation zone and subsequently average the data (II, III). Alternatively, an objective lens with a smaller magnification may be used for haze sampling. The second main limitation of CMTF haze assessment is related to the currently used 8-bit video camera that may be saturated by highly reflective structures within the haze region, leading to the underestimation of the actual amount of haze. One way to solve this problem is to shift the detector sensitivity (i.e. the dynamic range) during CMTF-scanning by lowering the gain setting to predefined levels, and subsequently adjust the data by correction factors. More elegantly, a photomultiplier tube with a higher dynamic range (e.g. 16 bit) may be attached next to video camera to provide more sensitive measurements of both lower and higher grades of corneal opacities (Yu et al. 2000).

Results with discussion

Human studies

The clinical part of this thesis included a 1-year prospective study of 17 eyes of 17 myopic patients (mean -6 ± 2 D; range -3 to -9 D) that received a standardized 6-mm diameter PRK (aiming
18 at emmetropia) and were examined using *in vivo* CMTF before surgery and at 1, 3, 6, and 12 months post-treatment (II, VII).

Assessment of photoablation depth

Among these PRK-treated individuals, the amount of central corneal thinning by 1 month was found to correlate closely with the achieved refractive change with a relationship of $14{\cdot}3\,\mu m$ corneal thinning per diopter (II). This finding is in reasonable agreement with the theoretical value of $13\,\mu m$/D (for a 6-mm zone) (Munnerlyn et al. 1988; Colliac et al. 1994), thereby validating the fundamental concept of PRK that graded amounts of anterior stromal thinning can induce predictable changes in corneal refractive power. By 1 month post-treatment, there was a substantial range of residual refractive error (from $-0{\cdot}5$ to $+7$ D). This low predictability appeared to be directly related to a considerable variation in intended versus achieved photoablation depth (II), emphasizing the need for a more accurate control of the photoablative removal of corneal tissue. A complete control of the desired stromal thinning will require a dynamic, intraoperative assessment of the actual photoablation rate (tissue removed per pulse) and profile in individual patients. At present, such a method is not available but it may be presented in the future as the technology continues to evolve.

Characteristics of haze development

In all PRK-treated individuals, the cornea showed an enhanced light backscattering (haze) that appeared to originate predominantly from high numbers of brightly reflecting wound-healing keratocytes (with increased reflectivity of both nuclei and cell bodies) within the anterior, approximately $100\,\mu m$ of the stroma (II, VII). By contrast, the more posteriorly located keratocytes appeared quiescent (with low-reflecting nuclei and invisible cell processes) at all time-points. These findings suggest that cellular-based

reflections (as opposed to extracellular matrix deposition) are a main biological explanation for haze development in humans. Correspondingly, the gradual normalization of keratocyte morphology and cellular reflectivity was associated temporally with restoration of corneal clarity and disappearance of haze. No inflammatory cells, major keratocyte losses (acellular zones in the anterior stroma), or spindle-shaped (migratory) fibroblasts were observed at any time-point but may have been present in the immediate postoperative period, since the subjects were first examined at 1 month post-PRK. In the majority of the patients (15 of 17), corneal haze peaked at 3 months, 548 ± 435 U, and gradually declined over time to 196 ± 156 U at 1 year. In these 15 individuals, a cumulative measure of corneal haze over the 12-month period correlated significantly ($r = 0{\cdot}68$) with the depth of photoablation (as assessed by 1 month post-PRK). This finding suggests that higher corrections (i.e. deeper photoablations) generally are associated with development of greater corneal opacification over time. Unsuspectedly, two patients developed corneal light backscattering of about 4000–6000 U (corresponding to clinical haze grade 3–4) that persisted throughout the 1-year study. The explanation for profound and prolonged haze in these two subjects remains to be fully elucidated (II, VII), but it clearly demonstrates the high patient-to-patient variability and the relatively high risk of inducing an undesirable aggressive wound-healing response after PRK in otherwise healthy eyes. Valid predictors for the magnitude of corneal haze development in single patients still need to be identified.

Mechanisms of refractive instability

From 1 to 12 months post-PRK, considerable refractive instability was detected among the treated patients with an average refractive shift of $+0{\cdot}84 \pm 1{\cdot}23$ D ranging from $-1{\cdot}63$ D (hyperopic shift) to $+3{\cdot}38$ D (myopic regression) (VII). The observed changes in ocular refraction correlated closely with the concurrent changes in corneal thickness with a linear relationship of 10 μm corneal re-thickening per diopter of (myopic) regression for all patients and 15 μm/D for a subgroup of eight patients with myopic regression higher than 1 D. No association was observed between the refractive changes (from 1 to 12 months) and the concurrent epithelial regrowth that averaged 7 μm (from 45 to 52 μm, compared to 51 μm preoperatively), demonstrating that epithelial re-thickening occurred without compensatory hyperplasia and had no measurable systematic impact on the refractive regression after PRK. These findings are in accordance with previous observations by Gauthier et al. using optical pachymetry (Gauthier et al. 1997). Thus, it appears that the corneal epithelium after a 6·0-mm diameter PRK may recover uniformly over the entire photoablated area (without transient or persistent hyperplasia) and thereby generate a plano lens with limited effect on corneal refraction.

From 1 to 12 months post-PRK, regrowth of the photoablated stroma averaged 6 ± 12 μm (ranging from 27 μm thinning to 22 μm re-thickening) and correlated closely ($r = 0{\cdot}84$) with the concurrent loss of photorefractive effect. The average stromal re-thickening was 8 μm/D of refractive regression for all patients and 11 μm/D for the eight patients with myopic regression higher than 1 D. Interestingly, a profound stromal thinning of 27 μm was detected in one patient that developed a postoperative hyperopic shift of $-1{\cdot}63$ D. Taken together, it appears that refractive changes after PRK are directly associated with dynamic and parallel changes in stromal thickness within the photoablation center. Interestingly, the compensatory stromal wound repair after PRK appeared to be regulated in a graded manner as suggested by the finding of a differential refractive instability with higher corrections (i.e. deeper excisions) generally leading to more stromal tissue addition (from 1 to 12 months) and thereby more refractive regression. Thus, depth of photoablation appears to be the principal factor determining both the degree of stromal re-thickening as well as the magnitude of corneal haze development after PRK. Most interestingly, stromal regrowth was not correlated with formation of corneal light scattering over time, suggesting that haze and regression were caused by two independent wound-healing mechanisms.

Rabbit eye model

The experimental part of this thesis included studies of rabbit corneal wound healing following various injuries including epithelial denudation, PRK, and transepithelial photoablation (III–VI).

Corneal response to epithelial abrasio

Three hours following epithelial denudation of rabbit corneas by gentle scraping, condensed and shrunken keratocyte nuclei were seen in the anterior stroma (IV). By day 1, this zone contained pyknotic and fragmented nuclei, but no inflammatory cells. By 1 week, an acellular zone with a thickness of 108 ± 14 μm had developed in the anterior stroma, demonstrating a massive keratocyte loss, consistent with previous histologic findings (Dohlman et al. 1968; Nakayasu 1988; Crosson 1989; Hanna et al. 1989; Fantes et al. 1990; Campos et al. 1994a, b; Szerenyi et al. 1994). Subsequently, the underlying intact keratocytes became activated and transformed into spindle-shaped cells that, over time, repopulated the acellular zone. These migratory fibroblasts displayed increased light scattering (from both nuclei and cell bodies) that induced a nine-fold increase in total corneal light reflectivity (1442 ± 630 U; corresponding to slit-lamp detectable haze grade-1). As repopulation proceeded, the migratory cells (and thereby the haze) moved anteriorly towards the surface. Repopulation was completed by 3 weeks and moderately reflective, quiescent keratocytes now started to appear in the anterior stroma with gradual normalization of the morphology. Over time, corneal light reflectivity diminished and had reached the preoperative level by 2–3 months post-injury. Thus, the slight and transient haze detected after epithelial abrasio in rabbit corneas appears to be mediated predominantly by increased cellular-based reflections from the directional migration of spindle-shaped, wound-healing keratocytes undergoing repopulation of the acellular anterior stroma (IV). Following disappearance of the initial edema, no major increase in stromal thickness was detected apart from the expected physiological growth of approximately 1 μm per week. Restoration of the normal architecture was not associated with accelerated stromal growth. As for the corneal surface, reepithelialization was complete by day 3 and the preoperative epithelial thickness was restored by 2 weeks with no changes thereafter.

Corneal response to photoablation

Although there are general similarities between rabbit corneal wound healing after photoablation versus epithelial scraping, several important differences were noted (III, IV). During the first 24–48 h post-ablation, profound inflammation was detected in the treated stroma. Even after transepithelial photoablation including only a shallow (14 μm) stromal keratectomy, an intense inflammatory response was seen (IV). This finding is contrasted by the total absence of inflammation following manual epithelial debridement and brings into question the importance of the integrity of the first layer of keratocytes (or the basal membrane) for coordinating chemotaxis and eliciting inflammation. The inflammatory response seen immediately after photoablative procedures may play a 24
22 critical role for amplifying the subsequent wound repair through the release of specific growth factors and regulatory cytokines at the site of injury. Identification of these factors and insight into their patterns, concentration ranges, and kinetics will be of major interest.

Both PRK and transepithelial photoablation (including a 14–44 μm stromal keratectomy) were associated with profound keratocyte losses in the anterior stroma. The depth of keratocyte killing showed no dependence on the photoablation depth, and the acellular zone after photoablation was generally less
23 than following epithelial abrasio (IV). Moreover, all photoablated corneas developed more profound and prolonged light backscattering (haze) over time as compared with manually debrided corneas. Taken together, these findings suggest that the initial keratocyte loss after PRK in the rabbit is unrelated to the magnitude of the subsequent wound-healing response. The hypothesis proposed by Wilson et al. (based on *ex vivo* histology) that a decreased keratocyte loss may lead to diminished corneal haze and wound repair (Wilson 1997; Helena et al. 1998; Kim et al. 1998; Wilson & Kim 1998) can therefore not be supported by these *in vivo* observations (III, IV).

In the rabbit eye model, total corneal reflectivity had increased about 15 times by 1 week post-PRK. This increase in light scattering was related to an increased reflection from the photoablated stromal surface (1745 ± 262 U) in addition to increased reflections from activated fibroblasts in the deeper stroma (713 ± 607 U) (III). As these spindle-shaped cells migrated towards the surface, they became substantially more reflective than following scraping. Moreover, as repopulation became complete (by 3 weeks), the migratory fibroblasts transformed into a 3D interwoven meshwork of highly reflective, stellate myofibroblasts, a repair phenotype expressing α-smooth-muscle actin (III–V). By contrast, myofibroblastic cells were not detected following epithelial abrasio (IV). In PRK-treated corneas, myofibroblast transformation was associated with prolonged enhancement of corneal haze that peaked at 3 weeks (4648 ± 1263 U) and decreased linearly over time to 889 ± 700 U by 6 months post-PRK. This decline in light reflectivity appeared to be related to remodelling of the photoablated surface in addition to the disappearance of the highly reflective myofibroblasts. A minor contribution to haze may come from the initial deposition of new extracellular matrix (stromal regrowth during week 3), but the overall effect of new matrix formation (from week 4 and beyond) was a progressively diminishing reflectivity (i.e. transparent regression). Overall, these findings in rabbits support the observations in humans (II, VII) that cellular-based reflections from wound-healing keratocytes (as opposed to extracellular matrix deposition) may be the main biological explanation for haze development following PRK-procedures.

Interestingly, the magnitude of corneal haze development increased linearly with the photoablation depth up to about 45 μm with apparently no further effect of deeper ablations on maximal light scattering (IV). Thus, there may exist a keratectomy depth beyond which the rabbit cornea may develop maximal haze. This finding in rabbits supports the clinical observation that higher corrections (i.e. deeper photoablations) generally are associated with more corneal light scattering. However, no apparent limit for maximal haze development could be identified in humans within the range of 20–154 μm stromal photoablation (II, VII).

After the initial corneal swelling had resolved by 2 weeks, the photoablated stroma underwent a progressive re-thickening. After a 120 μm PRK (corresponding to a -9 D correction), the stromal regrowth rate was highest during the third week, 21 ± 9 μm/week, declining to 6 ± 5 μm/week by week 7, and to $1{\cdot}5 \pm 1$ μm/week by 6 months post-treatment (III). At this time-point, the stroma had regained more than 90% of the preoperative thickness, demonstrating a remarkable wound-healing response with nearly complete regression of the initially achieved photoablative effect. By contrast, the central epithelial thickness had normalized by 2 weeks with no significant changes thereafter (III, IV). Thus, it appears that keratocyte-mediated re-thickening of the photoablated stroma is the major repair mechanism by which the rabbit cornea regains thickness and curvature after a 6-mm diameter PRK, which is analogous to the observations in humans (VII). The low stability of PRK in rabbits is contrasted by the much higher stability of PRK in humans, which brings into question the use of rabbits as test models for corneal refractive surgery. However, it should be noted that one over-corrected PRK-patient developed a 50 μm stromal regrowth during a period of 8 months leading to +6 D myopic regression (VII). Thus, the human cornea does have a potential for severe stromal re-thickening similar to that of the rabbit.

Stromal regrowth in rabbit corneas occurred predominantly by regeneration, defined as growth by expansion of scarless tissue underlying the photoablated stromal surface (III, V). Regeneration appeared to account for approximately 75% of the total re-thickening, whereas only about 25% of the growth was mediated by fibrosis or scarring, defined as the deposition of new (fibrotic) tissue above the photoablated stromal surface. This interesting ability of the rabbit cornea to perform regeneration or scarless healing has not previously been recognized (and accounted for) in wound-healing studies that solely have focussed on the fibrotic component.

Anti-TGF_β treatment

Early inhibition of the concentration of TGF_β by topical application of neutralizing antibodies (for 3 days until reepithelialization) had a profound effect on 120 μm PRK-treated rabbits (V). Anti-TGF_β treatment significantly reduced both the extent and the duration of increased corneal scattering (haze) to a level nearly similar to that following manual epithelial debridement (IV). This reduction in early development of light reflecting structures and

the more rapid decline in haze appeared related to anti-TGF_β-mediated reduction of keratocyte activation and proliferation, inhibition of myofibroblast transformation, and total abolishment of stromal fibrosis. Thus, it appeared that corneal haze development after PRK in rabbits is critically related to TGF_β-mediated activation of corneal keratocytes in the early phase, leading to myofibroblast transformation and deposition of fibrotic extracellular matrix. Anti-TGF_β therapy, on the other hand, provided an effective means of modulating myofibroblast function and reducing post-PRK haze development in rabbit corneas. Most interestingly, anti-TGF_β treatment appeared to abolish stromal fibrosis without reducing or delaying stromal regrowth (i.e. regression of photoablative effect) that instead occurred entirely by regeneration independent of the anti-TGF_β treatment (IV).

Keratocyte reflectivity and corneal crystallins

To investigate the basis for cellular transparency, normal keratocytes (which do not scatter light) were isolated from transparent rabbit corneas and were found to contain two water-soluble crystallin-like proteins, transketolase and aldehyde dehydrogenase class 1 (VI). The proteins were found in high concentrations, corresponding to about 28% of the total soluble protein content. These corneal crystallins are equivalent to those found in high concentrations in the intraocular lens, where they are known to have structural functions related to maintenance of the optical properties and transparency of the lens. By analogy with the lens, the level of soluble crystallins within the keratocyte cytoplasm may play a role in the regulation of the refractive and optical properties of the individual cells. In accordance, a marked reduction (>50%) in crystallin levels was detected in scleral fibroblasts (isolated from opaque rabbit sclera) as well as in highly reflective migratory keratocytes (isolated from the central hazy portion of freeze-wounded rabbit corneas 2 weeks post-injury). A full-thickness freeze-injury was studied (instead of PRK) because freezing leads to acellularity of the entire stroma and thereby ensures collection of predominantly migratory fibroblasts without contaminating normal keratocytes. However, decreased keratocyte crystallin mRNA levels have recently been identified in PRK-treated rat corneas up to 3 months post-surgery (Schultz et al. 2001). Taken together, these findings lead to the novel hypothesis that high concentrations of crystallin proteins in normal rabbit keratocytes may contribute to the cellular transparency by minimizing the refractive index homogeneities in the keratocyte cytoplasm (VI). Correspondingly, the selective reduction of crystallin levels in migratory fibroblasts may lead to an increased cellular reflectivity by increasing the magnitude of refractive index fluctuation within the cytoplasm.

Conclusions with perspectives

This thesis has provided evidence supporting the hypothesis of a close relationship between structural changes within the corneal stroma and changes in the optical clarity and refractive stability of the cornea following excimer laser photoablative procedures.

The main structure responsible for corneal haze development after PRK appears to be high numbers of stromal wound-healing keratocytes with enhanced cellular-based reflections from their normally invisible (stealth-like) cytoplasm. A minor contribution to haze may come from the photoablated stromal surface, whereas the overall effect of new extracellular matrix deposition is a progressively diminishing reflectivity. The highly reflective wound-healing keratocytes seem to be able to dynamically alter the optical properties of their cell processes relative to the surrounding extracellular matrix. Each layer of keratocytes may thereby act as a semi-transparent mirror, promoting an increase in the total light backscattering of the cornea. Multiple factors may be associated with the maintenance of the cellular transparency. It is proposed that the level of soluble crystallin proteins within the keratocyte cytoplasm may be involved in the regulation of the cellular reflectivity and thereby the amount of corneal haze. However, many questions still need to be answered before this novel hypothesis can be accepted. Of specific interest will be identification of how the crystallins and other intracellular proteins may interact and promote or inhibit light scattering. Obviously, these studies also need to be extended from rabbit corneas to humans. However, further insight into this issue may lead to new and effective strategies for pharmacologic manipulation of corneal clarity not only following various keratorefractive procedures but also in many corneal diseases associated with development of stromal opacities.

The main biological cause of refractive instability after excimer laser PRK appears to be dynamic changes in stromal thickness within the photoablation center. Myopic regression seems to be related to central stromal re-thickening, whereas hyperopic shift after PRK appears to be a consequence of central stromal thinning. The data show a linear relationship of 10–15 μm corneal re-thickening per diopter of (myopic) regression mediated almost solely by stromal regrowth, whereas only a minor contribution may originate from restoration of the preoperative epithelial thickness. These data are almost identical to the fundamental PRK-treatment algorithm of 13 μm central stromal photoablation per diopter of refractive power change (for a 6-mm diameter PRK), suggesting a direct causal relationship between central stromal regrowth and regression. Thus, deposition of new stromal extracellular matrix seems to play a direct and decisive role for development of refractive 25
regression by being the main repair mechanism by which the cornea regains thickness and curvature after a 6-mm diameter PRK. When a lenticular stromal volume (with a central thickness of 13 μm/D) is removed by PRK, subsequent stromal wound healing tends to reverse the profile change by adding new tissue 'filling in' the ablated region, leading to central re-steepening and refractive (myopic) regression.

The photoablation depth appears to be the main factor determining both the degree of stromal regrowth as well as the magnitude of corneal haze development after PRK. However, stromal re-thickening is not correlated with formation of corneal light scattering over time, suggesting that haze and (myopic) regression are caused by two independent wound-healing mechanisms. This finding in humans is supported by the interesting observation in rabbits that approximately 75% of post-PRK stromal re-thickening is mediated by regeneration, defined as 26

growth by expansion of scarless tissue underlying the photoablated stromal surface. By contrast, only about 25% of the re-thickening is caused by fibrosis (scarring) within the photoablated region. In essence, regeneration represents accelerated normal physiological growth of the stroma without activation of reflective wound-healing keratocytes. The clinical finding that some PRK-patients show significant but almost completely transparent myopic regression (VII) suggests the existence of a similar regenerative growth mechanism in humans.

Another important observation in rabbits was the finding that topical treatment with neutralizing antibodies to TGF_{β} completely abolished post-PRK stromal fibrosis (scarring) without reducing or delaying the total stromal re-thickening, that instead occurred entirely by regeneration (scarless healing). This finding suggests the existence of unknown regulatory mechanism(s) that tightly and dynamically control corneal thickness in the rabbit. There may be at least two underlying pathways: (1) a TGF_{β}-mediated pathway controlling stromal fibrosis (scar formation); and (2) a non-TGF_{β}-mediated pathway controlling total stromal thickness by regeneration (scarless healing). An increased understanding of these regulatory pathways will be of interest. Further insight into the feedback mechanisms that control corneal growth and maintain corneal thickness and curvature may have direct implications for our ability to treat many corneal diseases in humans including ametropia and keratoconus.

There is currently no way of identifying those patients who will have exaggerated wound healing after photoablation and develop severe haze or profound refractive regression. However, the cell of prime focus is the keratocyte, since it may control both corneal clarity (through cellular reflections) and refractive regression (through stromal regrowth). Therapeutic, interventional strategies designed to control and eliminate corneal haze and modulate more precisely the refractive outcome of laser vision correction should therefore specifically target the keratocyte and its phenotypic transformations. So far, little is known about the complex interacting environment leading to keratocyte-mediated wound repair. The nature of the cellular reactions and signalling pathways should therefore be further characterized at the molecular level. Due to this lack of specific knowledge, none of the currently available pharmacological agents can sufficiently control corneal wound repair. Presumably, the complexity will require the use of combination therapies instead of a single agent. Due to the high patient-to-patient variability, therapeutic wound-healing modulation should probably have an individualized approach in order to be maximally effective. This will require identification of reliable predictors to monitor and relate the individual wound-healing ability to the desired final dioptic result and optical outcome. So far, no obvious predictors have been identified. However, the preoperative density, morphology, and spatial distribution of the stromal keratocytes may represent simple estimates of the corneal wound-healing capacity (Møller-Pedersen & Ehlers 1995; Møller-Pedersen 1997, 1999; Prydal et al. 1998; Patel et al. 1999). Other possible candidates include cytokine and growth factor levels in tear fluid (Vesaluoma & Tervo 1998) and messenger RNA levels in the epithelium (Tomas-Barberan et al. 1998). Undoubtedly, the future success of excimer laser correction will depend on our ability to accurately monitor, control, and manipulate corneal wound healing in individual patients to improve the predictability and permanency of the procedures. This is even more relevant now that wavefront-guided excimer lasers have become available starting a quest for supernormal visual acuity (Møller-Pedersen et al. 2002). Until a better understanding of the keratocyte-mediated wound repair has been provided, corneal refractive surgery will probably never achieve predictable and safe visual and refractive results.

Danish summary (Dansk resumé)

Refraktionsanomalier (optiske brydningsfejl) skyldes en ubalance mellem øjets længdeakse og brydningsstyrken af hornhinden henholdsvis øjets linse. Nærsynethed er den mest almindelige brydningsfejl med en prævalens på *ca.* 20 til 25% i den europæiske befolkning. Traditionelt er brydnings-og bygningsfejl blevet korrigeret med briller eller kontaktlinser. Men i de senere år er en række refraktionskirurgiske alternativer blevet introduceret. Interessen har primært samlet sig om at ændre hornhindens forflade-krumning, som er ansvarlig for *ca.* 70% af øjets samlede brydningsstyrke. Normalt har hornhinden en tykkelse på *ca.* 500 μm bestående af et overfladeepithel på *ca.* 50 μm, et kollagent bindevæv kaldet stromaet på *ca.* 450 μm, samt et endothel på *ca.* 5 μm på hornhindens bagside. Nærsynethed kan således korrigeres ved en kontrolleret affladning af de centrale dele af hornhinde-stromaet.

Den for tiden fremherskende refraktionskirurgiske teknik til behandling af nærsynethed er excimer laser photoablation, hvor 193 nm ultraviolet lys anvendes til at bryde intermolekylære bindinger i hornhindens overflade. Herved fjernes små mængder af vævet, så der indslibes en ny og svagere brydende optik. Behandlingen kan gives enten som PRK (photorefraktiv keratektomi) direkte i hornhinde-stromaets overflade forudgået af epithelfjernelse eller som LASIK (laser *in situ* keratomileusis) intrastromalt under en 130–160 μm hængslet vævslap, som initielt skæres med en mikrokeratom. Fælles for de to teknikker er, at der af hornhinde-stromaet skal fjernes væv svarende til en linse med en central tykkelse på *ca.* 13 μm for hver dioptri (D) nærsynethed ved en behandlingszone på 6 mm i diameter. Reduceres behandlingszonen til 5 mm skal der centralt fjernes *ca.* 9 μm/D. Excimer laser behandling af hornhinden er imidlertid forbundet med en række væsentlige problemer.

For det første er præcisionen af excimer laser behandling ikke på højde med det at få et par nye briller eller kontaktlinser, idet den inducerede ændring i brydningsstyrken ikke præcist kan forudsiges. For lavere grader af nærsynethed op til *ca.* −6 D opnår mellem 70 til 100% af patienterne en brydningsstyrke, som ligger indenfor ±1 D af det tilsigtede, mens kun en mindre del vil ligge indenfor ±0·5 D. Ved behandling af højere grader af nærsynethed (over −6 D) stiger variationen på slut-refraktionen markant. Den forholdsvis beskedne præcision af excimer laser behandling er relateret til en manglende kontrol over den præcise photoablations-dybde hos det enkelte individ. Man kan således ikke direkte måle, hvor meget væv der reelt fjernes under operationen. Det antages derfor, at alle hornhinder principielt reagerer ens på laser-impulserne, hvilket ikke er tilfældet. Konsekvensen kan være

en betydelig forskel mellem den tilsigtede og den reelle mængde fjernede væv; og dermed en utilsigtet over- eller underkorrektion.

Hertil kommer, at excimer laser behandling igangsætter et sårhelingsrespons, som varer op til 12 måneder eller længere. I denne periode gendannes en del af det tabte hornhindevæv i det behandlede område, hvorved brydningsstyrken specielt i de første 3 til 6 måneder kan svinge betydeligt i retningen af mere nærsynethed (betegnet regression). Nogle personer taber mere af operationseffekten end andre, da der er stor biologisk variation i sårhelingsevnen. Graden af regression ligger typisk i størrelsesordenen 0·8 til 3·0 D, især efter behandling af større grader af nærsynethed. Det er endvidere karakteristisk, at de to øjne heler nogenlunde ens. Brydningsstyrken stabiliseres oftest indenfor det første år, men nogle studier finder, at regressionen kan fortsætte i flere år efter både PRK henholdsvis LASIK. De specifikke årsager til det gradvise tab af operationseffekt er fortsat uafklarede. Hvilke af hornhindens cellelag gendannes og med hvilken hastighed? Hvilke regulatoriske vækstfaktorer spiller en rolle? Hvorledes kan man opnå en bedre kontrol over helingsprocessen?

Excimer laser behandling bevirker endvidere, at den transparente hornhinde taber noget af sin optiske gennemskinnelighed og udvikler diffuse uklarheder (kaldet haze) i det behandlede område. Graden af haze udvikling er størst efter PRK sammenlignet med LASIK. Haze udviklingen tiltager i de første 3 til 6 måneder, hvorefter hornhinden gradvist klarer op i løbet af 9 til 12 måneder. I denne helingsperiode kan synsfunktionen og især kontrast-følsomheden være påvirket. Der er stor variation i graden og varigheden af haze blandt forskellige individer, men *ca.* 5% af alle behandlede hornhinder får permanente optiske uklarheder, især efter korrektion af større grader af nærsynethed. Det er i øjeblikket ikke muligt før operationen at forudsige hvilke patienter, som vil have et accentueret sårhelingsrespons og udvikle svær haze. Til trods for den hyppige forekomst og kliniske betydning af haze har der været en mangel på studier af de tilgrundliggende strukturelle årsager. Der er således ikke formuleret nogen tilfredsstillende hypotese omkring haze udvikling, og mange vigtige spørgsmål er uafklarede. Hvad er de biologiske årsager? Hvilke celletyper er involveret? Hvad er de underliggende biokemiske mekanismer? Hvorledes kan haze udvikling behandles og forebygges?

Udfra ovenstående problemstillinger og overvejelser blev en række kliniske og eksperimentelle studier af excimer laser opererede hornhinder indledt. Det overordnede mål var at bidrage til identifikation af de biologiske mekanismer og strukturelle årsager til: (1) det gradvise tab af initiel induceret refraktiv effekt (regression); og (2) det delvise tab af hornhindens optiske klarhed (haze udvikling) efter excimer laser PRK. Undersøgelsernes specifikke formål var:

(1) at udvikle en ny biometrisk metode baseret på *in vivo* konfokal mikroskopi til kvantitativ evaluering af sårhelingen i hornhindens forskellige cellelag via præcise målinger af epithel og stroma tykkelsen, samt objektiv bestemmelse af hornhindens lysreflektivitet og optiske klarhed (haze).
(2) at måle den reelle photoablationsdybde hos PRK-patienter, og bestemme betydningen af epithelets henholdsvis stromaets revækst for regression af refraktiv effekt.
(3) at identificere den præcise lokalisation og strukturelle basis for haze udvikling ved at korrelere *in vivo* histopatologiske fund med dybde (z-akse) lokalisationen af hornhindens lysreflektivitet over tid hos både PRK-patienter samt i en eksperimentel dyremodel (kanin).
(4) at fastlægge den tidsmæssige sammenhæng efter PRK mellem ændringer i kanin hornhindens tykkelse, tabet af keratocytter i stromaet, keratocyt aktivering og migration, epitheliel regeneration, samt stromal revækst.
(5) at karakterisere kanin hornhindens sårheling efter afskrabning af epithelet (abrasio), samt bestemme hvorvidt en transepitheliel photoablation kan reducere det initielle keratocyt tab.
(6) at etablere sammenhængen mellem tabet af keratocytter, volumet af den fjernede mængde stroma, graden af det efterfølgende sårhelingsrespons samt haze udviklingen hos excimer laser behandlede hornhinder.
(7) at evaluere betydningen af vækstfaktoren Transforming Growth Factor Beta (TGFβ) for kanin hornhindens sårheling via applikation af specifikke TGFβ-neutraliserende antistoffer efter PRK.
(8) at undersøge den strukturelle basis for hornhindens cellulære transparens ved at analysere ekspressionen af specifikke vandopløselige krystallinproteiner i cytoplasmaet hos keratocytter isoleret fra normalt væv henholdsvis hornhinder med haze.

Sammenfattende viser disputatsarbejdet, at der er en direkte sammenhæng mellem strukturelle ændringer i hornhindens stroma og ændringer i hornhindens optiske klarhed og refraktive stabilitet efter excimer laser behandling.

Den mest betydningsfulde strukturelle komponent for haze udvikling efter PRK er de stromale keratocytter, som optræder i det behandlede område med forøgede cellulære reflektioner fra deres normalt lav-reflekterende (usynlige) cytoplasma. Et mindre bidrag til haze kommer fra den photoablerede stromale overflade, hvorimod den generelle effekt af deponering af ny extracellulær matrix i stromaet er en aftagende lys-reflektivitet. De involverede reflektive keratocytter synes at være i stand til dynamisk at ændre de optiske egenskaber af deres stjerneformede cytoplasmatiske udløbere, relativt til den omgivende extracellulære matrix. Hver enkelt af hornhindens *ca.* 200 lag af keratocytter kan dermed opfattes som et semireflektivt spejl, som kan forøge hornhindens samlede lys-reflektion. Mange faktorer er potentielt associerede med opretholdelse af keratocyttens cellulære transparens. Der fremføres her en hypotese om, at niveauet af opløselige krystallin-proteiner i keratocyttens cytoplasma er involveret i regulationen af den cellulære transparens og dermed i haze udvikling. Mange vigtige spørgsmål skal dog besvares førend en sådan hypotese kan accepteres. Af specifik interesse vil være identifikation af, hvorledes krystalliner og andre intracellulære proteiner samarbejder om at fremme henholdsvis hæmme cellens lysreflektion. Yderligere skal studierne udvides til også at omfatte keratocytter fra humane donorer. En øget indsigt i disse forhold vil lede hen imod udvikling af nye og effektive metoder til farmakologisk manipulation af hornhindens transparens, ikke bare efter refraktiv kirurgi men generelt ved de mange hornhindesygdomme, som er forbundet med tab af vævets optiske klarhed.

Den væsentligste årsag til refraktionsændringer efter PRK er dynamiske ændringer i stromaets tykkelse indenfor det laser behandlede område. Regression mod nærsynethed er relateret til revækst af det behandlede stroma, mens et refraktivt skift mod langsynethed er relateret til en central udtyndning. Der synes at være en lineær sammenhæng på 10 til 15 μm central stromal revækst per dioptri regression (ved en 6 mm behandlingszone). Deponering af ny extracellulær matrix i det behandlede stroma er dermed den primære sårhelingsmekanisme, hvormed hornhinden delvist genopretter sin tykkelse og krumning efter PRK.

Den primære faktor, som er relateret til både graden af haze udvikling og graden af stromal revækst hos PRK-patienter, er photoablations-dybden. Men haze udvikling og regression er ikke direkte korrelerede, hvilket tyder på, at der er tale om to forskellige regulatoriske mekanismer. Dette understøttes af, at hornhinde-stromaet hos kaninen er i stand til at hele dels ved fibrose (arvævs-dannelse) dels ved regeneration (arvævs-fri heling). Regeneration repræsenterer således en accelereret fysiologisk vækst med ekspansion af det normale stroma uden ledsagende aktivering af reflektive keratocytter og dermed haze udvikling. Et andet vigtigt fund er, at lokal behandling med neutraliserende antistoffer mod TGFβ er i stand til at hæmme fibrose-udviklingen uden at reducere eller forsinke stromaets revækst efter PRK. Dette fund peger i retningen af eksistensen af ukendte regulatoriske mekanismer, som dynamisk kontrollerer hornhindens tykkelse og krumning hos kaninen. Der synes at eksisterer mindst to forskellige regulatoriske veje: (1) en TGFβ-medieret mekanisme, som styrer fibrose-udvikling i stromaet (arvævsdannelse); og (2) en ikke-TGFβ-medieret mekanisme, som styrer hornhindens samlede tykkelse via regeneration (arvævs-fri heling). En øget indsigt i disse forhold omkring regulationen af hornhindens vækst, krumning og tykkelse er særdeles vigtig for vores mulighed for at forebygge og behandle mange øjensygdomme, herunder specielt udvikling af nærsynethed, langsynethed og keratoconus.

References

Allemann N, Chamon W, Silverman RH, Azar DT, Reinstein DZ, Stark WJ & Coleman DJ (1993): High-frequency ultrasound quantitative analyses of corneal scarring following excimer laser keratectomy. Arch Ophthalmol **111**: 968–973.

Amm M, Wetzel W, Winter M, Uthoff D & Duncker GIW (1996): Histopathological comparison of photorefractive keratectomy and laser in situ keratomileusis in rabbits. J Refract Surg **12**: 758–766.

Andrade HA, McDonald MB, Liu JC, Abdelmegeed M, Varnell R & Sunderland G (1990): Evaluation of an opacity lensometer for determining corneal clarity following excimer laser photoablation. Refract Corneal Surg **6**: 346–351.

Andresen JL & Ehlers N (1998): Chemotaxis of human keratocytes is increased by platelet-derived growth factor-BB, epidermal growth factor, transforming growth factor-alpha, acidic fibroblast growth factor, insulin-like growth factor-I, and transforming growth factor-beta. Curr Eye Res **17**: 79–87.

Auran JD, Koester CJ, Kleiman NJ, Rapaport R, Bomann JS, Wirotsko BM, Florakis GJ & Koniarek JP (1995): Scanning slit confocal microscopic observation of cell morphology and movement within the normal human anterior cornea. Ophthalmology **102**: 33–41.

Benedek GB (1997): Cataract as a protein condensation disease: the Proctor Lecture. Invest Ophthalmol Vis Sci **38**: 1911–1921.

Berns MW, Chao L, Giebel AW, Liaw LH, Andrews J & VerSteeg B (1999): Human corneal ablation threshold using the 193-nm ArF excimer laser. Invest Ophthalmol Vis Sci **40**: 826–830.

Beuerman RW, McDonald MB, Shofner RS, Munnerlyn CR, Clapham TN, Salmeron B & Kaufman HE (1994): Quantitative histological studies of primate corneas after excimer laser photorefractive keratectomy. Arch Ophthalmol **112**: 1103–1110.

Binder PS, Bosem M & Weinreb RN (1996): Scheimpflug anterior segment photography assessment of wound healing after myopic excimer laser photorefractive keratectomy. J Cataract Refract Surg **22**: 205–212.

Böhnke M & Masters BR (1999): Confocal microscopy of the cornea. Prog Retin Eye Res **18**: 553–628.

Braunstein RE, Jain S, McCally RL, Stark WJ, Connolly PJ & Azar DT (1996): Objective measurement of corneal light scattering after excimer laser keratectomy. Ophthalmology **103**: 439–443.

Campos M, Cuevas K, Garbus J, Lee M & McDonnell PJ (1992): Corneal wound healing after excimer laser ablation. Effects of nitrogen gas blower. Ophthalmology **99**: 893–897.

Campos M, Raman S, Lee M & McDonnell PJ (1994a): Keratocyte loss after different methods of de-epithelialization. Ophthalmology **101**: 890–894.

Campos M, Szerenyi K, Lee M, McDonnell JM, Lopez PF & McDonnell PJ (1994b): Keratocyte loss after corneal deepithelialization in primates and rabbits. Arch Ophthalmol **112**: 254–260.

Carson CA & Taylor HR (1995): Excimer laser treatment for high and extreme myopia. The Melbourne Excimer Laser and Research Group. Arch Ophthalmol **113**: 431–436.

Cavanagh HD, Petroll WM, Alizadeh H, He YG, McCulley JP & Jester JV (1993): Clinical and diagnostic use of in vivo confocal microscopy in patients with corneal disease. Ophthalmology **100**: 1444–1454.

Chang S, Maurice DM & Ramirez-Florez S (1996): Quantitative measurement of corneal haze after myopic PRK. J Refract Surg **12**: 412–416.

Chayet AS, Assil KK, Montes M, Espinosa-Lagana M, Castellanos A & Tsioulias G (1998): Regression and its mechanisms after laser in situ keratomileusis in moderate and high myopia. Ophthalmology **105**: 1194–1199.

Colliac JP, Shammas HJ & Bart DJ (1994): Photorefractive keratectomy for the correction of myopia and astigmatism. Am J Ophthalmol **117**: 369–380.

Crosson CE (1989): Cellular changes following epithelial abrasion. In: Beuerman RW, Crosson CE, Kaufman HE, eds. *Healing Processes in the Cornea*. The Woodlands, Texas: Portfolio Publishing Co, 3–14.

Del Pero RA, Gigstad JE, Roberts AD, Klintworth GK, Martin CA, L'Esperance FAJ & Taylor DM (1990): A refractive and histopathologic study of excimer laser keratectomy in primates. Am J Ophthalmol **109**: 419–429.

Dohlman CH, Gasset AR & Rose J (1968): The effect of the absence of corneal epithelium or endothelium on the stromal keratocytes. Invest Ophthalmol **7**: 520–534.

Dougherty PJ, Wellish KL & Maloney RK (1994): Excimer laser ablation rate and corneal hydration. Am J Ophthalmol **118**: 169–176.

Dutt S, Steinert RF, Raizman MB & Puliafito CA (1994): One-year results of excimer laser photorefractive keratectomy for low to moderate myopia. Arch Ophthalmol **112**: 1427–1436.

Ehlers N & Hjortdal JØ (1992): Excimer laser refractive keratectomy for high myopia. 6-month follow-up of patients treated bilaterally. Acta Ophthalmol Scand **70**: 578–586.

Epstein D, Fagerholm P, Hamberg Nystrom H & Tengroth B (1994): Twenty-four-month follow-up of excimer laser photorefractive keratectomy for myopia. Refractive and visual acuity results. Ophthalmology **101**: 1558–1563.

Fantes FE, Hanna KD, Waring GO, Pouliquen Y, Thompson KP & Savoldelli M (1990): Wound healing after excimer laser keratomileusis (photorefractive keratectomy) in monkeys. Arch Ophthalmol **108**: 665–675.

Fitzsimmons TD, Fagerholm P, Harfstrand A & Schenholm M (1992): Hyaluronic acid in the rabbit cornea after excimer laser superficial keratectomy. Invest Ophthalmol Vis Sci **33**: 3011–3016.

Gartry DS, Larkin DF, Hill AR, Ficker LA & Steele AD (1998): Retreatment for significant regression after excimer laser photorefractive keratectomy. A prospective, randomized, masked trial. Ophthalmology **105**: 131–141.

Gauthier CA, Holden BA, Epstein D, Tengroth B, Fagerholm P & Hamberg Nystrom H (1997): Factors affecting epithelial hyperplasia after photorefractive keratectomy. J Cataract Refract Surg **23**: 1042–1050.

Girard MT, Matsubara M & Fini ME (1991): Transforming growth factor-beta and interleukin-1 modulate metalloproteinase expression by corneal stromal cells. Invest Ophthalmol Vis Sci **32**: 2441–2454.

Grant MB, Khaw PT, Schultz GS, Adams JL & Shimizu RW (1992): Effects of epidermal growth factor, fibroblast growth factor, and transforming growth factor-beta on corneal cell chemotaxis. Invest Ophthalmol Vis Sci **33**: 3292–3301.

Han HS, Song JS & Kim HM (2000): Long-term results of laser in situ keratomileusis for high myopia. Korean J Ophthalmol **14**: 1–6.

Hanna KD, Pouliquen YM, Savoldelli M, Fantes F, Thompson KP, Waring GO & Samson J (1990): Corneal wound healing in monkeys 18 months after excimer laser photorefractive keratectomy. Refract Corneal Surg **6**: 340–345.

Hanna KD, Pouliquen Y, Waring GO, Savoldelli M, Cotter J, Morton K & Menasche M (1989): Corneal stromal wound healing in rabbits after 193-nm excimer laser surface ablation. Arch Ophthalmol **107**: 895–901.

Hanna KD, Pouliquen YM, Waring GO, Savoldelli M, Fantes F & Thompson KP (1992): Corneal wound healing in monkeys after repeated excimer laser photorefractive keratectomy. Arch Ophthalmol **110**: 1286–1291.

Harrison JM, Tennant TB, Gwin MC, Applegate RA, Tennant JL, Van den Berg TJTP & Lohmann CP (1995): Forward light scatter at one month after photorefractive keratectomy. J Refract Surg **11**: 83–88.

Haviv D, Hefetz L, Krakowsky D, Abrahami S, Kibarski U & Nemet P (1997): For how long can regression continue after photorefractive keratectomy for myopia. Ophthalmology **104**: 1948–1951.

Helena MC, Baerveldt F, Kim WJ & Wilson SE (1998): Keratocyte apoptosis after corneal surgery. Invest Ophthalmol Vis Sci **39**: 276–283.

Hersh PS, Brint SF, Maloney RK, Durrie DS, Gordon M, Michelson MA, Thompson VM, Berkeley RB, Schein OD & Steinert RF (1998): Photorefractive keratectomy versus laser in situ keratomileusis for moderate to high myopia. A randomized prospective study. Ophthalmology **105**: 1512–1522. discus.

Hersh PS, Stulting RD, Steinert RF, Waring GO, Thompson KP, O'Connell M, Doney K & Schein OD (1997): Results of phase III excimer laser photorefractive keratectomy for myopia. The Summit PRK Study Group. Ophthalmology **104**: 1535–1553.

Huebscher HJ, Genth U & Seiler T (1996): Determination of excimer laser ablation rate of the human cornea using in vivo scheimpflug videography. Invest Ophthalmol Vis Sci **37**: 42–46.

Ichijima H, Jester JV, Petroll WM & Cavanagh HD (1994): Laser and tandem scanning confocal microscopic studies of rabbit corneal wound healing. Scanning **16**: 263–268.

Ichijima H, Petroll WM, Jester JV & Cavanagh HD (1992): Confocal microscopic studies of living rabbit cornea treated with benzalkonium chloride. Cornea **11**: 221–225.

Ivarsen A, Stultiens BAT & Møller-Pedersen T (2002): Validation of confocal microscopy through focusing (CMTF) for corneal sublayer pachymetry. Cornea **21**: 700–704.

Jester JV, Barry-Lane PA, Cavanagh HD & Petroll WM (1996): Induction of alfa-smooth muscle actin expression and myofibroblast transformation in cultured corneal keratocytes. Cornea **15**: 505–516.

Jester JV, Barry-Lane PA, Petroll WM, Olsen DR & Cavanagh HD (1997): Inhibition of corneal fibrosis by topical application of blocking antibodies to TGF-beta in the rabbit. Cornea **16**: 177–187.

Jester JV, Petroll WM, Barry PA & Cavanagh HD (1995): Temporal, 3-dimensional, cellular anatomy of corneal wound tissue. J Anat **186**: 301–311.

Jester JV, Petroll WM, Garana RM, Lemp MA & Cavanagh HD (1992): Comparison of in vivo and ex vivo cellular structure in rabbit eyes detected by tandem scanning microscopy. J Microsc **165**: 169–181.

Kim JH, Kim MS, Hahn TW, Lee YC, Sah WJ & Park CK (1997): Five year results of photorefractive keratectomy for myopia. J Cataract Refract Surg **23**: 731–735.

Kim WJ, Shah S & Wilson SE (1998): Differences in keratocyte apoptosis following transepithelial and laser-scrape photorefractive keratectomy in rabbits. J Refract Surg **14**: 526–533.

Kremer I, Kaplan A, Novikov I & Blumenthal M (1999): Patterns of late corneal scarring after photorefractive keratectomy in high and severe myopia. Ophthalmology **106**: 467–473.

Krueger RR, Talamo JH, McDonald MB, Varnell RJ, Wagoner MD & McDonnell PJ (1995): Clinical analysis of excimer laser photorefractive keratectomy using a multiple zone technique for severe myopia. Am J Ophthalmol **119**: 263–274.

Latvala T, Tervo K, Mustonen R & Tervo T (1995): Expression of cellular fibronectin and tenascin in the rabbit cornea after excimer laser photorefractive keratectomy: a 12 month study. Br J Ophthalmol **79**: 65–69.

Li J, Jester JV, Cavanagh HD, Black TD & Petroll WM (2000): On-line 3-dimensional confocal imaging in vivo. Invest Ophthalmol Vis Sci **41**: 2945–2953.

Lohmann CP, Fitzke F, O'Brart D, Muir MK, Timberlake G & Marshall J (1993): Corneal light scattering and visual performance in myopic individuals with spectacles, contact lenses, or excimer laser photorefractive keratectomy. Am J Ophthalmol **115**: 444–453.

Lohmann C, Gartry D, Kerr Muir M, Timberlake G, Fitzke F & Marshall J (1991): Haze in photorefractive keratectomy: its origins and consequences. Laser Light Ophthalmol **4**: 15–34.

Lohmann CP, Reischl U & Marshall J (1999): Regression and epithelial hyperplasia after myopic photorefractive keratectomy in a human cornea. J Cataract Refract Surg **25**: 712–715.

Lohmann CP, Timberlake GT, Fitzke FW, Gartry DS, Muir MK & Marshall J (1992): Corneal light scattering after excimer laser photorefractive keratectomy: the objective measurements of haze. Refract Corneal Surg **8**: 114–121.

Maguen E, Salz JJ, Nesburn AB, Warren C, Macy JI, Papaioannou T, Hofbauer J & Berlin MS (1994): Results of excimer laser photorefractive keratectomy for the correction of myopia. Ophthalmology **101**: 1548–1556.

Maldonado MJ, Arnau V, Martinez Costa R, Navea A, Mico FM, Cisneros AL & Menezo JL (1997): Reproducibility of digital image analysis for measuring corneal haze after myopic photorefractive keratectomy. Am J Ophthalmol **123**: 31–41.

Malley DS, Steinert RF, Puliafito CA & Dobi ET (1990): Immunofluorescence study of corneal wound healing after excimer laser anterior keratectomy in the monkey eye. Arch Ophthalmol **108**: 1316–1322.

Maurice DM (1957): The structure and transparency of the cornea. J Physiol Lond **136**: 263–286.

McCarty CA, Aldred GF & Taylor HR (1996): Comparison of results of excimer laser correction of all degrees of myopia at 12 months postoperatively. The Melbourne Excimer Laser Group. Am J Ophthalmol **121**: 372–383.

McLaren JW & Bourne WM (1999): A new video pachometer. Invest Ophthalmol Vis Sci **40**: 1593–1598.

Mishima S & Hedbys BO (1968): Measurement of corneal thickness with the Haag-Streit pachometer. Arch Ophthalmol **80**: 710–713.

Møller-Pedersen T (1997): A comparative study of human corneal keratocyte and endothelial cell density during aging. Cornea **16**: 333–338.

Møller-Pedersen T (1999): Corneal keratocyte density – aspects of methodology, physiology and pathophysiology. PhD-thesis. Faculty of Health Sciences, University of Aarhus. Acta Ophthalmol Scand 77: 366–367 (Summary).

Møller-Pedersen T & Ehlers N (1995): A three-dimensional study of the human corneal keratocyte density. Curr Eye Res **14**: 459–464.

Møller-Pedersen T, Ehlers N & Hjortdal JØ (2002): Supersyn (Super Vision). Ugeskr Laeger **164**: 619–623.

Munnerlyn CR, Koons SJ & Marshall J (1988): Photorefractive keratectomy: a technique for laser refractive surgery. J Cataract Refract Surg **14**: 46–52.

Nakayasu K (1988): Stromal changes following removal of epithelium in rat cornea. Jpn J Ophthalmol **32**: 113–125.

O'Brart DP, Corbett MC, Verma S, Heacock G, Oliver KM, Lohmann CP, Kerr Muir MG & Marshall J (1996): Effects of ablation diameter, depth, and edge contour on the outcome of photorefractive keratectomy. J Refract Surg **12**: 50–60.

Ohji M, SundarRaj N & Thoft RA (1993): Transforming growth factor-beta stimulates

collagen and fibronectin synthesis by human corneal stromal fibroblasts in vitro. Curr Eye Res **12**: 703–709.

Olsen T (1982): Light scattering from the human cornea. Invest Ophthalmol Vis Sci **23**: 81–86.

Olsen T & Ehlers N (1984): The thickness of the human cornea as determined by a specular method. Acta Ophthalmol Scand **62**: 859–871.

31 Olsen T, Nielsen CB & Ehlers N (1980a): On the optical measurement of corneal thickness. II. The measuring conditions and sources of error. Acta Ophthalmol Scand **58**: 975–984.

32 Olsen T, Nielsen CB & Ehlers N (1980b): On the optical measurement of a corneal thickness. I. Optical principle and sources of error. Acta Ophthalmol Scand **58**: 760–766.

Patel S (1987): Refractive index of the mammalian cornea and its influence during pachometry. Ophthalmic Physiol Opt **7**: 503–506.

Patel S, Marshall J & Fitzke FW (1995): Refractive index of the human corneal epithelium and stroma. J Refract Surg **11**: 100–105.

Patel SV, McLaren JW, Camp JJ, Nelson LR & Bourne WM (1999): Automated quantification of keratocyte density by using confocal microscopy in vivo. Invest Ophthalmol Vis Sci **40**: 320–326.

Perez Santonja JJ, Ayala MJ, Sakla HF, Ruiz Moreno JM & Alio JL (1999): Retreatment after laser in situ keratomileusis. Ophthalmology **106**: 21–28.

Petroll WM, Cavanagh HD & Jester JV (1993): Three-dimensional imaging of corneal cells using in vivo confocal microscopy. J Microsc **170**: 213–219.

Petroll WM, Jester JV & Cavanagh HD (1996): Quantitative three-dimensional confocal imaging of the cornea in situ and in vivo: system design and calibration. Scanning **18**: 45–49.

Piebenga LW, Matta CS, Deitz MR & Tauber J (1995): Two-zone excimer photorefrective keratectomy for moderate myopia. Ophthalmology **102**: 1054–1059.

Prydal JI, Franc F, Dilly PN, Kerr Muir MG, Corbett MC & Marshall J (1998): Keratocyte density and size in conscious humans by digital image analysis of confocal images. Eye **12**: 337–342.

Ramirez-Florez S & Maurice DM (1996): Inflammatory cells, refractive regression, and haze after excimer laser PRK. J Refract Surg **12**: 370–381.

Rawe IM, Zabel RW, Tuft SJ, Chen V & Meek KM (1992): A morphological study of rabbit corneas after laser keratectomy. Eye **6**: 637–642.

Reinstein DZ, Silverman RH, Rondeau MJ & Coleman DJ (1994): Epithelial and corneal thickness measurements by high-frequency ultrasound digital signal processing. Ophthalmology **101**: 140–146.

Salz JJ, Azen SP, Berstein J, Caroline P, Villasenor RA & Schanzlin DJ (1983): Evaluation and comparison of sources of variability in the measurement of corneal thickness with ultrasonic and optical pachymeters. Ophthalmic Surg **14**: 750–754.

Schallhorn SC, Blanton CL, Kaupp SE, Sutphin J, Gordon M, Goforth H & Butler FK (1996): Preliminary results of photorefractive keratectomy in active-duty United States navy personnel. Ophthalmology **103**: 5–21.

Schultz GS, Varela JC, Goldstein MH & Baker HV (2001): Gene chip microarray analysis of rat corneas following excimer laser photorefractive keratectomy. Invest Ophthalmol Vis Sci **42**: S587. (Abstract) nr 3152.

Seiler T (1999): Iatrogenic keratectasia: academic anxiety or serious risk? J Cataract Refract Surg **25**: 1307–1308.

Seiler T, Holschbach A, Derse M, Jean B & Genth U (1994): Complications of myopic photorefractive keratectomy with the excimer laser. Ophthalmology **101**: 153–160.

Seiler T, Koufala K & Richter G (1998): Iatrogenic keratectasia after laser in situ keratomileusis. J Refract Surg **14**: 312–317.

Shah S, Chatterjee A & Smith RJ (1998): Predictability of spherical photorefractive keratectomy for myopia. Ophthalmology **105**: 2178–2184.

Shah M, Foreman DM & Ferguson MWJ (1994): Neutralising antibody to TGF-beta1,2 reduces cutaneous scarring in adult rodents. J Cell Sci **107**: 1137–1157.

Shah M, Foreman DM & Ferguson MWJ (1995): Neutralisation of TGF-beta1 and TGF-beta2 or exogenous addition of TGF-beta3 to cutaneous rat wounds reduces scarring. J Cell Sci **108**: 985–1002.

Sher NA, Hardten DR, Fundingsland B, DeMarchi J, Carpel E, Doughman DJ, Lane SS, Ostrov C, Eiferman R, Frantz JM et al. (1994): 193-nm excimer photorefractive keratectomy in high myopia. Ophthalmology **101**: 1575–1582.

Somodi S & Guthoff R (1995): Darstellung der keratozyten in der humanen kornea mittels fluoreszensmikroskopie. Ophthalmologe **92**: 452–457.

Stulting RD, Carr JD, Thompson KP, Waring GO, Wiley WM & Walker JG (1999): Complications of laser in situ keratomileusis for the correction of myopia. Ophthalmology **106**: 13–20.

Szerenyi KD, Wang X, Gabrielian K & McDonnell PJ (1994): Keratocyte loss and repopulation of anterior corneal stroma after de-epithelialization. Arch Ophthalmol **112**: 973–976.

Talley AR, Hardten DR, Sher NA, Kim MS, Doughman DJ, Carpel E, Ostrov CS, Lane SS, Parker P & Lindstrom RL (1994): Results one year after using the 193-nm excimer laser for photorefractive keratectomy in mild to moderate myopia. Am J Ophthalmol **118**: 304–311.

Tomas-Barberan S, Schultz GS, Tarnuzzer RW & Fagerholm P (1998): Messenger RNA levels for genes involved in extracellular matrix from human corneal scrapings before and after photorefractive keratectomy. Acta Ophthalmol Scand **76**: 568–572.

Tuft SJ, Zabel RW & Marshall J (1989): Corneal repair following keratectomy. A comparison between conventional surgery and laser photoablation. Invest Ophthalmol Vis Sci **30**: 1769–1777.

Tuunanen TH & Tervo TT (1998): Results of photorefractive keratectomy for low, moderate, and high myopia. J Refract Surg **14**: 437–446.

Vesaluoma MH & Tervo TT (1998): Tenascin and cytokines in tear fluid after photorefractive keratectomy. J Refract Surg **14**: 447–454.

Weber BA, Fagerholm P & Johansson B (1997): Colocalization of hyaluronan and water in rabbit corneas after photorefractive keratectomy by specific staining for hyaluronan and by quantitative microradiography. Cornea **16**: 560–563.

Wiegand W, Thaer AA, Kroll P, Geyer OC & Garcia AJ (1995): Optical sectioning of the cornea with a new confocal in vivo slit-scanning videomicroscope. Ophthalmology **102**: 568–575.

Williams DK (1997): Multizone photorefractive keratectomy for high and very high myopia: long-term results. J Cataract Refract Surg **23**: 1034–1041.

Wilson SE (1997): Molecular biology for the refractive corneal surgeon: Programmed cell death and wound healing. J Refract Surg **13**: 171–175.

Wilson SE, He YG, Weng J, Li Q, Mcdowall AW, Vital M & Chwang EL (1996a): Epithelial 33 injury induces keratocyte apoptosis: Hypothesized role for the interleukin-1 system in the modulation of corneal tissue organization and wound healing. Exp Eye Res **62**: 325–337.

Wilson SE & Kim WJ (1998): Keratocyte apoptosis: implications on corneal wound healing, tissue organization, and disease. Invest Ophthalmol Vis Sci **39**: 220–226.

Wilson SE, Li Q, Weng J, Barry-Lane PA, Jester JV, Liang Q & Wordinger RJ (1996b): The fas–fas ligand system and 34 other modulators of apoptosis in the cornea. Invest Ophthalmol Vis Sci **37**: 1582–1592.

Wilson G, O'Leary DJ & Henson D (1980): Micropachometry: a technique for measuring the thickness of the corneal epithelium. Invest Ophthalmol Vis Sci **19**: 414–417.

Yaylali V, Kaufman SC & Thompson HW (1997): Corneal thickness measurements with the Orbscan topography system and ultrasonic pachymetry. J Cataract Refract Surg **23**: 1345–1350.

Yu AA, Li J, Jester JV, Cavanagh HD & Petroll WM (2000): The use of a photomultiplier tube for signal detection during confocal microscopy through focusing. Invest Ophthalmol Vis Sci **41**: S258 (Abstract No. 1356).

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