Corneal Optical Regularization and Biomechanical Stabilization in Keratoconus and Irregular Astigmatism by Use of Topography-Guided Custom Ablation and Corneal Cross-Linking

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University of Oslo, SynsLaser Kirurgi, Norway

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University of Oslo, SynsLaser Kirurgi,
Oslo, Norway
2016
## 2. ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>AL</td>
<td>Axial length</td>
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<tr>
<td>AS-OCT</td>
<td>Anterior segment optical coherence tomography</td>
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<tr>
<td>BAC</td>
<td>Benzalkonium chloride</td>
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<tr>
<td>CA</td>
<td>Custom ablation</td>
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<td>CDVA</td>
<td>Corrected distance visual acuity</td>
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<tr>
<td>CH</td>
<td>Corneal hysteresis</td>
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<td>CK</td>
<td>Conductive keratoplasty</td>
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<tr>
<td>CRF</td>
<td>Corneal resistance factor</td>
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<tr>
<td>CorVis ST</td>
<td>Corneal visualization Scheimpflug technology</td>
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<tr>
<td>CXL</td>
<td>Corneal collagen crosslinking</td>
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<tr>
<td>D</td>
<td>Diopter</td>
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<td>DA</td>
<td>Deformation amplitude</td>
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<tr>
<td>FLEX</td>
<td>Femtosecond lenticule extraction</td>
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<tr>
<td>FS</td>
<td>Femtosecond</td>
</tr>
<tr>
<td>FS-LASIK</td>
<td>Femtosecond laser assisted-laser in situ keratomileusis</td>
</tr>
<tr>
<td>HOAs</td>
<td>Higher-order aberrations</td>
</tr>
<tr>
<td>IA</td>
<td>Irregular astigmatism</td>
</tr>
<tr>
<td>ICC</td>
<td>Intraclass correlation coefficient</td>
</tr>
<tr>
<td>ICRS</td>
<td>Intrastromal corneal ring segments</td>
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<tr>
<td>IOP</td>
<td>Intraocular pressure</td>
</tr>
<tr>
<td>IOPg</td>
<td>Goldmann-correlated IOP</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>IOPcc</td>
<td>Corneal compensated IOP</td>
</tr>
<tr>
<td>IVCM</td>
<td>In vivo confocal microscopy</td>
</tr>
<tr>
<td>LASIK</td>
<td>Laser in situ keratomileusis</td>
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<tr>
<td>LASEK</td>
<td>Laser-assisted subepithelial keratectomy</td>
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<tr>
<td>LogMAR</td>
<td>Logarithm of the minimum angle of resolution</td>
</tr>
<tr>
<td>LOAs</td>
<td>Lower-order aberrations</td>
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<tr>
<td>MMC</td>
<td>Mitomycin-C</td>
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<tr>
<td>ORA</td>
<td>Ocular response analyzer</td>
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<tr>
<td>PIOL</td>
<td>Phakic intraocular lenses</td>
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<tr>
<td>PRK</td>
<td>Photorefractive keratectomy</td>
</tr>
<tr>
<td>ReLEx</td>
<td>Refractive lenticule extraction</td>
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<tr>
<td>RGP</td>
<td>Rigid gas permeable lens</td>
</tr>
<tr>
<td>RMS</td>
<td>Root mean square</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SE</td>
<td>Spherical equivalent</td>
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<tr>
<td>SimK</td>
<td>Simulated keratometry</td>
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<tr>
<td>SMILE</td>
<td>Small incision lenticule extraction</td>
</tr>
<tr>
<td>TGCA</td>
<td>Topography-guided custom ablation</td>
</tr>
<tr>
<td>T-PTK</td>
<td>Transepithelial phototherapeutic keratectomy</td>
</tr>
<tr>
<td>UDVA</td>
<td>Uncorrected distance visual acuity</td>
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3. LIST OF PAPERS

Wavefront optimized versus custom-Q treatments in surface ablation for myopic astigmatism with the WaveLight Allegretto Laser.

II. X Chen, A Stojanovic, Y Liu, Y Chen, Y Zhou, TP Utheim
Postoperative changes in corneal epithelial and stromal thickness profiles after photorefractive keratectomy in treatment of myopia.

III. X Chen, A Stojanovic, X Wang, J Liang, D Hu, TP Utheim.
Epithelial Thickness Profile Change after Combined Topography-guided Transepithelial Photorefractive Keratectomy and Corneal Crosslinking in Treatment of Keratoconus
*J Refract Surg.* (accepted for publication)

IV. A Stojanovic, S Chen, X Chen, F Stojanovic, J Zhang, T Zhang, TP Utheim.
One-step transepithelial topography-guided ablation in the treatment of myopic astigmatism

V. X Chen, A Stojanovic, D Sminonsen, X Wang, Y Liu, TP Utheim.
Topography Guided Transepithelial Surface Ablation in Treatment of Moderate to High Astigmatism

Transepithelial, topography-guided ablation in treatment of visually disturbing irregular astigmatism and/or scattering in LASIK-flap/interface complications
VII. X Chen, A Stojanovic, Y Hua, JR Eidet, D Hu, J Wang, TP Utheim. 
Reliability of Corneal Dynamic Scheimpflug Analyser Measurements in Virgin and 
Post-PRK Eyes
eCollection 2014.

VIII. A Stojanovic, X Chen, N Jin, T Zhang, F Stojanovic, S Ræder, TP Utheim. 
Safety and efficacy of epithelium-on corneal collagen cross-linking using a 
multifactorial approach to achieve proper stromal riboflavin saturation

IX. A Stojanovic, J Zhang, X Chen, TA Nitter, S Chen, Q Wang. 
Topography-guided Transepithelial Surface Ablation Followed by Corneal Collagen 
Crosslinking Performed in a Single Combined Procedure for the Treatment of 
Keratoconus and Pellucid Marginal Degeneration
2010 Feb 12.
4. LIST OF FIGURES AND TABLES

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Figure 2. Five distinct layers of the cornea. A sixth layer (Dua’s layer) has recently been proposed. Courtesy of Dr. Magnus Fritzvold, Akershus University Hospital, Norway.

Figure 3. The X, Y, Z hypothesis of corneal epithelial maintenance. The desquamated cells (Z component) are continuously replaced not only by the basal cells (X) that divide, but also by cells migrating in from the periphery (Y). Courtesy of Dr. Magnus Fritzvold, Akershus University Hospital, Norway.

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**Figure 11.** Topography-guided surface ablation in treatment of subepithelial irregularity masked by epithelial remodelling. (a) Epithelium masking a stromal irregularity; (b) Mechanical or alcohol-aided epithelial removal exposes irregular surface; (c) When topography representing the epithelial surface is used as the basis for the treatment planning, and the treatment itself is performed on the stroma after the epithelial removal, a new irregular surface is produced; (d) When the same topography is used for planning of transepithelial ablation that removes both the epithelium and the stroma as a single entity, a regularized surface shape will be “transferred” to the stroma below the irregularity (dotted line).

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### 4.2 Tables

**Table 1.** Amsler-Krumeich Classification for Grading Keratoconus.

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5. GENERAL INTRODUCTION

5.1 The Ocular Surface

5.1.1 Structure and Function

The ocular surface comprises cornea and bulbar and tarsal conjunctiva, extending to the mucocutaneous junctions of the lid margins. It protects the ocular structures from the external world and optimizes the optical properties of the eye. The ocular surface is covered with tear film secreted by the lacrimal and meibomian glands and conjunctiva. The tear film consists of three layers: the outermost lipid layer produced by the meibomian glands, the intermediate aqueous layer secreted by the main and accessory lacrimal glands, and the innermost mucin layer secreted by the surface epithelium and the conjunctival goblet cells (Figure 1).

Figure 1. The tear film. It consists of three layers: lipid, aqueous, and mucin. Courtesy of Haakon Raanes, The Oslo School of Architecture and Design, Norway.

The intermediate aqueous layer is the largest (7 µm), followed by the lipid (0.1 µm) and the mucin (0.02 to 0.05 µm) layers (Figure 1). Tears are also composed of enzymes, immunoglobulins, various metabolites, and exfoliated epithelial and polymorphonuclear cells. By serving as a permeability barrier to the corneal epithelium and by cleaning, lubricating and nourishing the ocular surface, and providing physical and immune protection against
infection, tear film ensures the normal function and structure of the ocular surface to maintain a clear cornea for vision.\textsuperscript{3,5-8} Most of the refractive power of the eye is derived from the air-tear film interface where the greatest change in the refractive index resides. The pre-corneal tear film also fills and flattens the microscopic depressions of the corneal surface created by the reticulations on the epithelial surface,\textsuperscript{9} playing a key role in the maintenance of a smooth and regular optical surface.\textsuperscript{4,10} Disturbance of the tear film can affect the optical quality and visual performance of the eye.\textsuperscript{4,11,12}

Tear film maintenance is dependent on adequate tear production and distribution. Qualitative and quantitative alterations in the volume, composition, and structure of the tear film can cause dry eye disease. In 2007, the Dry Eye Workshop (DEWS) defined dry eye as “a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface, accompanied by increased osmolarity of the tear film and inflammation of the ocular surface.\textsuperscript{13} The two major subtypes of dry eye disease are aqueous-deficient dry eye disease, resulting from a decrease in lacrimal gland secretion, and evaporative dry eye disease, in which there is excessive evaporative water loss. Dry eye disease affects 5% to 34% of all people globally, and prevalence increases with age.\textsuperscript{14}

The status of the ocular surface and tear film before refractive surgery can impact surgical outcomes in terms of potential complications during and after surgery, refractive outcome, optical quality, and postoperative dry eye.\textsuperscript{15} Postoperative dry eye is one of the most common complaints after corneal refractive surgery.\textsuperscript{16-23} It is believed to have a multifactorial aetiology, consisting of neurotrophic epitheliopathy, altered tear film coverage of the changed corneal contour, and an inflammatory desiccation of ocular surface.\textsuperscript{24} Although usually transient, it negatively affects the quality of life and is the primary reason for patient dissatisfaction with the refractive surgery outcome.\textsuperscript{25-28} Patients with dry eye after refractive surgery also have higher risks for refractive regression and ocular surface damage.\textsuperscript{22,29,30} Identifying patients with dry eye disease prior to surgery and treating them pre- and postoperatively can lead to improved outcomes in refractive surgery.\textsuperscript{31-35}

5.1.2 The Cornea

5.1.2.1 Structure and function

The cornea consists of transparent avascular tissue that acts as the primary infectious and
structural barrier of the eye. The average adult cornea is 0.52 mm thick in the center and about 0.65 mm thick at the periphery. The horizontal diameter of the cornea is 11.5 to 12 mm, and it is approximately 1 mm larger than the vertical diameter.\textsuperscript{36, 37}

From anterior to posterior, the human cornea has long been believed to consist of five distinct layers (Figure 2); however, a sixth layer (Dua’s layer) has recently been proposed.\textsuperscript{38}

![Figure 2. Five distinct layers of the cornea. A sixth layer (Dua’s layer) has recently been proposed. Courtesy of Dr. Magnus Fritzvold, Akershus University Hospital, Norway.]

### 5.1.2.2 The epithelium

The corneal epithelium is a non-keratinized, stratified squamous epithelium of four to six cell layers and represents approximately 10\% of the corneal thickness.\textsuperscript{39} The mean epithelial thickness at the corneal vertex is 53.4±4.6 μm, and it is thicker inferiorly than superiorly and thicker nasally than temporally.\textsuperscript{40} It is continuous with the conjunctival epithelium at the corneoscleral limbus.\textsuperscript{41} The corneal epithelium is composed of a basal layer of column-shaped cells, a suprabasal layer of cuboid wing cells, and a superficial layer (1–3 layers) of flat squamous cells.\textsuperscript{42} There is general agreement that the stem cells of the corneal epithelium in humans are located in the periphery of the cornea, although other locations recently have been suggested.\textsuperscript{43-45} The limbal stem cells give rise to basal corneal epithelial cells that migrate centrally. These basal cells generate suprabasal cells,\textsuperscript{42} which in turn give rise to differentiated squamous cells with numerous finger-like projections or microvilli on their surface.\textsuperscript{46} The microvilli increase the cell surface area, allowing close association with the tear film that traps tear fluid, thus preventing desiccation of the ocular surface.\textsuperscript{47} Moreover, there are tight junctions between the cells that form the corneal barrier,\textsuperscript{48} which prohibit tears, toxins, and microbes from entering deeper corneal layers. Individual epithelial cells are connected to each other and to the basement membrane by desmosomes and
hemidesmosomes, respectively. These structures are important in mediating cell migration in response to epithelial injury. Under normal conditions, the corneal epithelium is renewed every 9–12 months. This contrasts with the human epidermis, where the replacement takes place approximately once a month.

Thoft and Friend reported the ‘The X, Y, Z hypothesis of corneal epithelial maintenance’ (Fig. 3). The hypothesis proposed that the sum of the proliferation of basal cells (X) and the centripetal migration of cells (Y) equals the epithelial cell loss from the corneal surface. Thoft and Friend were unable to rule out the involvement of the neighbouring bulbar conjunctiva. Later, mathematical analysis indicated that the corneal epithelial cell mass could be renewed by cells from the limbal epithelium alone. Limbal stem cells can self-renew and give rise to fast-dividing progenitor cells, which are called transit amplifying cells. Transit amplifying cells undergo a limited number of cell divisions before they become terminally post-mitotic cells and, eventually, terminally differentiated cells. Terminally differentiated cells are shed from the ocular surface during normal wear and tear, which stimulates the cycle of cell division, migration, and differentiation.

Various cytokines have been shown to be important in the maintenance and wound healing of the cornea. These factors are supplied, in part, by the adjacent tear film and the aqueous humour. Other growth factors are produced by keratocytes in the supporting stroma, or by corneal epithelial cells themselves.
5.1.2.3 Bowman’s membrane

Bowman’s membrane is a clear acellular layer consisting of collagen fibrils with a diameter of 20-25 nm and proteoglycans. These fibrils are not ordered in bundles; individual fibrils run in various directions to form a thick, dense, felt-like sheet that is about 8-12 μm thick. The collagen fibrils in the posterior layer of the Bowman’s sheet are gradually assembled in bundles and merge into the collagen lamellae of the stroma.58 The Bowman’s membrane is more resistant to damage than the corneal epithelium.47 However, unlike the corneal epithelium, it cannot regenerate after injury.47 Instead, fibrous scar tissue is formed, resulting in opacity. Bowman’s membrane is not necessary for the formation of normal epithelium, and its physiological role remains somewhat unclear.47

5.1.2.4 The stroma

The stroma accounts for about 90% of the total corneal thickness.42 The parallel arrangement of lamellae formed from heterodimeric complexes of type I and type V collagen fibrils maintains the transparency of the cornea.41 The organization of collagen fibrils in the human cornea has been studied using electron microscopy,58 X-ray diffraction or scattering,59,60 and second harmonic generation imaging microscopy.61 The collagen fibrils have a diameter of 25-35 nm, and are packed in parallel-arranged layers or lamellae.62 Adjacent lamellae lie at different angles, between 0 and 90°.63 The human corneal stroma consists of over 300-stacked lamellae through its central thickness. As the cornea thickens away from the vertex, the number of lamellae increases, reaching about 500 throughout the stromal thickness at the limbus.64 The collagen lamellae are thin (about 0.2-1.2 μm) and narrow (about 0.5-30 μm) in the anterior third of the stroma, run mostly obliquely to the corneal surface, and are more irregularly interwoven. Furthermore, parts of the anterior lamellae are inserted into Bowman’s layer.65-67 In the posterior stroma, collagen lamellae become thicker (0.2-2.5 μm) and wider (100-200 μm) and tend to be arranged parallel to the corneal surface, preferentially oriented along the superior-inferior and nasal-temporal corneal meridians.58,59,61,68

It is envisaged that these properties of the anterior lamellae contribute to balance the intraocular pressure, maintain corneal curvature, make stronger transverse shear properties and increase tensile strength compared to the posterior stroma; while the preferentially aligned fibrils in the posterior stroma take up the additional tensile stress along the superior-inferior and nasal-temporal meridians exerted by the rectus muscles and the orbicularis.69-73
The collagen lamellar arrangement has also been reported to be different between the central and limbal area of the cornea in the posterior third of the cornea. The central cornea maintained the preferred superior-inferior and nasal-temporal orientation of collagen to within about 1 mm from the limbus, where a circular or tangential disposition of fibrils interlaced with significant numbers of mature elastic fibres occur. The circumferential reinforcement of fibrils is thought to help to withstand the increased tension in that region brought about by the differing curvatures of the cornea and sclera. Furthermore, while the mean fibril diameter remains constant across all corneas, the mean fibril spacing across the central cornea measured 5-7% lower than in the peripheral cornea.

Keratocytes, which represent the main cell type in the stroma and mostly reside in the anterior stroma, are involved in maintaining the extracellular matrix environment. They are able to synthesize collagen molecules and glycosaminoglycans, as well as matrix metalloproteases — all of which are crucial in maintaining stromal homeostasis. Keratocytes also produce crystalline proteins that maintain corneal transparency.

5.1.2.5 *Descemet's membrane*
Endothelial cells continuously “secrete” Descemet’s membrane. It is about 3 µm thick at birth but increases in thickness throughout life, reaching 10-12 µm in adulthood. It functions as a protective barrier against infections and eye injuries.

5.1.2.6 *The endothelium*
The intact human endothelium is a monolayer, a honeycomb-like appearing mosaic when viewed from the posterior side. It is responsible for maintaining the stroma in a relatively dehydrated state. Human endothelial cell density is approximately 3500 cells/mm² at birth and decreases at an average rate of 0.6% per year throughout life. The number of endothelial cells also decreases with trauma, inflammation, and other pathological processes. Endothelial cells have no mitotic capability *in vivo*; however, when the cell density decreases the remaining cells have the ability to “stretch” and take over the space of the degenerated endothelial cells.

5.1.2.7 *Dua’s layer*
A recently described corneal layer was named after Professor Harminder S. Dua, who
discovered it.\textsuperscript{38} It is a 15 microns thick, well-defined, acellular layer in the pre-Descemet's cornea. The membrane is sufficiently strong to withstand a pressure of approximately 700-950 mm Hg. Its recognition may have considerable impact on posterior corneal surgery and understanding of corneal biomechanics and posterior corneal pathology.\textsuperscript{38}

\section*{5.2 Corneal Biomechanics}

Biomechanical properties refer to the dynamic response of the biological tissue to mechanical stress, and the resulting deformation after stress. It is related to the structure and function of the tissue. Elasticity represents how a material deforms in response to an external stress and returns to its original shape along the same stress-strain pathway when the imposed stress is removed. Viscosity is the resistance of a fluid to flow. It is determined by water content, macromolecular components, and interactions between macromolecules. In highly viscous or gel-like substances, molecules are strongly connected to each other and, thus, are not very flexible.

Biomechanically, the cornea can be considered a bi-composite material consisting of collagen fibrils and the ground substance (proteoglycans and glucosaminoglycans), in which the fibers are embedded. The collagen fibrils provide elastic reinforcing structure while the ground substance provides viscoelasticity.\textsuperscript{77} When loaded, the cornea demonstrates some instantaneous deformation (purely elastic behaviour) followed by progressive deformation (viscoelastic behaviour).\textsuperscript{78} It is important to keep in mind that the corneal stiffness and viscoelasticity are not directly related, and alterations in tissue structure can lead to independent changes in both.\textsuperscript{78}

The highly anisotropic and distinctive arrangement of collagen fibers and the specific collagen-matrix interaction are important factors for the anisotropic biomechanical properties of the cornea. The biomechanics of the corneal stroma have been investigated by the measurement of pulling force of corneal strips,\textsuperscript{79,80} mercury droplet markers’ displacement with increasing intraocular pressure,\textsuperscript{81} optical coherence tomography elastography,\textsuperscript{82} ultrasonic techniques,\textsuperscript{83} supersonic shear wave elastography,\textsuperscript{84} corneal hysteresis,\textsuperscript{85-87} as well as Brillouin microscopy\textsuperscript{88,89} and atomic force microscopy.\textsuperscript{90-92} These studies have revealed that the anterior stroma is stiffer than the posterior stroma. The anterior stroma has also been found not to swell in response to an artificial edema-inducing condition.\textsuperscript{69} Dowson et al.\textsuperscript{93} demonstrated increasing tensile strength moving from the central cornea towards the periphery. \textit{In vitro} pressure-induced regional mechanical performance of the cornea and
limbus measurements by Hjortdal et al.\textsuperscript{81} showed that the highest elastic moduli were found at the center and para-center in the meridional direction, and at the limbus in the circumferential direction.

Changes in the collagen fibrillar morphology and arrangement, as well as changes in the collagen-matrix interaction, may lead to alterations in corneal biomechanical properties in certain conditions. During ageing, there is an increase in the cross-linking among collagen molecules, an increase in the diameter of collagen fibrils, glycation-induced expansion of intermolecular spacing, as well as a decrease in the interfibrillar spacing of the corneal collagen.\textsuperscript{94, 95} Therefore, one would expect a tendency toward biomechanical strengthening of the cornea with ageing. Corneal stiffness of diabetic eyes may increase due to increased cross-linking through glycosylation and lysyl oxidase enzymatic activity.\textsuperscript{96}

In keratoconus corneas, reduced mean diameter and interfibrillar spacing of the collagen fibrils,\textsuperscript{97} slippage of collagen lamellae,\textsuperscript{98, 99} as well as a loss of the normal interwoven structure of the lamellae,\textsuperscript{100} may result in biomechanical instability of the tissue.\textsuperscript{73} Corneal refractive surgery may also induce corneal biomechanical changes.\textsuperscript{101, 102} Finally, the photo polymerization effect of riboflavin-ultraviolet radiation A (UVA) corneal cross-linking (CXL) induces cross-links at the collagen fibril surface and in the protein network surrounding the collagen.\textsuperscript{103} Nevertheless, the biophysical and biochemical factors determining corneal stiffness, elasticity, viscosity, and damping are not fully understood,\textsuperscript{72} and further studies are warranted.

5.3 Corneal Optics

In addition to functioning as a barrier, the cornea is the most important optical component of the eye, refracting the light and focusing it onto the retina with minimum scatter and optical degradation.

5.3.1 Eye Optics

5.3.1.1 Refractive components and accommodation

The human eye is similar to a camera, consisting of: the main refracting component (the cornea), a variable aperture (the pupil), adjustable focusing (the crystalline lens) and a dark (posterior) chamber. These optical elements form images on a layer of photosensitive retinal tissue, which converts patterns of light into neuronal signals. Many attempts have been made to simplify the optical system of the human eye. For instance, in Gullstrand’s eye model, the total refractive power of the eye is 58.64 diopters (D), with 43 D contributed by the cornea.
and 19 D contributed by the lens, while the axial length of the eye is 24 mm. The eye changes its refractive power to focus on near objects by a process called accommodation, which is a result of the optical power change of the crystalline lens. According to the Helmholtz theory of accommodation, contraction of the ciliary muscle leads to thickening and increased curvature of the crystalline lens, due to relaxation of the lenticular capsule.

In a relaxed accommodative state, the combined optical power of the cornea and crystalline lens must match the axial length of the eye in order to provide a sharp focus of a distance object on the retina (emmetropia). When a mismatch occurs, the images will be out of focus, which is commonly called ammetropia (refractive error).

5.3.1.2 Myopia
Myopia is the type of defocus where the total refractive power is too high relative to the axial length of the eye, leading to images of distant objects focusing in front of the retina. Myopia is the most common eye disorder worldwide. The prevalence and progression seem to be affected by many variables such as ethnicity, sex, familial disposition, age of onset, degree of myopia, near-reading activities, outdoor activities, as well as education level. Midelfart and colleagues found the prevalence of myopia in Norway to be 35.0% in the young adult population and 30.3% in the middle-aged group in 1996-1997.

5.3.1.3 Hyperopia
In contrast to the myopia, the total refractive power in hyperopia is too low relative to their axial length. Consequently, the images of distant objects are focused behind the retina. However, young hyperopes are usually not bothered by their hyperopia because they can easily “move” the defocused images to the retina by using their accommodation, which is still of large amplitude. Midelfart et al. found the prevalence of hyperopia in Norway to increase with age from 13.2% (20–25 years) to 17.4% (40–45 years).

5.3.1.4 Astigmatism
An eye with astigmatism produces two orthogonally symmetrical lines of foci instead of a focal point. The focal distance between the lines decides the amount of astigmatism. Astigmatism cannot be corrected by changing viewing distance or accommodation. Lower order aberrations (LOAs), defocus and regular astigmatism can be corrected by spectacles, contact lenses, and refractive surgery.
5.3.2 Corneal Optics

Although the cornea is represented in Gullstrand’s model as one refractive surface with 43 D power, the light entering the eye is actually refracted by both the anterior and the posterior surface of the cornea. The anterior corneal surface is flatter than the posterior surface. The mean radius of curvature of the anterior and posterior surface is 7.8 mm and 6.8 mm, respectively. However, the anterior surface has much greater optical power (48.8 D) than the posterior surface (-5.8 D) due to larger differences in refractive indices between the air and the cornea than between the cornea and the aqueous.\textsuperscript{110}

The curvature of the anterior surface of the cornea normally decreases from the vertex to the periphery; i.e. the cornea is not spherical. The term asphericity (Q-value) is used to define the shape of the cornea in terms of change in curvature. $Q = \frac{a^2}{b^2} - 1$, where $a$ and $b$ represent the radius of curvature of central and peripheral cornea, respectively. A spherical cornea that has the same radius over the whole area will generate a field of focal points instead of one point, because the peripheral light rays (that are not perpendicular to the cornea) are refracted more than the central rays. This phenomenon is called spherical aberration. If the spherical shape is changed to prolate, where the radius of the curvature is increasing towards periphery ($Q<0$), the peripheral light rays are relatively less refracted, and the spherical aberration is reduced. On the contrary, the spherical aberration is increased with oblate shape, where the radius of curvature becomes smaller towards the periphery ($Q>0$) and the peripheral light rays are more refracted. The ideal asphericity for the anterior surface of the cornea to eliminate spherical aberration for a distant object would be a prolate ellipsoid with $Q = -0.528$. However, several investigators have shown that the mean Q-value of the anterior cornea over a 6-mm zone is -0.26 (0.00 to-0.50).\textsuperscript{111} The normal human cornea with a Q-value of -0.26 is rarely free of spherical aberration itself, but in balance with the slightly negative asphericity of the crystalline lens, it contributes to achieve a minimal spherical aberration of the total optical system of the eye.\textsuperscript{112}

5.3.3 Irregular Astigmatism

Since the anterior cornea provides almost two-thirds of the eye’s focusing power, imperfections of the corneal surface will have a significant impact on retinal image quality,\textsuperscript{113} causing optical anomalies known as aberrations, which are responsible for inferior optical performance of the eye.\textsuperscript{114} Corneal irregular astigmatism is a type of refractive anomaly, in which the orientation of the principal meridians and/or the power change across the cornea
from point to point. It occurs primarily in keratoconus, or secondary to iatrogenic keratectasia, decentered or otherwise complicated corneal refractive surgery, corneal scarring (post traumatic, post keratitis) etc.115 The irregularities cause distortedly projected images on the retina, perceived as visual disturbances such as glares, halos, starburst, multiple images, and reduced contrast sensitivity.116

To analyze and define the irregular astigmatism, a decomposition of the complex optical irregularities may be performed and then represented in terms of wavefront aberrations that correlate to the quality of vision. Wavefront is an imaginary three-dimensional surface representing corresponding points of the light rays vibrating in unison. The difference between the wavefront from the optical system with refractive anomalies, and the wavefront from the ideal optical system defines wavefront aberrations. A typical way to describe the wavefront aberration is in terms of a Zernike polynomial series.117, 118 Each Zernike polynomial can describe only a limited family of shapes. For example, one Zernike polynomial may describe how much the surface tilts, while another may describe how much the edge may turn up or down, etc. Hence, Zernike decomposition can provide a good description of the cornea’s optical properties. Zernike coefficients represent the weight of each of the components on the wavefront aberrations. The aberration components represented by Zernike terms are grouped into lower order aberrations (LOAs) and higher order aberrations (HOAs). The higher the order is, the more subtle the irregularities are that can be described. The lower order terms correspond to conventional refractive errors: first order terms represent prism, while the second order terms represent defocus and astigmatism. Higher order terms include other more complex monochromatic aberrations, like coma (the 3rd order), with the opposite power at the opposite ends of one meridian, spherical aberration (4th order), etc. Generally speaking, odd-order higher order aberrations are non-rotationally symmetric, while even-order are rotationally symmetric aberrations. Wavefront aberrometry, which measures the ocular wavefront aberration, as well as the corneal topography, are the most effective means to evaluate the corneal irregular astigmatism.

5.3.4 Treatment of Irregular Astigmatism

Correction of irregular astigmatism remains a difficult challenge. Spherical or sphero-cylindrical spectacle glasses can only help to move the images onto the retinal plane, but they cannot resolve the visual disturbances caused by distorted images. Soft contact lenses provide marginally better visual acuity than spectacle correction, with the level of residual aberrations
still remaining high due to modelling of the soft contact lens to the corneal surface. Rigid gas permeable (RGP) contact lenses have been reported to be able to provide a significant improvement in visual acuity for patients with irregular astigmatism. The shape of RGP is unaffected by the underlying corneal surface. The tear fluid (n=1.336) formed beneath the RGP lens has a similar refractive index to the cornea (n=1.376) and therefore neutralizes most (1.336/1.376=97%) of the aberrations of the anterior corneal surface. However, discomfort often associated with the use of RGP can have a negative impact on the patient’s quality of life. For these patients, surgical intervention becomes a necessity.

Excimer laser surgery on the cornea, guided by topography or wavefront, is now a method of choice for the treatment of corneal irregular astigmatism. This will be elaborated in chapter 5.6.1. In addition, there are several other possible options for reshaping the irregular cornea and improving the regularity of the corneal surface.

Thermal keratoplasty is based on shrinkage of the stromal collagen to achieve a change in corneal shape. The thermal energy can be applied by a Holmium-laser, or by a technique called conductive keratoplasty (CK), where resistance to the electrical current flow through the tissue generates thermal energy. The CK-technique has been reported to be effective in reshaping the cornea in eyes with keratoconus, without serious complications. However, the mentioned methods did not show a predictable dose response between the applied energy and the induced refractive change, but they showed considerable regression. Lately, a new device and technique that involves the application of microwaves to the corneal surface was used to change the refractive state of the cornea by changing the pathway and thereby the curvature of the collagen fibers. The changes in refraction were reported to be more predictable, but were also temporary, regressing over the course of 3 months. Vega-Estrada A and his colleagues combined the microwave thermal keratoplasty with corneal collagen cross-linking (CXL) to smoothen the irregular anterior cornea in keratoconic eyes.

The use of intrastromal corneal ring segments (ICRS) is another way to reshape the cornea and mostly improve the irregular component of astigmatism and corrected distance visual acuity (CDVA). The concept was introduced in 1978, and the ICRS were first implanted in human eyes in 1991 to correct myopia. They are currently used to improve vision in keratoconus and corneal ectasia, mostly in combination with spectacles, contact lenses, or phakic intraocular lenses (PIOL). The tunnels for implantation of ICRS could be created mechanically or by femtosecond (FS) laser. Several studies have demonstrated no significant difference in terms of visual, refractive, and keratometric outcomes between the two tunnelling techniques. Mechanical tunnelling, however,
seems to result in more complications, including increased incidence of epithelial defects and greater risk of corneal perforation.\textsuperscript{135, 136} There are three commonly used models of ICRS: Intacs (Additional Technology, Inc.), Kerarings (Mediphacos Ltda.), and Ferrara rings (Mediphacos, Inc., Belo Horizonte, Brazil). The models differ in cross-sectional shape, diameter, arc length, and thickness. In general, for a greater degree of flattening, thicker ring segments and closer placement of the ring to the visual axis\textsuperscript{142} are used. Whereas some studies reported stable long-term outcomes of ICRS implantation in keratoconic eyes,\textsuperscript{143, 144} Vega-Estrada et al.\textsuperscript{143, 145} found that the short-term improvement achieved by ICRS implantation was lost after 5 years in progressive keratoconic eyes. The authors therefore suggested that the implantation of ICRS might not arrest the progression of keratoconus.

5.4 Diagnostics – Assessment of Various Technologies for Measurement and Analysis of Corneal Optical Properties and Morphology

Current corneal assessment technologies make the process of corneal evaluation fast and precise. The most commonly used instruments, including aberrometers, corneal topographers (either Placido-disk-, slit-scanning- or Scheimpflug-imaging-based), and optical coherence tomography (OCT), will be introduced in this chapter.

5.4.1 Wavefront Aberrometry

Measuring and representing the optical irregularities in term of wavefront aberrations is one way to assess the quality of the eye’s optical system. Wavefront aberrometers perform a high-resolution spatial auto refraction across the area of the pupillary opening, rendering a map of wavefront aberrations expressed in micrometers of deviation (root-mean-square [RMS]) from the ideal wavefront plane.\textsuperscript{146} The principles used in the commercially available devices include ray tracing,\textsuperscript{147} automatic retinoscopy,\textsuperscript{148} Hartmann-Shack\textsuperscript{149} and Tscherning.\textsuperscript{150} Because of the dynamic nature of the accommodation and the age-related physiological changes in crystalline lens, considerable variation in the aberrometry results may occur from exam to exam during the same session and from exam to exam over a period of time.\textsuperscript{151, 152} For this reason and due to the “crossover effect” (local optical distortion exceeding a certain magnitude registered by a “wrong” lenslet of the lenslet array sensor in a Hartman-Shack aberrometer)\textsuperscript{146}, as well as due to the limitations of the pupil-dependent measurement area,
unreliable aberrometry results are quite frequent in eyes with very distorted corneal optics. Hence, the optical irregularities residing within the cornea are registered more reliably by the use of corneal topography, rather than ocular aberrometry.

5.4.2 Corneal Topography and Imaging

5.4.2.1 Placido-based corneal topography

The most commonly used topographers are based on Placido-disk principle. A Placido-disk consists of multiple concentric light and dark rings. The reflection of the rings on the cornea will appear non-circular if any anterior corneal surface irregularity is present. There are many Placido-disk based devices that are currently available, and the term “videokeratoscopy” represents the technique employed. There are several limitations in reconstructing the corneal surface with Placido-based technology. First of all, the area of corneal coverage is limited to about 60%, thus excluding the peripheral and the area of the very center of the cornea. Secondly, it lacks information from the posterior corneal surface, which is believed to be an important indicator for ectatic diseases such as keratoconus. Finally, the Placido-derived topographic curvature maps are reference-axis based, and extrapolated three-dimensional information about the corneal physical shape will be bound to the rotational position of the fixating eye. Therefore, the cases with displaced corneal apex and the cases with great corneal asymmetry are prone to pattern errors because the reference axis does not go through the corneal apex and the consequently tilted reflective images are analyzed by the system.

5.4.2.2 Elevation-based corneal topography

While Placido-disk-based topography calculates anterior corneal curvature by analyzing reflected images from the Placido disk, elevation-based topography directly measures the x, y, and z coordinates of more than 20,000 points on the corneal surface by triangulation. Optical slits are projected onto the cornea and the data points acquired from each slit are used to reconstruct the true topography of the anterior segment surfaces as well as the thickness of the cornea.

Currently, there are five elevation-topography systems available: Orbscan (Bausch & Lomb, Rochester, NY), Pentacam (Oculus Optikgerate GmbH, Wetzlar, Germany), Galilei (Ziemer Ophthalmic Systems AG, Zurich, Switzerland), Sirius (Costruzione Strumenti Oftalmici, Florence, Italy), and Precisio (iVIS Technology, Taranto, Italy). Orbscan utilizes
transversal scanning-slit technology, while the other four utilize rotating slit, Scheimpflug imaging. Scheimpflug imaging is based on the Scheimpflug principle, where the planar subject is not parallel to the image plane. In this way, a wide depth-of-focus is achieved, with sharp images from the anterior through the posterior corneal surface and crystalline lens. The devices share many of their features and measure the same basic corneal parameters, but they are not always interchangeable in clinical practice.

Elevation-based topography offers important advantages over Placido-based devices, the ability to image the posterior cornea and to produce an accurate pachymetry map being the most significant. For this reason, it is often referred to as corneal topo/tomography. The primary elevation data accurately represent the corneal morphology, unlike the secondary derived data from curvature information acquired by Placido-based systems using the so-called arc step method (a method prone to cumulative error). Primary elevation data are not based on any assumed axis either, and therefore will not be influenced by the displaced corneal apex that is common in optically irregular corneas. The advantage of the morphology-derived pachymetry map over ultrasound pachymetry is that it can accurately identify the value and location of the thinnest point, as well as the corneal thickness distribution. This has found application in assessment of the progression of keratoconus.

5.4.2.3 Interpretation of corneal topographic maps

Corneal curvature is mostly calculated by two algorithms: 1) The axial (also called sagittal) curvature, which measures the curvature at a certain point on the corneal surface in axial direction relative to the center, and 2) The tangential (also called local, or instantaneous) curvature, which measures the curvature at a certain point on the corneal surface in meridional direction relative to the other points on the particular Placido ring. Simulated keratometry (SimK) is derived from the curvature topography data and is used to characterize corneal curvature in the central 3 mm area using keratometric index of 1.3375 instead of the true refractive index of the anterior cornea (1.376) in order to compensate for the contribution of the posterior corneal surface. The steep SimK and flat SimK give an average curvature of the points along the steepest and flattest meridians within the central 3 mm area. Elevation maps display the height of each point on the corneal surface (in µm) relative to a reference surface, called best-fit surface (usually sphere or aconic), which is a mathematical approximation of the actual corneal elevation calculated by the instrument’s software for each topography.

Both curvature and elevation-based topography provide colour-coded maps.
Warmer colours (reds, oranges) represent steeper corneas (higher dioptric power) in the curvature map, points above the reference surface in the elevation map, and areas with thinner cornea in the pachymetric map. Cooler colours (blues, violets), however, represent flatter corneas (lower dioptric power), points below the reference surface, and area with thicker cornea in respective maps. Greens and yellows represent medium values in all of the maps. However, different topographers use different numbers of steps and colour coding as their default, making it difficult to compare the results from different topographers.

Anterior corneal optical irregularities measured either by Placido-disk- or elevation-based-topography may be analyzed, decomposed and presented as wavefront data, for the diagnosis and definition of irregular astigmatism. Higher amounts of vertical coma and larger values of odd-order RMS have been reported in patients with keratoconus or keratoconus suspect.

5.4.2.4 Topography in keratoconus

Keratoconus shows a characteristic topographic pattern. The anterior corneal surface is characterized by a focal steepening over the inferior mid-peripheral zone, which is surrounded by a zone of progressively decreasing curvature, described as asymmetric bowtie with skewed axis or asymmetric bowtie with inferior steepening (Figure 4). The anterior elevation map shows the physical location of the cone as a focally increased elevation, located mostly in the inferotemporal quadrant. Various topographers feature a plethora of keratometric indices for the easy identification of keratoconus patterns. Some indices describing anterior corneal surface irregularity derived from Scheimpflug images such as index of height decentration, and index of surface variance, have been identified as robust indicators for keratoconus severity and progression.
Figure 4. Placido based topography (left, axial map; right, instantaneous map) showing inferior steepening in a keratoconic eye.

Figure 5. Elevation-based topo/tomography showing increased maximum elevation in the anterior (upper left) and posterior surfaces (upper right) in the same keratoconic eye as demonstrated in Figure 4. The locations of these two points coincide with the point with thinnest pachymetry (lower right).

Figure 6. The thickness profile of the total cornea (left) and the epithelium (right) in the same keratoconic eye as demonstrated in Figure 4 and 5, measured by RTVue OCT.

In accordance with the early pathogenesis of keratoconus, the posterior corneal protrusion is reflected on the corneal posterior elevation and consequently on the corneal pachymetry, both of which can be registered by elevation topo/tomography, as suggested by
numerous studies. It was reported that the maximum posterior elevation and posterior elevation of the thinnest corneal point was significantly higher, and the central pachymetry and thinnest pachymetry are significantly thinner in keratoconus than in normal eyes, and that these points coincide, even in the mildest forms of keratoconus (Figure 5). This suggests that the first detectable sign of keratoconus is a bowing of the posterior corneal surface detected by tomography. The annular pachymetric distribution was also demonstrated to be a sensitive parameter for distinguishing even the mildest form of keratoconus from normal eyes.

5.4.3 OCT and OCT-based Topography
Apart from scanning-slit tomography and rotating Scheimpflug imaging, the optical coherent tomography is another non-contact 3-dimensional (3-D) optical imaging technology that can be used for assessment of the cornea and the anterior segment. OCT is an optical method based on low coherence interferometry. It compares the time-delay of infrared light reflected from the anterior segment structures against a reference reflection. This interference pattern leads to a cross-sectional image of the anterior segment of the eye with a high resolution. After the images have been captured and saved to the computer, various parameters can be measured including corneal thickness, anterior chamber depth, anterior chamber angle, and angle-to-angle distance.

There are currently two different types of OCTs applied in ophthalmology: time-domain OCT, in which varying the position of the reference mirror produces cross-sectional images, and Fourier-domain OCT, in which the reference mirror is fixed and Fourier transformation of the spectral interferogram generates the cross-sectional images. Because the Fourier-domain systems, such as the RTVue 100 (Optovue, Inc., Fremont, CA), do not depend on mechanical movement of a reference mirror and detect signals from the entire depth range in parallel rather than serially, they achieve higher speed without losing signal-to-noise ratio.

OCT technology can provide corneal structural analysis and may be used to assess a wide range of anterior segment features from the cross-sectional images, including evaluation of the flap- and the residual stromal bed thickness and keratectasia after laser in situ keratomileusis (LASIK), as well as localization of the demarcation line within the stroma after CXL. Anterior segment OCT (AS-OCT) has demonstrated a good repeatability and reproducibility of the central and peripheral cornea thickness mapping.
mapping with the AS-OCT has been suggested as a helpful screening and early diagnostic tool for keratoconus.\textsuperscript{180, 182}

Recently, a new, high speed, swept-source anterior segment spectral domain OCT based corneal topo/tomographer, CASIA (Casia SS-1000; Tomey, Nagoya, Japan), has been developed.\textsuperscript{183-185} Its high speed scanning (0.34 second in corneal map mode) may contribute to minimization of the artefacts caused by ocular movements during the examination,\textsuperscript{186} while its 1310 nm wavelength of the light source allows better penetration into the opaque tissues such as cloudy corneas compared to visible light,\textsuperscript{187, 188} making it a better suited tool for the examination of corneal pathology than the existing corneal topo/tomographers. Both the short duration of the exam and the use of infrared instead of visible light significantly increase the comfort of examination and allows the patients to hold their eyes widely open, contributing to better coverage of the examined cornea. It has been reported that the success rate of precisely digitizing the corneal surfaces in keratoconic eyes was 95% using the AS-OCT-based corneal topo/tomography.\textsuperscript{183}

The most unique application of OCT in diagnosis of keratoconus is mapping of the corneal epithelial thicknesses. The corneal epithelium is a mouldable and active corneal layer, being regulated by the blinking action and the force applied by the eyelid. It maintains the optical quality of the eye by remodelling itself to compensate for any changes in the stromal surface shape,\textsuperscript{189} such as those occurring after myopic and hyperopic laser ablation,\textsuperscript{190, 191} orthokeratology,\textsuperscript{192} secondary stromal irregularities\textsuperscript{193} and in keratoconus.\textsuperscript{194-196} Therefore, information about the thickness distribution of the corneal epithelium may help to identify irregularity of the stromal surface, as in subclinical keratoconus, before it is detectable on corneal topo/tomography. The recent studies of clinical \textit{in vivo} epithelial mapping by OCT demonstrated a typical epithelial remodelling pattern with keratoconus, with the epithelium being thinner inferotemporally and thicker supranasally, compared to that of normal eyes (Figure 6). This suggests that the AS-OCT-derived epithelial mapping has a critical potential in the diagnosis of early and progressive keratoconus.\textsuperscript{194, 195} The concept of use of epithelial mapping as a part of structural analysis of cornea and its application to various diagnostic and even surgical purposes has been introduced and developed by Reinstein using high frequency ultrasound technology.\textsuperscript{196} However, that technology did not reach the level of commercial availability. AS-OCT proved to be more practical in that respect, primarily due to its ease of use and the comfort of non-contact, quick and easy to perform examinations.

\textbf{5.4.4 \textit{In Vivo} Confocal Microscopy (IVCM)}
The modern *in vivo* confocal laser scanning microscopy employs a laser light source that focuses on one point of the object through a pinhole diaphragm. The reflected laser light is separated by a beam splitter from the incident laser beam path and is deflected through a second confocal diaphragm to reach a photosensitive detector. In this way, the scattered light from outside the focal plane is highly suppressed, and only the objective layer located at the focal plane contributes to the image, enabling imaging in high resolution.

The IVCM can be used to analyze the images of the corneal structures. Its non-invasive nature enables real-time *in vivo* evaluation of the cornea at the cellular level. The z-axis position of these images can be used to calculate corneal sublayer’s thicknesses. Clinically, the *in vivo* confocal microscopy has been used to study normal and diseased corneas, and corneas following surgery or contact lens use.

Corneal wound healing after refractive surgery is a complex cascade. During laser refractive surgery, damage to the epithelium has been shown to cause the release of various kinds of cytokines and chemokines, inducing keratocyte apoptosis and modulating the subsequent stromal repair response. An intensified corneal wound healing reaction can occasionally lead to undesirable complications, such as regression of the refractive outcome and haze. One important feature of IVCM is its ability to objectively quantify corneal backscatter, which is used to define stromal reaction, keratocyte activation, and objective haze grading. The IVCM has also been applied to evaluate corneal nerve regeneration after corneal refractive surgery.

Only a small area of the cornea is imaged with confocal microscopy, and a tracking system is not present in current devices. As a result, positional repeatability is low. Therefore, the reliability of the data obtained with confocal microscopy should be facilitated by repeated measurements.

5.4.5 Corneal Biomechanical Measurements

Despite the great needs for the corneal biomechanical measurements at the individual level, most of our current knowledge comes from *ex vivo* experimental studies performed with stress-strain and other mechanical tests. *In vitro* measurements have proven to be largely dependent on the technique used and experimental conditions (time post-mortem, hydration conditions, storage solutions, etc.). As the compelling clinical need for biomechanical information has increased, various *in vivo* techniques have been under active development.
The ocular response analyzer (ORA; Reichert Inc., Buffalo, NY, USA) described by Luce et al. in 2005, is the first device to allow *in vivo* measurements of corneal biomechanical properties. Recently, a dynamic Scheimpflug analyzer (CorVis ST; Oculus, Wetzlar, Germany) has been developed. Both ORA and CorVis ST apply a rapid air-puff onto the anterior surface of the cornea that causes the cornea to deform. The air puff indents the cornea, passing from its original state, through first applanation and into slight concavity. Then, as the pressure decreases, the cornea rebounds through a second applanation, back to its original shape. Due to its biomechanical properties, the cornea resists the air puff, causing delays in the inward and outward applanation events. The ORA utilizes an electro-optical system to record the signal intensity of the reflected infrared light from the central 3 mm area through the deformation process and generates a waveform. Information from the electro-optical system is processed, analyzed, and presented as a corneal deformation signal (Figure 7). When the cornea undergoes applanation, the reflected light is maximally aligned with the detector, generating a signal peak. Once the device detects the first applanation event, the piston producing the air jet receives a signal to shut down to allow the air pressure to dissipate and the cornea to recover its shape. In the contrary, the CorVis ST employs a fixed profile air pulse with a fixed internal pump pressure. Furthermore, it uses Scheimpflug imaging and a high-speed camera (4,330 frames per second) to record images of the horizontal 8 mm cross-section of the cornea as it deforms, providing quantitative information of the corneal displacement.

![Signal Time Response](Figure 7. The output of ORA measurement: the signal peak (the red and blue curves) in the left side presents the moment where the first inward applanation was reached, whereas the peak in the right side represents the moment where the second outward applanation was reached during the measurement. The green curve shows the change of the pressure of the air pulse in time.)
In ORA, the first inward applanation pressure is called “P1” and the second outward applanation pressure is called “P2” (Figure 7). The average of P1 and P2 provides a Goldmann-correlated intraocular pressure (IOPg). The difference between P1 and P2 is termed corneal hysteresis (CH=P1-P2). Corneal hysteresis (CH) represents the ability of the cornea to absorb and dissipate energy (damping capacity), which is in contrast to its stiffness, elasticity, or rigidity. It is the result of the viscous damping within corneal tissues that is created by the viscosity of glycosaminoglycans and proteoglycans, as well as by a collagen matrix interaction. Corneal resistance factor (CRF) is derived from the formula (P1-kP2), where k is a constant that is strongly associated with CCT. The CRF is a parameter that was empirically developed to be strongly associated with corneal stiffness and is believed to be a measurement of the overall corneal resistance (total viscoelastic properties) of the cornea during measurement. Corneal compensated IOP (IOPcc) is an empirical IOP measurement (IOPcc=P2 -c1xP1+c2, where c1 and c2 are constants), which is less affected by corneal biomechanical properties on IOP measurement compared with other tonometry techniques.

The CH and CRF are two most commonly analyzed metrics in the ORA to describe the biomechanical properties of the cornea. The repeatability and reproducibility of the measurements have been proven to be good for both CH and CRF. In addition, the Waveform Score may provide information on the reliability of the signals in ORA. Lam et al. proposed that a score < 3.50 might indicate an unreliable signal that should be discarded.

In published data, the CH and CRF showed a positive correlation with CCT. It is postulated that a thicker healthy cornea contains more collagen fibers and ground substance, resulting in a greater resistance against deformation and a higher damping capacity. The IOP represents an additional force that restores the cornea to its original position. There is inverse correlation between IOPcc and CH. In contrast, there is positive correlation between IOPcc and CRF, indicating that resistance against deformation of the cornea is higher in eyes with higher IOP values. As a consequence of age-related collagen cross-linking, corneal stiffness increases with ageing, whereas simultaneous and gradual diminution of proteoglycan and glycosaminoglycans of the viscous ground substance occurs, resulting in decreased CH. Whether other factors may affect CH and CRF, such as ethnicity, gender, diurnal variation, glaucoma, diabetes, the eyes refractive status, and corneal curvature, remains controversial.
The CH and CRF decreases in keratoconic eyes.\textsuperscript{86, 261, 262} It may be partially due to the decrease in corneal thickness, but may be primarily due to the altered structure of proteoglycans and glycosaminoglycans in keratoconic eyes. As the keratoconus progresses, the proteoglycan content of the stroma increases, whereas fibril diameter is reduced, leading to weakening lateral cohesion.\textsuperscript{97} Still, there is a large overlap between normal eyes and keratoconic eyes in CH and CRF,\textsuperscript{263} and it was demonstrated that CH and CRF alone might not be sufficient to identify keratoconus suspect cornea.\textsuperscript{262}

The CH and CRF decrease after corneal refractive surgery, and greater attempted corrections correlate with greater reductions in CH and CRF.\textsuperscript{264-267} A combination of thickness reduction and change in the viscoelastic properties of the cornea may be responsible for the decrease. In LASIK, the flap creation itself causes a reduction in CH.\textsuperscript{231} Other studies, however, found no difference between the surface ablation procedure and LASIK;\textsuperscript{101, 231, 265} therefore, the authors suggested that it was mainly the tissue removal that accounted for the induced changes.\textsuperscript{101, 268, 269}

Recently, it has been postulated that the shape of the applanation signal (the elevation of the first and second peak, as well as undulation of the signal) may yield important information in addition to CH and CRF,\textsuperscript{269-271} and it should be considered in the interpretation of results. The waveform analysis might have increased sensitivity to biomechanical changes in the cornea. For example, no statistically significant changes in CH and CRF measurements were detected after CXL in patients with keratoconus and post-LASIK ectasia,\textsuperscript{272-274} whereas analysis of the waveform of the ORA signal showed a statistically significant increase of the P2 area after CXL.\textsuperscript{261, 274} The waveform parameters may be useful to differentiate between healthy and diseased biomechanical conditions.\textsuperscript{268, 269} They demonstrated a good ability to distinguish between keratoconus and normal eyes, or between keratoconus and post-LASIK eyes.\textsuperscript{268} Nevertheless, how these parameters represent the biomechanical properties of the cornea is still unknown and further studies are needed to evaluate the biomechanical relevance and clinical importance of these parameters.

Unlike ORA, in which the signal intensity of the reflected infrared light is used for analysis, the CorVis ST utilizes the data acquired from the Scheimpflug camera during the measurement. The measured parameters can be grouped by three distinct phases: first inward applanation, highest concavity (maximal deformation), and second outward applanation. At the two applanation phases, values of length of the flattened cornea (A1L, A2L), time elapsed to reach applanation (A1T, A2T) and the velocity (A1V, A2V) of the cornea at those moments are registered. At the phase of highest concavity, it records the deformation
amplitude (DA) at the corneal apex, the distance of the two apexes (peak distance) of the cornea, radius of curvature, and the time taken to reach it (Figure 8 and Figure 9). At the time of the first applanation, the strength of the air pulse at the time of the first applanation is determined, based on which a calibration factor is used to calculate an IOP value.

Figure 8. The CorVis ST utilizes the Scheimpflug camera to record the dynamic procedure of the corneal response to an air puff. A) The first applanation is achieved. B) The cornea reaches its highest concavity. C) The second applanation is achieved when the cornea rebounds to its original position from the highest concavity.
Figure 9. A representative output of the CorVis ST measurement showing the dynamic deformation amplitude (upper left), applanation length (upper middle) and corneal velocity (upper right) during the course of deformation and recovery, as well as the parameters measured at the time when first and second applanation, and highest concavity is reached (the lower table).

The measurement of CCT, IOP, A1T, and DA with the CorVis ST has been reported to be reliable,\textsuperscript{275-278} while the other parameters demonstrated relatively large deviation. One study showed that in healthy eyes, the IOP measured with CorVis ST did not differ statistically from the Pascal dynamic contour tonometry (DCT; Swiss Microtechnology AG, Port, Switzerland), whereas it provided statistically higher IOP values compared to both Goldmann applanation tonometry and ORA. The difference was influenced by CCT and age, but not affected by corneal curvature or spherical equivalent.\textsuperscript{279} However, another study showed that IOP measured with CorVis ST was on average 2.2 mmHg lower compared to ORA-derived IOPg or IOPcc.\textsuperscript{280} Although both use an air-puff approach, the CorVis parameters showed poor correlation with CH and CRF obtained by ORA measurements in healthy eyes,\textsuperscript{280} indicating that they are fundamentally different.

Shorter A1T and larger DA in CorVis ST measurements may indicate less rigid corneas. Studies showed that the DA is strongly affected by the IOP,\textsuperscript{281} but there are disagreement on whether the DA and AIT are affected by age\textsuperscript{276, 280, 282-284} or diabetes.\textsuperscript{253}

In a recent paper, Hassan et al. carried out a comparison of the corneal biomechanical parameters variation between PRK and LASIK using the CorVis ST; they observed that most of the biomechanical parameters were unchanged one month after LASIK and PRK compared to the preoperative data.\textsuperscript{285} Another study\textsuperscript{286} showed that during the small incision lenticule extraction (SMILE) procedure, the deformation parameters (A1T, A2T, DA, and IOP) did not change after stromal-lenticule creation with FS laser, but changed significantly after the
lenticule extraction. Mastropasqual et al.\textsuperscript{287} reported increased DA and A1T seven days after SMILE procedure; however, at 1 and 3 months, these values did not show statistically significant alterations. The authors thereby hypothesized that a substantial modification of corneal biomechanics occurs in the very first follow-up time after the surgery and that the new biomechanical balance is relatively quickly established. Furthermore, one study compared the corneal deformation parameters after SMILE, laser-assisted subepithelial keratectomy (LASEK), and FS-LASIK reported significant higher DA, and shorter A1T after FS-LASIK compared to LASEK. No significant differences were detected in these two variables between LASEK and SMILE groups, or between the SMILE and FS-LASIK groups.\textsuperscript{288}

The standard Corvis ST parameters are different in groups of KC and normal subjects, but they are widely dependent on IOP and CCT.\textsuperscript{289} When IOP was excluded from analysis, greater DA correlated significantly with thinner CCT in both healthy eyes and KC eyes. In pachymetry and IOP-matched comparison, the DA was statistically greater in KC eyes,\textsuperscript{276, 290, 291} whereas A1T was shorter in KC eyes.\textsuperscript{289, 290} However, they did not reach the level of good predictive accuracy for the detection of keratoconus. Temporal symmetry factor (T\textsubscript{sym}) is the ratio of loading and unloading areas under the corneal deformation against the curve.\textsuperscript{292} T\textsubscript{sym} was shown to be highly sensitive to the CXL treatment in the \textit{in vitro} porcine eye study by Kling et al.\textsuperscript{292} However, an \textit{in vivo} human eye study by Tian et al. did not show a significant difference between the KC group and control group.\textsuperscript{291}

It has been shown that the type of mount used in an experimental setting affected the corneal deformation response to an air puff. The A1T was shorter, and the DA was higher under the same internal pressure in human donor corneas mounted intact as a whole-globe compared to when mounted in an artificial anterior chamber. Therefore, \textit{in vivo} air-puff examinations may be affected by scleral stiffness in addition to the cornea.\textsuperscript{293} In a recent study, the impact of orbital muscles was found to have a greater impact on deformation than the sclera.\textsuperscript{281} Therefore, some parameters focusing on delineating the whole globe motion from the corneal deformation were introduced. For example, the corneal contour deformation (CCD) was introduced as a measure of the recoil effect of the eye caused by the air pulse indentation,\textsuperscript{291} and deflection amplitude as the deformation amplitude corrected for whole eye movement at the highest concavity.\textsuperscript{289}

The corneal dynamic deformation recorded by the CorVis ST has the potential to provide useful information on corneal biomechanics. As the CorVis ST is a relatively new technology, studies with CorVis ST are limited, and the reliability of the measurements needs
to be improved. Further improvements in reliably measured relevant CorVis ST parameters and exploration of the applicability and capabilities of this technique in characterizing corneal biomechanics are warranted.

Optical coherence elastography has also been used for the assessment of corneal biomechanics. Generally, this technique employs a loading device to induce tissue deformation and utilize the OCT-based displacement-detection technique to monitor the dynamic response of the tissue.\textsuperscript{294, 295} Recently, shear wave imaging OCT (SWI-OCT) is utilized to image the low-amplitude Lamb wave (elastic wave whose particle motion lies in the plane perpendicular to the object) that is induced using a focused air-puff device with short-duration and low-pressure air stream.\textsuperscript{296} The phase velocities of the Lamb wave at the major frequency components were quantified, and related to the biomechanical properties (for example, higher stiffness has higher phase velocity at major frequency components). Hence, they were considered to be an effective indicator of corneal elastic properties. Its application in vivo clinical studies remains to be explored.

The above-mentioned measurements involving physical deformation of the cornea are inherently coupled with IOP and other geometrical factors. Furthermore, the measurements of ORA and CorVis ST rely on large magnitude (millimeter-scale) global deformations of the eye, which causes a non-linear corneal response to the mechanical stimulation, leading to inaccurate measurements. Finally, the lack of depth-resolved detection limits the clinical usefulness of these techniques.

A recent technique named Brillouin microscopy claims to be able to determine intrinsic viscoelastic properties decoupled from the structural information and applied pressure.\textsuperscript{297, 298} Brillouin light-scattering is an inelastic scattering process arising from the interaction between incident light and spontaneous acoustic phonons in the sample material. As the propagation speeds of acoustic phonons are related to the material’s mechanical properties, measuring the frequency shift induced by the acousto-optic interaction allows the elastic properties to be determined. Brillouin microscopy measures the frequency shift by employing ultrahigh-resolution spectrometry. The frequency shift $\Omega$ is related to the longitudinal elastic modulus $M'$ by the expression $M' = 1/4 \Omega^2 \lambda^2 (\rho/n^2)$, where $\lambda$ is the optical wavelength in air, $\rho$ is the mass density, and $n$ is the refractive index. Studies by Scarcelli et al.\textsuperscript{299} demonstrated a good log-log linear relationship between the longitudinal modulus $M'$ determined from the Brillouin shift and conventional Young’s (or shear) moduli $E'$ measured at low frequencies: $\log (M') = a \cdot \log (E') + b$, where $a$ and $b$ are material-dependent coefficients. Brillouin scattering microscopy is a novel optical technology that enables three-
dimensional mechanical imaging. Bovine eye\textsuperscript{297} and human cornea studies\textsuperscript{89,300} demonstrated that in healthy corneas, the Brillouin shift was highest in the anterior corneal region and decreased gradually toward the endothelium, with much less variation laterally at the same depth. The anterior cornea showed moderate decay, and the posterior stroma showed steep decline over depth. In the cone region of the keratoconus eyes, the anterior portion had lower Brillouin shift, and the shift decayed in a more rapid fashion across the corneal depth compared to that of the healthy corneas. In addition, much higher cornea-to-cornea variability in terms of Brillouin shift and changes through depth was presented in the cone region of the keratoconus cornea compared with healthy controls. The profiles measured outside the cone region in the periphery were different from those of the cone region, and rather similar to those of healthy corneas. The CXL procedure resulted in a substantial increase of Brillouin modulus in the stroma by 10\%.\textsuperscript{297} The measurement is performed optically without the need for acoustic transducers or physical contact with the cornea, enabling \textit{in vivo} study of the human eye.\textsuperscript{300} One of the limitations of the current Brillouin instruments for use in the clinic is its relatively long acquisition time. A single full axis scan across the eye takes about 1 minute, although the scan time could be reduced to $<10$ seconds at the expense of the scan range or sampling intervals.\textsuperscript{300} The speed improvement in the future will facilitate its clinical application.

With the increased focus on the biomechanics of the cornea in recent years, the development of new instrumentation that might aid in the diagnosis of ocular disease and clinical assessment of corneal biomechanical properties is of great interest. Understanding of corneal biomechanical properties is extremely important to improve clinical diagnostic procedures, as well as to optimize treatment modalities. The development of biomechanical measurement techniques will be of great help in setting up optical models for refractive surgery.

5.5 Keratoconus

5.5.1 Definition, Etiology and Epidemiology

Keratoconus is a disease caused by weakening of the cornea due to abnormalities in its structure and/or composition. The internal pressure in the eye causes the weakened cornea to bulge from its normal shape, and the resultant conical protrusion of the cornea causes significant irregular astigmatism and visual impairment. Keratoconus is usually a bilateral, though often asymmetrical condition. After the initial diagnosis of keratoconus in one eye, it
may take years for the condition to become apparent in the fellow eye.\textsuperscript{301} It has been suggested that the term “forme fruste keratoconus” be used for such less affected fellow eyes that display no clinical findings except for certain topographic changes. In contrast, the term “keratoconus suspect” should be reserved for eyes with suspicious topographic patterns, wherein the fellow eye of the individual does not have keratoconus.\textsuperscript{302}

The prevalence of keratoconus in the general population has been reported to range from 57–229 per 100,000\textsuperscript{170, 303-305} and the incidence is higher among Asians than Caucasians.\textsuperscript{304, 306} Since more young adults are having screening topographies taken upon corneal refractive surgery evaluation, it is likely that the incidence and prevalence of keratoconus in recent years has increased most likely due to improved and earlier diagnostics. The exact etiology of keratoconus is not well understood yet, but it was found to be associated with atopic diseases,\textsuperscript{307} vernal keratoconjunctivitis,\textsuperscript{308} eye rubbing,\textsuperscript{309} floppy eyelids,\textsuperscript{310} Down syndrome\textsuperscript{311} and non-inflammatory connective tissue disorders, such as Ehlers–Danlos syndrome\textsuperscript{312} and osteogenesis imperfecta.\textsuperscript{313} The relationship between the cause and the effect is often unclear in the etiology of keratoconus. Some associations like environmental influence and mechanical trauma may potentially lead to the condition, but others may point towards a common genetically determined cause. A recent article systematically reviewed the current knowledge on genetic risk factors associated with keratoconus and concluded that a number of genetic susceptibility loci are implicated and that there is a genetic heterogeneity rather than a single major gene-effect responsible for development and progression of keratoconus.\textsuperscript{314} An international group of 36 experts published a global consensus on keratoconus and ectatic corneal disease. They concluded that the pathophysiology of keratoconus is likely to include environmental, biomechanical, genetic, and biochemical disorders, but that there is no primary pathophysiologic explanation for keratoconus.\textsuperscript{315}

5.5.2 Pathogenesis

5.5.2.1 Biochemical and inflammatory factors

It has been suggested that an abnormality in the degradative pathway of macromolecules may be responsible for keratoconus. Focus was initially centered on an imbalance between matrix metalloproteinases and their endogenous inhibitors, tissue inhibitors of matrix metalloproteinases. The matrix metalloproteinases are a family of enzymes that degrade a wide variety of extracellular materials, including collagens, proteoglycans, fibronectin, laminin and elastin. Chan and colleagues,\textsuperscript{316} in a recent review, concluded that an up-
regulation of selected matrix metalloproteinases and cathepsins, as well as decreased level of their inhibitors such as tissue inhibitors of matrix metalloproteinases, play a role in corneal thinning in keratoconus. The prominent up-regulated matrix metalloproteinases and cathepsins include matrix metalloproteinase-14, matrix metalloproteinase-13 and cathepsin K, B and G.

Keratoconus has conventionally been held to be a non-inflammatory condition. However, recent studies on cytokines and enzyme levels suggest that inflammation likely plays a greater role in the progression and development of keratoconus than previously assumed. It has been confirmed that rather than a global increase in proinflammatory cytokines, it is a complex imbalance between proinflammatory and anti-inflammatory molecules that disrupts the corneal homeostasis in eyes with keratoconus. Studies that analyzed inflammatory molecules in the tears of patients with keratoconus have found that interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-alpha) are overexpressed, indicating that the pathogenesis of keratoconus may involve chronic inflammatory events.

The up-regulated degradative enzymes and overexpressed inflammatory molecules may result in tissue damage and further weakening in keratoconic cornea, but the source of those components remains unclear. One theory suggests that the components are released upon keratocyte apoptosis, as a reaction to chronic triggers such as ongoing epithelial injury. This theory is consistent with the findings of keratoconus association with contact lens wearing and eye rubbing.

5.5.2.2 Structural and compositional changes

Biomechanical strength of the cornea is maintained by the intricate composition of the collagen network. Anterior stroma seems to have the most important role in keeping the unique shape of the cornea because of the higher degree of lamellar interweaving and a distinct population of lamellae inserting into Bowman’s layer. In eyes with keratoconus, the structure and composition of the collagen network is disrupted. It has been pointed out that the development of keratoconus involves a high degree of inter- and probably intra-lamellar displacement and slippage that leads to stretching and thinning of the central cornea and associated changes in corneal curvature. A marked loss or decrease in anterior lamellar interweaving and the sutural lamellae that are inserted into Bowman’s layer has been detected in keratoconic corneas. Furthermore, a reduction in the overall amount of protein in the keratoconic corneas compared with normal controls has been reported. This is further
supported by Koa and his colleagues, who demonstrated that the total collagen content in keratoconic corneas was about 50% of the normal. In addition, they observed a substantial increase in collagenase activity in keratoconic corneas grown in organ culture. In conclusion, keratoconus is a multifactorial disorder, with environmental, behavioural, and multiple genetic components contributing to its development. However, all of the findings point to essential pathology, which is a reduced mechanical stability of the cornea. A number of studies compared mechanical properties, including rigidity, stress-to-strain and strain-to-failure in extracted corneal tissue, and indicated that the keratoconic corneas are both weaker and less elastic compared to the normal corneas. The loss of biomechanical stability is thought to result in decreased resistance to intraocular pressure, which gives keratoconic corneas their characteristic protruded shape and consequent morphologic and optical changes.

5.5.3 Staging

5.5.3.1 Classic clinical diagnosis and staging

Slit-lamp biomicroscopy still serves as a means for examining gross tissue changes in keratoconus, such as the Fleischer ring (iron deposits around the base of the cone), Vogt’s striae, paracentral corneal thinning and protrusion, rupture in Bowman’s layer, and stromal scars beneath the breaks in Bowman’s layer. In the past, the diagnosis and grading of keratoconus were commonly based on central corneal thickness measurements, keratometric readings, and the degree of myopization, as in the Amsler-Krumeich classification (Table 1). In addition to slit-lamp biomicroscopy, ultrasonic pachymetry and videokeratoscopy were the most commonly used screening tools. The aforementioned global consensus group summarized the definition and diagnostic of keratoconus as follows:

- The findings mandatory to diagnose keratoconus:
  - Abnormal posterior elevation
  - Abnormal corneal thickness distribution
  - Clinically non-inflammatory corneal thinning

- Diagnosis of Keratoconus:
  - Full tomographic corneal thickness map
  - Slit-lamp examination
  - Anterior curvature map
Anterior and posterior elevation maps

Posterior corneal elevation abnormalities must be present to diagnose early or subclinical keratoconus. Central pachymetry is the least reliable indicator (determinant) for diagnosing keratoconus (keratoconus can be present in a cornea with normal central thickness)

- Ectasia progression (defined by a consistent change in at least 2 of the following parameters, where the magnitude of the change is above the normal noise of the testing system):
  - Progressive local protrusion and steepening of the anterior corneal surface
  - Progressive local protrusion of the posterior corneal surface with coinciding location with the anterior protrusion
  - Progressive thinning and/or an increase in the rate of corneal thickness change from the periphery to the thinnest point
  - Although progression is often accompanied by a decrease in CDVA, a change in both uncorrected visual acuity (UDVA) and CDVA is not required to document progression

- **Risk factors for keratoconus**:
  - Down syndrome
  - Family history
  - Ocular allergy
  - Ethnic factors (Arabian and Asian)
  - Mechanical factors, e.g., eye rubbing and floppy eyelid syndrome
  - Atopy
  - Connective tissue disorders (Marfan syndrome)
  - Ehlers–Danlos syndrome
  - Leber’s congenital amaurosis
  - Pregnancy

<p>| Table 1. Amsler-Krumeich Classification for Grading Keratoconus |
|----------------------|---------------------|
| STAGE | FINDINGS |
| 1 | Eccentric steepening |</p>
<table>
<thead>
<tr>
<th></th>
<th>Myopia, induced astigmatism, or both &lt;5.00 D</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Mean central K readings &lt;48 D</td>
</tr>
<tr>
<td>2</td>
<td>Myopia, induced astigmatism, or both from 5.00 to 8.00 D</td>
</tr>
<tr>
<td></td>
<td>Mean central K readings &lt;53.00 D</td>
</tr>
<tr>
<td></td>
<td>Absence of scarring</td>
</tr>
<tr>
<td></td>
<td>Corneal thickness &gt;400 micron</td>
</tr>
<tr>
<td>3</td>
<td>Myopia, induced astigmatism, or both from 8.00 to 10.00 D</td>
</tr>
<tr>
<td></td>
<td>Mean central K reading &gt;53.00 D</td>
</tr>
<tr>
<td></td>
<td>Absence of scarring</td>
</tr>
<tr>
<td></td>
<td>Corneal thickness 300-400 micron</td>
</tr>
<tr>
<td>4</td>
<td>Refraction not measurable</td>
</tr>
<tr>
<td></td>
<td>Mean central K readings &gt;55.00 D</td>
</tr>
<tr>
<td></td>
<td>Central corneal scarring</td>
</tr>
<tr>
<td></td>
<td>Corneal thickness&lt;200 micron</td>
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5.5.3.2 Modern imaging methods

The detection of moderate to severe keratoconus is fairly easy with the classic diagnostic tools; however, a variety of advanced imaging technologies are needed for discriminating eyes with early keratoconus or with risk factors for keratoconus from the normal population or other conditions. Corneal topography, one of the most important diagnostic imaging tools for keratoconus, has evolved from Placido-based- to scanning-slit- and to Scheimpflug-based imaging technology. Wavefront aberrometry also contributed to the early detection of keratoconus by detecting its characteristic optical properties and hence differentiating it from normal eyes. Also, optical coherence tomography (OCT) and OCT-based topography are becoming increasingly more useful in imaging the early indicators of keratoconus. A detailed presentation of modern imaging techniques is made in section 5.4.2 of this manuscript.

5.6 Interventions–Treatment of Refractive Errors and Keratoconus

5.6.1 Corneal Laser Refractive Surgery

5.6.1.1 Overview and history

The excimer laser reshaping of cornea was first introduced to ophthalmology and refractive
surgery over 25 years ago.\textsuperscript{330} Since then, the technique has been under development, and is now considered safe for vision correction. Excimer lasers are gas lasers that emit pulses of light within the UV spectral range. In corneal refractive surgery, the wavelength of 193 nm, obtained by mixing argon and fluorine gases (ArF-excimer), is most widely used. During excimer laser ablation, the UV light produces high-energy photons that break organic molecular bonds within corneal tissue at a precisely controlled x,y position on the corneal surface.\textsuperscript{331} Solid-state lasers are an alternative to excimer lasers, where a solid-state diode emits UV radiation at 210 or 213nm wavelength.\textsuperscript{332, 333}

Excimer laser is capable of “cold cutting” the corneal tissues. It causes a photochemical reaction resulting in the ablation of corneal tissues without thermal damage to the adjacent remaining structures. The method allows incisions of controlled depth and shape to be made in the cornea. A defined volume of a defined shape from a defined area of a cornea can be removed by ablating the tissue to a pre-determined depth.\textsuperscript{330}

To date, excimer laser systems have achieved important advances in the areas of beam profile refinement and homogenization, beam delivery (full beam, scanning slit, and flying spot delivery) and refinements of other laser beam parameters, such as pulse duration, frequency, energy, fluence and ablation rate. Eye tracking and iris recognition systems have been developed to compensate for eye movements during laser treatment (in the X–Y horizontal plane and cyclotorsion on lying supine).

5.6.1.2 Types of excimer laser ablation design
According to the laser ablation pattern design, two main modalities exist, the “standard” and the “custom” ablation (CA). The standard ablation involves central corneal flattening or steepening according to predetermined formula based only on the subject’s spherocylindrical refractive error (LOAs), while CA attempts to reshape the corneal surface to fit the individual eye’s optics and/or anatomy and that way it addresses the HOAs as well. In the current project, CA is based on the information derived from the topography of the anterior corneal surface, so called topography-guided custom ablation (TGCA). In theory, both the LOAs and HOAs of the whole eye could be corrected by using the information derived from the ocular wavefront aberrometry measurements, used in wavefront-guided CA. In practice, however, there are limitations to the latter technology: the eye is a dynamic optical system, while the ocular wavefront measurements are based on only one “snap-shot” of that dynamic system. Furthermore, the measurement range of ocular wavefront aberrometers is limited, making them unsuitable for measurements of larger amounts of aberrations originating from irregular
corneas. The TGCA may, in contrast, be used in highly irregular corneas and in cases with media opacities, as it is based on measurements of the corneal surface (corneal HOAs).

5.6.1.3 Surgical techniques

Surface Ablation

Excimer laser surgery for correcting refractive errors was originally introduced in the form of photorefractive keratectomy (PRK), which is performed after complete removal of the central epithelium using mechanical debridement or alcohol and application of the excimer laser to the denuded stroma for refractive correction (Figure 10). Published long-term outcomes for surface ablation show its safety and predictability in patients with the ranges of low and moderate myopia and thinner corneas. The main drawbacks of PRK are the significant postoperative pain, relatively slow visual recovery and postoperative haze development, especially in treatment of high myopia.

Healing of the cornea is followed by the release of inflammatory cytokines by the damaged epithelium which in turn results in apoptosis, as well as in the proliferation and degeneration of the underlying stromal keratocytes. Histologically, haze appears to be associated with highly reflective myofibroblastic cells and with disorganized collagen deposition of amorphous glycosaminoglycans. Modern PRK-techniques attempt to modulate the corneal wound healing reaction by aiming for a smooth post-ablation surface by use of high laser-frequency, small laser-beam diameter, randomized flying-spot delivery and/or various additional smoothing techniques that seem to be associated with reduced corneal haze. The postoperative application of mitomycin-C has been demonstrated to significantly delay keratocyte repopulation in the anterior stroma and to prevent corneal haze that way.

Various means for pain relief after PRK has also been developed over the years. The use of topical non-steroidal anti-inflammatory drugs (NSAIDs) and small amounts of topical anaesthetics seem to be safe and effective in improving postoperative comfort after PRK. Bandage contact lens use during reepithelialization and surface cooling before and immediately after the laser treatment seem to result in pain relief as well.

Disturbed integrity of the basement membrane underneath the basal epithelial cells has been proposed as a key factor in the corneal repair process and development of haze. Therefore, PRK modifications that preserve the epithelium as a flap were proposed to accelerate visual rehabilitation and reduce induced corneal haze. These treatments include
LASEK, where the corneal epithelial flap is separated manually from Bowman’s layer assisted by alcohol application and replaced after ablation\textsuperscript{352} and Epi-LASIK, where the epithelial flap is produced by an automated mechanical device (epikeratome) with a blunt blade.\textsuperscript{353} However, studies showed that there is no significant difference in refractive and corneal structural outcomes up to one year postoperatively between patients treated for myopia with PRK with MMC and LASEK/Epi-LASIK.\textsuperscript{354, 355}

Other modifications of the PRK procedure have also been attempted to improve the outcome. Transepithelial photorefractive keratectomy (T-PRK) uses an excimer laser to ablate the epithelium before the refractive ablation of the underlying stroma.\textsuperscript{356} The excimer laser epithelial removal in T-PRK can be performed separately by adding a phototherapeutic keratectomy (PTK) before the refractive ablation of the stroma, or as a part of integrated one-step transepithelial refractive procedure. The former has been reported to induce more postoperative discomfort and more intense wound healing in terms of haze and keratocyte apoptosis in the early postoperative period compare to LASEK.\textsuperscript{357} However, the latter was reported to result in faster epithelial healing, lower postoperative pain score and significantly less haze formation compared to alcohol-assisted PRK.\textsuperscript{358, 359} Possible explanations might be that the integrated ablation mitigates the risk of corneal hydration changes between epithelial removal and subsequent stromal ablation steps, as well as due to a smaller deepithelialization area.

In addition, T-PRK is the only type of surface ablation that can successfully deal with the consequences of epithelial remodelling by treating the epithelium and stroma as a single entity in an uninterrupted single ablation. This sets special requirements for the excimer laser with respect to the ablation speed, energy and fluence in order to achieve compatible ablation rate between the epithelium and the stroma. A detailed explanation will be presented in the next session.
LASIK

LASIK was introduced in early 1990s. Shortly after its introduction, it became the dominant procedure in corneal refractive surgery. In LASIK, a microkeratome or an FS laser is used to cut a hinged corneal flap, followed by excimer ablation on the stromal bed and finally repositioning of the flap (Fig 10). Keeping the central corneal epithelium intact increases comfort during the early postoperative period, and reduces the wound healing response, which correlates with a lower rate of haze development. Studies have shown that LASIK is safe, effective, and predictable for treating myopia and myopic astigmatism.

While the microkeratome performs the cutting, the FS laser creates incisions by both cleaving (blunt wedge dissection) and ablation (subtractive dissection). Peak laser energy forms plasma, which causes ablation, then the shock wave with a cavitation bubble causes cleavage. Thousands of laser pulses are connected together in a raster pattern to create a cleavage plane. The lowest possible energy from FS laser that still can produce plasma is preferred to avoid the side effects of heating. Reduction in pulse duration (higher frequency), reduction in beam diameter, and use of shorter wavelengths are preferred to achieve the
smoothest possible FS laser cuts. The application of FS laser to LASIK flap creation has increased greatly since its introduction. It has been suggested that the use of FS laser for flap creation has improved the safety and predictability of the lamellar incision step\textsuperscript{367} and it yields a more uniform interface and more adherent fit to stromal bed,\textsuperscript{368} resulting in a reduced risk of postoperative complications as well as reduced variability in the level of optical scattering.\textsuperscript{369} The use of the FS laser has also reduced the complications such as epithelial ingrowth and flap striae, however, other specific complications have emerged, such as vertical gas breakthrough, opaque bubble layer, and transient light-sensitivity syndrome.\textsuperscript{367}

Although rare, flap or interface complications such as incomplete, decentered, buttonholed or lacerated flaps, improperly replaced free-cap, striae, wrinkles or folds along with diffuse lamellar keratitis (DLK) or recurrent epithelial ingrowth, can lead to visually disturbing irregular astigmatism and/or optical scattering,\textsuperscript{370} thereby resulting in a reduced visual performance such as haloes/glare, multiplopia, starburst, and decreased CDVA.\textsuperscript{114, 371} Treatment of the inferior visual performance occurring with LASIK flap or interface complications remains one of the most difficult challenges in refractive surgery. Since the source of the inferior vision could be either irregular astigmatism caused by the underlying pathology (irregular flap, wrinkles, folds, scarring after DLK, epithelial ingrowths, etc.) or the optical scattering (within the flap, LASIK interface, or both), an ideal treatment will require: 1) Measurement technologies to map the location and the depth of the pathology to be ablated; and 2) Topography-guided treatment technology to regularize the corneal surface and treat the irregular corneal optics with minimal waste of corneal tissue.

Re-lifting of the original flap and performing laser ablation on the original stromal bed, which is the most commonly used and effective method for treatment of residual refractive errors after LASIK, will not be the right approach in cases where the pathology lies within the flap, because that pathology would remain untouched. Various types of traditional surface ablation (that include epithelial removal) on top of the flap have been used for the treatment of LASIK complications.\textsuperscript{372-374} However, they do not take into account one factor that may increase the unpredictability of the outcomes: epithelial remodelling. As described earlier, the epithelium grows thicker over depressed stroma and thinner over elevated stroma in an attempt to re-establish a smoother and more symmetrical corneal optical surface.\textsuperscript{189} This masking effect causes a discrepancy in morphology between the epithelial and stromal surface (Fig 11). Due to the mentioned discrepancy, topography-guided surface ablations, if performed after epithelial removal, will result in erroneous ablation, because the treatment plan is based on preoperative topography measured on the epithelium-covered corneal surface,
while the treatment itself is applied on the stromal surface.

Figure 11. Topography-guided surface ablation in treatment of subepithelial irregularity masked by epithelial remodelling. (a) Epithelium masking a stromal irregularity; (b) Mechanical or alcohol-aided epithelial removal exposes irregular surface; (c) When topography representing the epithelial surface is used as the basis for the treatment planning, and the treatment itself is performed on the stroma after the epithelial removal, a new irregular surface is produced; (d) When the same topography is used for planning of transepithelial ablation that removes both the epithelium and the stroma as a single entity, a regularized surface shape will be “transferred” to the stroma below the irregularity (dotted line).

Transepithelial phototherapeutic keratectomy (T-PTK) will “transfer” the epithelial shape to stroma and eliminate the discrepancy between epithelial and stromal surfaces.\textsuperscript{193}
under the assumption of similar ablation rate between epithelium and stroma. Topography-guided ablation combined with T-PTK, as Custom Transepithelial “no-touch” (cTEN) ablation, will treat the surface irregularities, as well as the possible refractive errors, to achieve the targeted corneal morphology and optics.\textsuperscript{375, 376} Structural imaging technology, such as OCT, which can precisely map the epithelial depth as well as the flap pathology, can further facilitate the topography-guided transepithelial ablation in treatment of post-LASIK corneal irregularities.\textsuperscript{377} In conclusion, a treatment combing T-PTK with topography-guided ablation with the assistance of corneal structural imaging system may be an ideal tool for the treatment of damaged corneal optics following LASIK-flap or interface complications.

Refractive Lenticule Extraction (ReLEx)
FS laser creates corneal incisions, which are lamellar (used for LASIK-flap creation), as well as vertical and curved. As previously mentioned, in recent years, FS laser has largely replaced mechanical microkeratome in LASIK-flap creation due to its high safety, efficiency, precision and versatility in creation of lamellar incisions.\textsuperscript{378}

Lately, a new application of the FS laser, termed refractive lenticule extraction (ReLEx),\textsuperscript{379} has been developed for corneal refractive surgery. In ReLEx, the FS laser is used to create an intra-stromal refractive lenticule. Depending on the technique for the subsequent lenticule removal, ReLEx can be performed by using two procedures: FS lenticule extraction (FLEX) and small-incision lenticule extraction (SMILE). In FLEX which is mostly considered an evolutionary step towards SMILE, a LASIK-like flap is created to access and remove the refractive lenticule. Whereas in SMILE, no flap is created and the lenticule is extracted through a 2 to 4 mm long side cut.\textsuperscript{380-383} (Figure 10)

To create a refractive-accurate lenticule, the accuracy of the FS laser interface cut must be sufficiently high. Currently, the ReLEx procedures can only be carried out with the VisuMax FS laser (Carl Zeiss Meditec AG, Jena, Germany) due to its high reproducibility, regularity and uniformity.\textsuperscript{384-387} Laser parameters (i.e., pulse energy, spot distance, frequency)\textsuperscript{388} and laser-firing patterns\textsuperscript{389, 390} in VisuMax have been refined to improve the surface quality and the optical quality of the lenticules to make procedures like ReLEx SMILE possible. More advanced surgical maneuvers are needed in SMILE compared to FLEX to separate the lenticule from overlying and underlying stroma through a small incision. Still, the SMILE is the procedure of choice with VisuMax due to its concept of keeping the anterior cornea untouched and minimizing trauma to the corneal surface.\textsuperscript{22, 225, 391, 392}
SMILE and LASIK have common advantages of minimal postoperative pain and relatively quick postoperative visual recovery, compared to surface ablation. Therefore, comparisons are frequently made between these two procedures. Unlike in LASIK, where the performance of the excimer laser ablation can be affected by the variation in ambient humidity and stromal hydration change during the surgery, the refractive lenticule cut by FS laser in SMILE is executed prior to any interference with stroma. This may contribute to a greater predictability in refractive outcomes. Long-term refractive and visual outcomes are comparable or in favour of SMILE compared to FS-LASIK; however, the visual recovery after SMILE is usually a little slower than after LASIK. The reason for the delayed recovery is still not clear. In vivo confocal microscopy demonstrated a greater increase of backscattered light intensity after SMILE compared to FS-LASIK at one week, one month, and three months, postoperatively. More micro distortions in Bowman’s layer were also observed by OCT after SMILE than after FS-LASIK. Both the interface scatter and micro distortions in Bowman’s layer may contribute to the slightly reduced visual acuity in the early postoperative period.

One potential limitation of the SMILE technique is that no automatic eye registration/tracking is used during the procedure, which may increase the risk of decentration and induction of higher order aberrations. Lack of torsional alignment control may also reduce the effect of astigmatic correction. By analyzing the pre-/postoperative anterior elevation difference maps, Li et al. reported a mean of 0.17± 0.09 mm decentration with SMILE, which was associated with induction of postoperative coma. Nevertheless, a study by Lazaridis et al. demonstrated that the centration of the treatment zone measured by a Pentacam thickness difference map was better for patient-controlled fixation during SMILE than active eye tracker-assisted FS-LASIK (0.315±0.211 mm vs. 0.516 ± 0.254 mm). Some studies found less induction of HOAs after FLEX and SMILE compared to FS-LASIK, and postoperative contrast sensitivity was reported to be better after SMILE compared to FS-LASIK. Regarding the myopic astigmatism correction, Ivarsen et al. reported a small under-correction of 13% per diopter of attempted correction in low astigmatism, and 16% per diopter of attempted correction in high astigmatism.

Biomechanical tensile strength of the anterior central corneal stroma was found to be nearly twice that of the posterior stroma. With SMILE, preservation of the most superficial stroma is expected to provide the least disturbance to corneal biomechanics, compared to both LASIK and surface ablation. Mathematical model by Reinstein et al. and a finite-element anisotropic collagen fiber-dependent model of myopic surgery by Sinha Roy
et al. demonstrated that the SMILE may cause less biomechanical weakening to the cornea than comparable corrections involving LASIK flaps. Clinical in vivo measurements with ORA failed to detect differences in induced changes in CH and CRF between SMILE and FS-LASIK, or between SMILE and FLEX. However, some studies found less of a decrease of CH, CRF after SMILE than LASIK. Another study comparing the corneal deformation parameters after SMILE, LASEK and FS-LASIK measured with CorVis dynamic Scheimpflug analyzer reported significant higher deformation amplitude, and shorter 1st applanation time after FS-LASIK compared to LASEK, whereas no significant differences were detected in these two variables between LASEK and SMILE groups, or between the SMILE and FS-LASIK groups. It remains to be explored whether the discretions between theoretical model and different clinical measurements were due to the insensitivity of the parameters used for measuring corneal biomechanics or the study population.

Disruption of the corneal nerves is one of the contributing factors for postoperative dry eye. The combination of a large circumferential cut through the corneal surface during flap making with subsequent excimer photoablation of the stroma in LASIK significantly severs the corneal nerves. The decrease in corneal sensation after FS-LASIK and FLEX has been reported to be similar, because they both require the creation of a flap. The substantially shorter side cut used for lenticule extraction in SMILE may lead to better preservation of anterior corneal nerves and reduce the incidence of postoperative dry eye symptoms. The SMILE technique resulted in less anterior stromal nerve plexus disruption and significantly less reduction in corneal sensation compared to FS-LASIK and FLEX. The other dry eye tests, such as tear break-up time (TBUT), Schirmer test, tear osmolarity, and subjective symptoms showed comparable results or in favour of SMILE.

One potential advantage of the SMILE technique is that the removed lenticule allows the possibility of re-implantation at a future time. Pradhan et al. first described endokeratophakia, in which a SMILE lenticule from a myopic patient was implanted into a recipient eye through a small incision to correct hyperopia. Thereafter, Ganesh et al. introduced the technique of cryopreservation of corneal lenticules extracted after SMILE, which can be implanted into an FS laser-created intra-stromal pocket for hyperopic correction.

Perioperative complications of SMILE include minor epithelial abrasions at the incision, difficulties in removing the lenticule, small tears at the incision, cap perforation, central epithelial abrasion, suction loss, and remnant intrastromal lenticule. The postoperative complications include haze, dryness of the corneal surface, interface...
inflammation, interface infiltrates, epithelial ingrowth, and irregular astigmatism.\textsuperscript{421} Loss in CDVA seemed to depend on laser settings (lower spot energy and closer spacing compared to higher spot energy and wider spacing has resulted in higher loss) and the skill of the surgeon.\textsuperscript{421} Although the percentage of eyes with loss in CDVA was relatively high after three months, CDVA was restored to preoperative values in all eyes after approximately one year. Retreatments after SMILE are currently performed with surface ablation, or with LASIK by converting the cap into a flap using a Circle-cut. Topography-guided PRK was used to successfully treat visually disturbing irregular astigmatism after SMILE in some cases.\textsuperscript{422} However, subepithelial haze development in PRK performed on SMILE-cap appears to be a challenge, even in cases with limited ablation depth. The authors suggested an extended time span between SMILE and PRK and perioperative application of mitomycin C in order to reduce the incidence of haze formation. Lately, Donate and Thaeron\textsuperscript{423} also introduced a sub-cap-lenticule-extraction technique that permits the surgeon to use the previously created interface as the new superior plane of the new lenticule for enhancement.

5.6.2 Treatment of Keratoconus

The treatment of keratoconus is aimed at halting the progression of the disease and achieving visual rehabilitation.

5.6.2.1 Conservative treatment

In the early stages, the refractive error in keratoconus can usually be corrected to a satisfactory level by the use of spectacles and/or soft contact lenses. As the disease advances, the changes in corneal shape and consequent irregular astigmatism result in suboptimal visual quality with spherocylindrical correction, necessitating the use of hard contact lenses. Hard lenses may be adequate to provide clear vision at that point, but when the cornea becomes scarred or too steep, as the condition progresses, special contact lens designs becomes a necessity, with so called intralimbal hard lenses or miniscleral lenses for moderate cases and scleral lenses for advanced cases with large, decentered cones.\textsuperscript{424, 425} Other designs include piggyback lenses that involve fitting of a soft lens underneath a hard lens, and hybrid contact lenses, which have a hard lens in the center and a soft skirt. A global consensus group\textsuperscript{315} made the following guidelines for nonsurgical management of keratoconus:

- Guidance should be given to patients regarding the importance of not rubbing one’s eyes, the use of topical anti-allergic medication in patients with allergy, and the use of
preservative-free topical lubricants in the case of ocular irritation to decrease the impulse to eye rub.

- Spectacle correction based on subjective refraction and aberrometry for optical correction in early disease.
- Contact and scleral lenses, although they do not slow or halt progression of keratectasia, are very important in visual rehabilitation in keratoconus:
  - Rigid contact lenses should be used in cases of unsatisfactory vision with glasses or conventional soft contact lenses. Rigid-gas-permeable lenses (RGP) are preferred and should be tried initially.
  - In a patient who has failed RGPs, alternative contact lens options would be: hybrid lens (rigid center, soft skirt); toric, bitoric, and keratoconus design soft contact lens; keratoconus design corneal rigid gas-permeable contact lens; piggy-back; corneoscleral, mini scleral, and semiscleral contact lens; and scleral lens.

5.6.2.2 Surgical options

Intracorneal Ring Segments (ICRS) Implantation
ICRS are implanted in order to improve the irregular astigmatism by “stretching” and levelling of the corneal surface. Clinical studies reported a significant flattening of the central cornea, with improvement in uncorrected and corrected distance visual acuity after implantation of ICRS. An improvement in contact lens-tolerance by regularization of the anterior corneal contour is another benefit that may be obtained from this procedure.

Corneal Transplantation
In advanced keratoconus with deep corneal scarring, very protuberant cones and very thin cornea, where CXL and/or ICRS are not viable and contact lenses not tolerated, corneal transplantation is eventually necessary for visual rehabilitation, especially if the vision in the other eye is significantly impaired as well. Penetrating keratoplasty (PKP), which involves the full-thickness removal of the patient’s central cornea and replacement with a graft, has been successfully used for several decades. Data from the Australian Corneal Graft Registry, one of the largest graft registries, show a 91% successful graft rate 1 year post PKP and a 74% success rate at 5 years for all diagnoses that are normally indicated for corneal graft. Deep anterior lamellar keratoplasty is a surgical procedure that involves removal of the
corneal stroma down to Descemet’s membrane and replacement with a donor cornea.\textsuperscript{435, 436} This technique offers an alternative to PKP with a lower risk of graft rejection, potentially earlier visual rehabilitation, and better wound strength.\textsuperscript{435-437}

**Low Invasive Option - Corneal Collagen Cross-linking (CXL)**

The decrease in rigidity of the keratoconic cornea is attributed to the decrease and disorganization of the collagen network within the stroma. Corneal collagen cross-linking with riboflavin/ultraviolet-A (UVA, 370 nm), introduced a decade ago, is a minimally invasive procedure for the treatment of keratectasia via increasing the mechanical and biomechanical stability of the stromal tissue.\textsuperscript{438-442} The aim of CXL is to halt or slow down the progression of keratoconus by creating additional chemical cross-links between collagen fibrils and other extracellular matrix proteins in the corneal stroma through localized photo polymerization.\textsuperscript{443-445} Lately, CXL has also been combined with surgical optical corrections, such as ICRS and photorefractive keratectomy (PRK), in order to halt the development of keratoconus and at the same time also to improve visual acuity rather than just preserve the current state.\textsuperscript{446, 447}

In CXL, exposure of the riboflavin to UVA-irradiation results in absorption of energy and its excitement into a triplet state that undergoes either an aerobic, type 2 reaction, or an anaerobic, type 1 reaction.\textsuperscript{448} According to Kamaev and colleagues,\textsuperscript{448} an oxygenated environment causes the formation of singlet molecular oxygen, which then acts on tissues to produce additional cross-linked bonds. After a quick consumption of oxygen, which occurs only within several seconds, depending on UV-power and temperature, among other factors, it is suggested that the main photochemical kinetics mechanism is the direct interaction between the riboflavin triplets and reactive groups of corneal proteins, which leads to cross-linking of the proteins, mainly through radical reactions.\textsuperscript{448} These then induce the formation of new covalent bonds between the amino acids among the neighbouring collagen molecules\textsuperscript{449, 450} and among proteoglycan (PG) core proteins, as well as limited linkages between collagen and PG core proteins.\textsuperscript{451} Concurrently, riboflavin also offers a shielding effect to the deeper ocular structures, such as the corneal endothelium, the lens and the retina.\textsuperscript{452}

The “standard protocol” of CXL described by Wollensak and colleagues\textsuperscript{450} consists of removal of the corneal epithelium in a diameter of 9 mm, and saturation of the cornea by applying drops of 0.1% isotonic riboflavin solution in 20% dextran solution. The cornea is then exposed to UVA light (365 nm) with an irradiance of 3 mW/cm\textsuperscript{2} for 30 minutes, which
corresponds to a total radiant exposure of the cornea of 5.4 J/cm². The corneal epithelium with its tight junctions and hydrophobic character is the most important barrier to penetration of hydrophilic macromolecules like riboflavin. Thus, with the conventional CXL, removal of the intact corneal epithelium before the application of riboflavin is considered crucial to enable sufficient intrastromal diffusion of riboflavin.

The safety and efficacy of the conventional CXL has been confirmed by numerous studies. However, the epithelial removal seems to be responsible for most of the complications reported to date with CXL. To avoid potential complications due to epithelial removal, Boxer-Wachler and Pinelli suggested a modification of the technique with the epithelium kept intact (epithelium on). Substances such as benzalkonium chloride, ethylenediaminetetraacetic acid (EDTA) and trometamol, especially when combined, enhance epithelial permeability of hydrophilic macromolecules, such as riboflavin. By adding enhancers to help riboflavin penetrate into the corneal stroma through the intact epithelium, CXL can be performed without epithelial debridement (transepithelial CXL). In a non-randomized comparative study, Pinelli and colleagues reported no significant difference in the analyzed parameters between the “epithelium-on” group and the standard one. Transepithelial CXL has been proposed (but not proven) to reduce early postoperative pain, temporary worsening of vision, as well as complications such as infectious keratitis after conventional CXL. Additionally, thinner corneas may be treated safer by transepithelial compared to the conventional CXL, since the endothelium is better protected by UVA-filtering effect of the intact epithelium. Meanwhile, strong doubts were raised concerning the efficacy of the transepithelial (“epithelium-on”) CXL.

The critical limitation of CXL in thin corneas is the lack of sufficient corneal thickness for the UVA-radiation to be absorbed and attenuated before it reaches the endothelium. The cell damage threshold of UVA-irradiation combined with riboflavin is 10 times higher than with UVA-irradiation alone. Wollensak et al. demonstrated that when the combination of UVA and riboflavin is used in corneas thinner than 400 µm, the cytotoxicity threshold of 0.35 mW/cm² for the endothelial cell damage can be reached. In conventional CXL procedure, the treatment parameters (0.1% riboflavin- dextran 20.0% solution and 3 mW/cm² of UVA for 30 minutes) are assumed to treat the anterior 300 µm of the corneal stroma. Hence, only patients with a de-epithelialized corneal thickness of at least 400 µm are subjected to this treatment. The downside of this limitation is that eyes with advanced stages of keratectasia often have corneas thinner than 400 µm. Populations of Asian and African origin with inherently thinner corneas may be especially affected by this limitation. Various
modifications have been suggested to circumvent that, including application of hypoosmolar riboflavin solution,\textsuperscript{471} transepithelial CXL,\textsuperscript{464,472} CXL with custom epithelial debridement,\textsuperscript{473} and contact lens-assisted CXL.\textsuperscript{474}

\textit{Conventional Collagen Cross-linking}

Kymionis et al.\textsuperscript{475} applied conventional CXL procedure in 14 thin corneas with minimum corneal thickness of less than 400 $\mu$m (range 340-399 $\mu$m) after epithelial removal. Improvement in UDVA, CDVA, and reduction in mean keratometry readings were recorded during the 12 months follow-up. However, despite the absence of clinically evident complications, significant reduction of endothelial cell density from 2733 to 2411 cells/mm\textsuperscript{2} was observed postoperatively. The film of 0.1\% isoosmolar riboflavin with 20\% dextran was measured to be approximately 70 $\mu$m thick after 1 minute of instillation and remained stable for 22 minutes.\textsuperscript{476} With the riboflavin-dextran film, the UVA irradiance in human corneal stroma at 400 $\mu$m was measured as 0.21 mW/cm\textsuperscript{2}, which is much lower than the previously mentioned cytotoxicity level on which the set limitation of minimal deepithelialized stromal thickness of 400 $\mu$m is based. Hence, the absorption and shielding of UVA by the riboflavin film may have prevented damage to the endothelium. Nevertheless, a longer follow-up and larger patient series are essential to evaluate the safety and efficacy of conventional CXL in clinical application in thin corneas.

\textit{Hypoosmolar Riboflavin Solution}

The cornea has an inert swelling pressure,\textsuperscript{477} meaning that the corneal stroma has the tendency to increase its volume in an isooncotic environment. The deepithelialized cornea can swell to double its normal thickness when irrigated with a hypoosmolar solution.\textsuperscript{478} Hafezi and co-workers\textsuperscript{479} applied this method to increase corneal thickness before CXL in thin corneas. After epithelial removal, 0.1\%-20\% dextran isoosmolar riboflavin was applied to the cornea for 30 minutes. The 0.1\% dextran-free hypoosmolar riboflavin was then administered until the corneal thickness at the thinnest point reached 400 $\mu$m, before the initiation of UVA irradiation. The authors reported a stabilization of keratectasia in 20 eyes treated with this approach. A later study by Raiskup et al.\textsuperscript{471} applied 0.1\% hypoosmolar riboflavin after epithelial debridement until the riboflavin saturated cornea reached the minimum of 400 $\mu$m. In this study, one year after the treatment, CDVA and keratometric value remained unchanged and no damage to the cornea in the form of detectable scarring lesions in the stroma was registered. Similar results were reported by Wu et al.\textsuperscript{480} On the contrary, in eyes treated with
isoosmolar riboflavin solution only, a permanent stromal scar tended to develop in thin corneas after CXL. Gu et al. used 0.1% hypoosmolar riboflavin solution as saturation and swelling solution in 8 thin corneas that underwent the CXL procedure. They reported a slight decrease of endothelial cell density 3 months after the treatment.

The preoperative swelling of the cornea broadens the spectrum of CXL indications to thinner corneas. However, Hafezi and colleagues reported a case where CXL could not stop the progression of keratoconus in a very thin cornea (minimal thickness of 268 µm after removal of the epithelium), despite the fact that swelling with hypoosmolar riboflavin solution increased the thickness to 406 µm and no adverse endothelial reaction was observed postoperatively. The authors, therefore, hypothesized that there is a minimal, yet to be determined stromal thickness necessary for effective CXL to occur. They suggested a minimal stromal thickness of 330 µm or more before swelling, when using hypoosmolar riboflavin solution.

Kaya et al. and Soeters et al. performed intraoperative corneal thickness measurements during CXL with hypoosmolar riboflavin solution in thin corneas. The authors found that the artificial swelling effect was transient, and the thinnest pachymetric readings decreased significantly after 10 and 30 minutes of isoosmolar riboflavin (with dextran) application, with or without UVA irradiation. They inferred that the reduction of the corneal thickness was induced by the hyperoncotic effect of the dextran. Concurrently, lower absorption and shielding effect of the thinner hypoosmolar riboflavin film on the cornea, by application of the hypoosmolar riboflavin without dextran alone, would increase irradiance level in the stroma, putting the endothelium at higher risk. Therefore, the cornea should be swollen to a thickness greater than 400 µm or concentration of riboflavin in the hypoosmolar solution could be increased. It was therefore suggested that the development of new riboflavin solutions with isooncotic properties to create a stable film could increase the safety of CXL. Moreover, a lack of evaporation resistance provided by the corneal epithelium, and/or an increase in endothelial pump activity may also contribute to corneal thinning. It was proposed that removal of the lid speculum during riboflavin saturation, and the use of irradiating devices with shorter irradiation time (and higher power) might be advantageous. Monitoring the corneal thickness throughout CXL treatment could also be important. CXL can be expected to have less of an effect on the biomechanics of artificially swollen corneas due to the lower relative concentration of collagen in the hydrated stroma. Long-term follow-up studies addressing this issue are warranted.
Transepithelial Collagen Cross-linking

In a bilateral study, Filippello et al. used trometamol and sodium EDTA as enhancers and applied transepithelial CXL in 20 keratectatic eyes with a mean corneal thickness (including epithelium) of 412±21 µm. The transepithelial CXL treatment appeared to halt the progression of keratoconus in all treated eyes over 18 months follow-up. It also yielded statistically significant improvements in all visual and topographic outcome measures, whereas the contralateral untreated eyes demonstrated worsening of all parameters. Spadea et al., who used a similar protocol in thin corneas, confirmed its effect in the stabilization of keratoconic eyes. However, the visual and topographic improvement was minimal. No endothelial cell damage was observed in either of the studies.

Wollensak et al. estimated a 64% increase in corneal rigidity in human corneas with transepithelial CXL using topical anesthetics and benzalkonium chloride as enhancers, versus a 320% increase when using CXL with de-epithelialization. The safety and reproducibility of the study by Filippello et al. have recently been questioned since the postoperative demarcation line depth in their study was only approximately 100 µm, in contrast to about 300 µm in conventional CXL with epithelial debridement. It is unclear whether the shallower demarcation line using the transepithelial approach was due to limited penetration of riboflavin into the stroma or that it was a result of reduced UVA-light penetration by shielding from riboflavin-impregnated intact corneal epithelium. Iontophoresis-assisted transepithelial CXL, using a non-invasive delivery system based on a small electric current, was recently designed to enhance the penetration of riboflavin into the corneal stroma. Preclinical results showed that the iontophoresis was able to increase the concentration of riboflavin in the corneal stroma compared to enhancer-assisted transepithelial CXL, but did not reach concentrations previously reached with conventional epithelium-off CXL. The demarcation line after iontophoresis-assisted transepithelial CXL appeared to be less easily distinguishable and shallower than in conventional CXL; however, it demonstrated features more similar to that after conventional CXL in terms of depth and visualization, compared to enhancer-assisted transepithelial CXL. In general, there is consensus within the scientific community that the current transepithelial CXL protocols are not as effective as conventional epithelium-off CXL.

Custom Epithelial Debridement Technique

Kymionis et al. performed CXL with custom pachymetry-guided epithelial debridement in one keratoconic eye and one post-LASIK keratectatic eye with the thinnest stroma of less than
400 µm. In this modified CXL approach, 8.0 mm diameter of corneal epithelium was removed, leaving a small, localized area of corneal epithelium corresponding to the thinnest area over the apex of the cone. The authors suggested the use of hypoosmolar riboflavin during UVA-irradiation to avoid corneal stromal dehydration as well as to maintain the stromal riboflavin concentration. Nine months postoperatively, the topography remained stable, and no endothelial cell density alteration was detected in the treated eyes. However, a later study by Kaya et al. suggested that the epithelium over the cone area spared the stroma underneath from the CXL effect. Four weeks after the treatment, stromal haze and demarcation line were detected in the corneal areas with epithelial debridement, but not in the areas with intact epithelium; de-epithelialized stroma outside the cone region displayed total keratocyte apoptosis and honeycomb-like edema, whereas it was minimal beneath the intact epithelium. In contrast, Mazzotta et al. demonstrated keratocyte apoptosis at an average depth of 160 µm under the epithelial island compared to 250 µm under the de-epithelialized area in 10 eyes with a 1-year follow-up.

A previous study demonstrated that the stromal uptake of riboflavin after grid pattern full-thickness epithelial debridement was heterogeneous, with full penetration to the stroma immediately beneath the areas of epithelial debridement and no penetration to the stroma beneath the intact epithelium. Inadequate riboflavin saturation together with the ability of the epithelium to absorb the UVA radiation may lead to reduced CXL effect in the cone area and affect the efficacy of the whole procedure. The long-term efficacy of this modified CXL procedure with a larger number of patients needs to be assessed.

Contact Lens-assisted Collagen Cross-linking

Contact lens-assisted CXL (CACXL) was introduced by Jacob et al. A Soflens daily disposable soft contact lens (14 mm diameter, 8.6 mm basal curvature; Bausch & Lomb) with a thickness of 90 µm, made of hilafilcon and without a UV filter, was immersed in isoosmolar riboflavin 0.1% in dextran for 30 minutes, before it was applied onto the de-epithelialized, riboflavin-saturated cornea. The UVA-radiation of 3.0 mW/cm² for 30 minutes was initiated after confirmation that the minimum corneal thickness including the contact lens and riboflavin film was greater than 400 µm. The riboflavin solution was instilled every 3 minutes during the UVA-radiation to maintain corneal saturation and to keep the pre-corneal and pre-contact lens riboflavin film uniform. The pre-corneal riboflavin film with contact lens created an absorption medium in the pre-corneal space by artificially increasing the thickness of the “riboflavin-filter”.

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In the 14 eyes treated with CACXL, the authors reported an average increase of the minimum corneal thickness by 108 µm if the contact lens and riboflavin film were included. At a mean follow-up time of 6.1±0.3 months (range: 6-7 months), the mean postoperative depth of the stromal demarcation line was measured at 252.9 µm. No significant endothelium loss or signs of postoperative endothelial damage were observed. No significant change in the CDVA, or mean maximum keratometric value was detected postoperatively, although 1 D decrease of maximum keratometric value was observed in 4 eyes (28.5%).

The advantage of CACXL is that it is not dependent on the swelling properties of the cornea and that the cornea is not subjected to edema, which may cause Descemet membrane folds and endothelial damage. However, the surface irradiance at the level of the corneal stroma is reduced by 40% to 50% in CACXL secondary to absorption by the riboflavin film and soaked contact lens. Furthermore, oxygen diffusion, which has been demonstrated to be crucial in the CXL process, might be hindered by the contact lens. As a result, the effect of CXL may be reduced. The small patient population, short follow-up and absence of a control group are the limitations of the study.

The overall safety of the protocols for CXL in thin corneas presented above is good, as most of them managed to halt the progression of keratectasia without postoperative complications. Furthermore, modification of the tocicity and concentration of the photosensitizing riboflavin and modification of the UV energy and/or power have been proposed. Iseli et al. suggested that a higher riboflavin concentration may be applied for improved protective screening of the endothelium in thin corneas. Accelerated CXL (UVA irradiation at 30 mW/cm² for 3 minutes) has recently been reported to stabilize the progression of keratoconus in 34 thin corneas, without endothelial cell density loss during the 12 months of follow-up. Furthermore, in accelerated CXL, pulsed UV light seems to result in a higher effect compared to continuous UV light, presumably due to the optimization of oxygen availability. Oxygen concentrations measured in the corneal stroma showed that the certain combination of “on” and “off” time would facilitate the continuous replenishment of oxygen, leading to an increased CXL effect without the necessity to increase UV energy. Thus, using the pulsed mode during UVA irradiation may maximize the efficacy of CXL, while maintaining or improving the safety profile of the procedure. This may be especially beneficial in treating thin corneas.

The evidence of safety and efficacy regarding the use of modified CXL protocols is still limited to a handful of studies. Future long-term follow-up studies with a larger number of participants are warranted.
5.6.3 CXL in Combination with Excimer Laser Custom Ablation

Labiris et al. 507 studied the negative impact of keratoconus on quality of life. They reported that keratoconus deteriorates vision even in stage one, with or without decreased best-corrected visual acuity due to coma-dominant irregular astigmatism, and light scatter. Therefore, besides the corneal biomechanical instability, the visual impact of the irregular astigmatism must be taken into consideration in the treatment of keratoconus.

Customized ablation has been shown to be promising in reducing irregular astigmatism and improving visual acuity in non-keratoconic eyes. 376, 508 It is generally considered that keratoconus or subclinical keratoconus are absolute contraindications to LASIK, 509 whereas some studies demonstrated that PRK in selected cases of latent and forme fruste keratoconus to a maximum grade two was safe and effective. 510-515 Khochtali et al. 516 reported a case where one eye of a patient underwent PRK, while keratoconus developed in the untreated eye. They postulated that: 1) the ablation of the central anterior stroma and Bowman’s layer, where histopathologic alterations are prevalently seen in keratoconus, may halt the disease; and 2) the excimer laser ablation of the cornea may have a similar stiffening effect to corneal collagen cross-linking. Nevertheless, concerns remain that tissue removal by PRK may further decrease the biomechanical strength of the already weakened keratoconic cornea, increasing the risk for progression of the disease. Since CXL has been shown to successfully retard or eliminate the progression of keratoconus, a combined treatment may achieve both optical regularization and biomechanical stabilization of the cornea.

The PRK and CXL can be performed separately with some time interval or simultaneously at the same day. Kanellopoulos et al. 517 compared CXL with subsequent topography-guided PRK performed 6 months later, with the CXL and PRK performed in a combined procedure on the same day. The authors concluded that same-day simultaneous topography-guided PRK and CXL performed superiorly with a better CDVA, greater spherical equivalent and keratometric reduction, and less corneal haze. Another two factors make a simultaneous combined procedure appealing: 1) the patients can avoid two separate surgeries and 2) subsequent PRK ablation of the previously cross-linked anterior cornea is avoided. 517

The simultaneous combination of PRK with CXL proved to result in better UDVA, lower refractive error, and lower keratometric values than the CXL alone. 518 Furthermore, the incidence of haze was reportedly minimal after this procedure, 519 due to depopulation of
keratocytes in the cross-linked anterior stroma, which may reduce the possibility of haze formation.\textsuperscript{520, 521}

PRK before CXL generally includes epithelial removal by using 20\% alcohol,\textsuperscript{517} a surgical brush,\textsuperscript{519} or an excimer laser,\textsuperscript{518, 522} and laser ablation itself with\textsuperscript{517, 519} or without\textsuperscript{522} the application of Mitomycin-C 0.02\% at the end. A limit of maximum ablation depth of around 50 \(\mu\)m is considered to ensure enough residual stromal thickness for a safe subsequent CXL procedure.\textsuperscript{517, 521, 523, 524}

Preliminary results suggest that when certain precautions are met, the safety and efficacy profiles of the simultaneous combined treatment of PRK with CXL are excellent.\textsuperscript{517-519, 521, 522, 525, 526} This technique is also applied to post-LASIK ectasia and pellucid marginal degeneration,\textsuperscript{527, 528} after excimer laser-assisted lamellar keratoplasty for keratoconus,\textsuperscript{529} immediately after\textsuperscript{530} or sequentially\textsuperscript{525, 531} after the implantation of ICRS.

The aforementioned global keratoconus consensus group summarized their recommendations for the surgical treatments of keratoconus as follows:\textsuperscript{315}

Different Case Scenarios:

- Young (e.g., 15-year-old) patient with \textit{stable} keratoconus with satisfactory vision with glasses: Prescribe glasses only or in combination with contact lenses or CXL

- Young (e.g., 15-year-old) patient with \textit{progressive} keratoconus with satisfactory vision with glasses: Perform CXL and prescribe glasses or contact lenses

- Older (e.g., 55-year-old) patient with \textit{stable} keratoconus with satisfactory vision with glasses: Prescribe glasses only or with contact lenses.

- Older (e.g., 55-year-old) patient with \textit{progressive} keratoconus with satisfactory vision with glasses: Perform CXL only or with prescription of glasses/contact lenses.

- Patient with \textit{stable} keratoconus with unsatisfactory vision with glasses but satisfactory vision with rigid contact lenses and tolerates them well: Prescribe contact lenses (including scleral lenses).
• Patient with stable keratoconus with unsatisfactory vision with glasses and contact and scleral lenses, or who does not tolerate contact or scleral lenses: Perform lamellar keratoplasty. Consider ICRS in eyes with adequate corneal thickness and minimal to no scarring.

• Patient with stable severe keratoconus with unsatisfactory vision with glasses and contact and scleral lenses (this patient has moderate anterior corneal scarring but no evidence of previous corneal hydrops): Perform lamellar keratoplasty

• Patient with severe keratoconus with unsatisfactory vision with glasses and contact and scleral lenses (this patient has moderate anterior and deep corneal scarring with evidence of previous corneal hydrops): penetrating keratoplasty.
6. AIMS OF THE PRESENT STUDY

6.1 Overall Aims of the Study

The primary overall objectives of the study were to (1) evaluate the effect of transepithelial TGCA in the treatment of refractive errors; (2) the effect of transepithelial TGCA in the treatment of irregular astigmatism; and (3) the combined treatment of transepithelial TGCA and CXL in optical regularization and biomechanical stabilization of keratoconus.

6.2 Aims of the Individual Studies

Study I  To explore the change in corneal asphericity after excimer laser treatments by comparing wavefront optimized and custom-Q ablations.

Study II  To assess the change of epithelial thickness profile after transepithelial TGCA treatment for myopia.

Study III To assess the change of epithelial thickness profile in keratoconic eyes after the combined procedure of central corneal regularization with transepithelial TGCA and accelerated CXL in the treatment of keratoconus.

Study IV  To evaluate the safety and efficacy of transepithelial TGCA in the treatment of myopia.

Study V  To evaluate the safety and efficacy of transepithelial TGCA in the treatment of moderate to high astigmatism.

Study VI  To evaluate the efficacy and safety of transepithelial TGCA in treating visually disturbing irregular astigmatism and/or scattering caused by various LASIK-flap/interface complications.

Study VII To assess the biomechanical properties in virgin eyes and eyes after transepithelial TGCA.
**Study VIII**  To evaluate the safety and efficacy of the “epithelium-on” CXL in arresting keratoconus.

**Study IX**  To evaluate a combination of topography-guided custom ablation and CXL in a single procedure for the treatment of keratectasia.
7. MATERIALS AND METHODS

7.1 Patients

The participants recruited in the studies were patients searching for/undergoing refractive surgery at SynsLaser Clinic, Tromsø/Oslo, Norway (Papers 1, 2, 4, 5, 6, and 7), and keratoconus patients who underwent “epithelium-on” CXL, or a combined procedure of transepithelial TGCA and CXL at the University Hospital of North Norway, Tromsø, Norway (Papers 3, 8, and 9).

Inclusion criteria for studies 1, 2, 4, 5, and 7 were: age ≥ 18 years; no soft contact lens wear for 1 week (hard contact lens for 4 weeks) before the baseline examination; SE between -0.5 and -10.00 diopters (D) with ≤ 6.00 D of refractive astigmatism; stable refractive error (change of SE ≤ 0.50 D) for ≥ 2 years; and CDVA of 20/25 or better. Exclusion criteria were: eye pathology, including keratoconus or keratoconus suspect (detected by corneal topography); previous eye surgery; glaucoma; diabetes; and systemic diseases that could affect corneal wound healing (e.g. collagen vascular diseases).

For study 6, the inclusion criteria were: symptomatic eyes with LASIK flap/interface complications, residual stromal thickness ≥ 300 microns and no topographic signs suggestive of keratectasia. Nine of these eyes had previous unsuccessful retreatments by commonly used techniques such as flap-re-lift or re-cut, followed by wavefront guided custom ablation. Inclusion criteria for studies 3, 8, and 9 were: 1) documented progression of keratoconus during the last 12 months before treatment (increase of astigmatism or myopia by 1.00 D or increase in average SimK by 1.50 D); and 2) minimum corneal thickness of no less than 400 μm at the thinnest point measured by ultrasound pachymetry. Exclusion criteria were: 1) history of herpes virus keratitis; 2) severe dry eye; 3) concurrent corneal infections; 4) previous ocular surgery; and 5) hard contact lens wear ≤ 4 weeks before the baseline examination.

7.2 Surgical Techniques

7.2.1 Transepithelial Topography-Guided Custom Ablation

The Corneal Interactive Programmed Topographic Ablation software (CIPTA, iVIS Technology, Taranto, Italy) was used to generate transepithelial custom ablation plans for each eye based on subjective refraction and corneal topography measured with the Precisio topo/tomographer (Precisio, iVIS Technology, Taranto, Italy). The optical zone size of the
ablation was suggested by the pMetrics dynamic pupillometry (iVIS Technology, Taranto, Italy). Preoperative topography was also used to customize transition zone size to provide a smooth transition between the ablated and non-ablated areas of the cornea. The CIPTA-planned transepithelial ablation consisted of refractive and lamellar components; the function of the latter was removal of the epithelium. The refractive component was derived by the intercept between a desired postoperative regular aconic surface and the preoperative corneal topography; with the tissue above the intersection to be ablated. The desired postoperative regular aconic surface represented a resolution of the vectors of the manifest and the preoperative corneal astigmatism. The default value for the lamellar component was 52µm, which could be adjusted based on preoperative measurements of epithelial thickness and/or clinical judgment. The refractive and lamellar components of the procedure were combined and executed as a single, uninterrupted ablation. The ablations were centered on the corneal vertex.

The ablation plan is executed by a 0.6 mm dual-flying-spot 1KHz (2 x 500Hz) excimer laser system (iRES, iVIS Technology, Taranto, Italy). Among others the laser employs automatic intra-operative illumination adjustment, so the light intensity is automatically modulated to achieve the pupil size registered during the acquisition of topography. This contributes to precise registration along with the iris/scleral vessel-based dynamic cyclotorsional-, in addition to synchronous x,y-pupil-tracking. After registration of the iris and scleral vessels by the laser’s x,y and cyclotorsional tracker, the corneal surface was gently dried with a lint-free, pre-soaked merocel to achieve a reflective, homogeneous, “dry” surface, upon which the laser ablation was performed. After the ablation, Mitomycin C 0.02% was applied to the cornea for 15 in eyes which maximum stromal ablation depth exceeding 100 µm. At the end of the surgery, 1-2 drops of Dexamethasone with Chloramphenicol mixture (Spersadex med kloramfenikol, Laboratoires Thea, Clermont-Ferrand, France) and 1 drop of Bromfenac 0.9% (Yellox, Croma-Pharma GmbH, Leobendorf, Austria) eye drops were applied, followed by a bandage contact lens (Acuvue Oaysis, Johnson & Johnson Vision Care, Inc., FL, USA). Bromfenac 0.9% BID was used 2 days before and 3 days after the surgery. Dexamathasone with Chloramphenicol QID was used the first 2 weeks, and then replaced by a low potency steroid Rimexolone 1% (Vexol, Alcon Laboratories, Surrey, United Kingdom) eye drops in tapering doses for another 3 weeks. The bandage contact lens was removed from the cornea between postoperative days 5 and 7.

7.2.2 Epithelium-On Corneal Collagen Cross-linking
To reduce the risk for UV exposure of retroiridal eye structures, miosis was induced by applying two drops of pilocarpine 2% (Pilokarpin, Ophtha AS, Norway). It was followed by the application of two drops of local anesthetic proparacaine 0.5%, (Alcaine, Alcon Norway AS), two drops of local antibiotic gentamycin 0.3% (Garamycin, Schering-Plough AS, Norway) followed by proparacaine again, with one drop every minute for five minutes. All drops were preserved by BAC (0.001% for Pilokarpin, 0.005% for Garamycin and 0.01% for Alcaine), aiming to increase the epithelial permeability by chemically disrupting the tight junction proteins. A round Merocel sponge (Medtronic, Inc., Minneapolis, MN) 5 mm in diameter was inserted into the conjunctival sac to provide a depot of riboflavin, and to produce micro-abrasions of the superficial epithelial layers caused by friction upon patient blinking. Thereafter, two drops of proparacaine and two drops of 0.5% aqueous riboflavin solution without dextran (Vitamin B2; Streuli, Uznach, Switzerland) were applied alternating every 30 seconds, until the riboflavin saturation was verified by the slit-lamp inspection of the cornea and by the determination of the presence of riboflavin-flare in the anterior chamber (Figure 12). Under the same examination, the staining of the epithelial micro-abrasions was verified. The initial slit-lamp saturation evaluation was performed 25 minutes after the first application of riboflavin and repeated every five minutes until the saturation was confirmed. During the premedication and riboflavin induction time, the patient was instructed to blink normally between eye drop instillation and to remain in a comfortable sitting position. The Merocel sponge was then removed and corneal thickness measured with ultrasound pachymetry, at which point the patient was placed in supine position. Irrigation with isotonic balanced salt solution (BSS) was performed before the UVA-irradiation in order to avoid the shielding effect of riboflavin covering the epithelium. An eyelid speculum was then inserted and a ring-shaped Merocel shield k20-5021 (Katena Products, Inc. Denville, NJ) was applied to protect the limbal region and its stem cells from UVA radiation.

The cornea was subjected to UVA radiation for 30 minutes with a wavelength of 365 nm at a working distance of 5 cm. The UV-X lamp (IROC AG, Zürich, Switzerland) provided an irradiance of 3 mW/cm² within a circular diameter of 9 mm. During the irradiation, BSS was applied every three minutes and proparacaine drops were added as needed. After the UVA irradiation, two drops of atropine 1% (Atropin minims, Chauvin, England) and 2 drops of gentamycin were applied. The cornea was protected with a soft bandage contact lens for 12-18 hours. Instructions were given to apply a mixture of 0.1% dexamethasone and 0.5% chloromycetin (Spersadex med Kloramfenikol, Novartis, Norway) eye drops four times daily for seven days, as well as to use artificial tears as needed.
7.2.3 Combined Topography-Guided Transepithelial Custom Ablation and Corneal Collagen Cross-linking

The TGCA part was performed as mentioned above. Immediately after ablation, the ultrasound pachymetry measurement was taken and the stroma was saturated by topically applied 0.17% riboflavin-5-phosphate, using one drop every 3 minutes. In cases where the measurements showed values <400 µm despite an estimated residual corneal thickness of at least 400 µm, the swelling with hypotonic 0.25% riboflavin solution was applied to induce a slight corneal edema until a thickness of 400-µm was achieved. The UVA light irradiation was then initiated with 18 or 12 mW/cm² power with effective irradiation time of 5 or 7.5 minutes, using either high intensity UVA illuminator (Peschke CCL-VARIO Meditrade GmbH) or KXL system (Avedro, Inc., MA). In both cases, the UV-radiation zone size was 9 mm. At the end of the surgery, 1–2 drops of a Dexamethasone with Chloramphenicol mixture (Spersadex med Kloramfenikol, Laboratoires Thea, Clermont-Ferrand, France) and 1 drop of Bromfenac 0.9% (Yellox, Croma-Pharma GmbH, Leobendorf, Austria) eye drops were applied, followed by a bandage contact lens (Acuvue Oaysis, Johnson & Johnson Vision Care, Inc., FL). Bromfenac 0.9% BID was used 2 days before and 3 days after the surgery. Dexamethasone with Chloramphenicol QID was used in the first 2 weeks, and then replaced by a low potency steroid Rimexolone 1% (Vexol, Alcon Laboratories, Surrey, UK) eye drops in tapering doses for another 3 weeks. The bandage contact lens was removed from the cornea between postoperative days 5 and 7.

7.3 Clinical Measurements
7.3.1 General Ophthalmologic Evaluation

All patients underwent complete ophthalmologic evaluation pre- and postoperatively, including slit lamp biomicroscopy, Scheimpflug-based corneal topo-/tomography (Precisio, iVIS Technology, Taranto, Italy), Placido-based corneal topography and wavefront aberrometry (Nidek OPD II, Nidek Co. Ltd, Aichi, Japan), eye tonometry (Icare tonometer, Revenio Group Corporation, Helsinki, Finland), subjective spectacle refraction, UDVA, and CDVA (Paper 2-9).

7.3.2 Corneal Epithelial and Stromal Thickness Profile Measurements

We employed the RTVue-100 SD-OCT system with a corneal adaptor module, running on software version A6 (9.0.27). It features a 26,000-Hz scanning with 5 μm axial resolution and 15 μm transverse resolution. The cornea was imaged using Pachymetry pattern (6-mm scan diameter, 8 meridians, 1024 axial-scans each) centered on the pupil. The 8 radial meridional scans were employed by the system software to produce three-dimensional thickness maps by interpolation. Data output included thickness maps of the total cornea and the epithelium, across a diameter of 6-mm. Each map is divided into 17 sectors: One central circle, centered around the pupil, which is 2-mm in diameter (centre), 8 octants within an annulus between 2 and 5 mm circles (paracentre), and 8 octants within an annulus between 5 and 6 mm circles (mid-periphery) (Figure 6). For each of these sectors, average thickness is displayed over the corresponding area (Papers 2 and 3).

Additionally, in study 3, epithelial thickness at the central, superior and inferior region, minimum (Min) and maximum (Max) values, the difference between minimum and maximum epithelial thickness (Min-Max), and map standard deviation (St Dev) were recorded from the output of the measurements. Furthermore, we defined a minimum epithelial thickness area (MinArea) as either the pupil central 2 mm diameter zone, or the continuous paracentral zones with epithelial thickness of $\geq 3$ μm thinner than the adjacent zones, depending on the location of the thinnest epithelium preoperatively. The area of the remaining zones in the paracentre was defined as ParaRest. The postoperative epithelial thickness measurements at the same areas were calculated and compared with those of the preoperative values. Three measurements were obtained on a single visit, of which the two best quality images were chosen, and the average value was used for further analysis.

7.3.3 Corneal Dynamic Scheimpflug Analyzer Measurements
The CorVis ST software version 1.00r30 rev. 771 was used in study 7. When the eye was aligned and the Scheimpflug image was in focus, the air puff was released automatically and the cornea was imaged during the deformation event. The air pulse (lasting approximately 20 ms) forced the cornea inwards through applanation until it achieved its highest concavity (concavity phase). On its return, the cornea underwent a second applanation before achieving its natural shape. In total, approximately 140 images of the two-dimensional cross-section of the cornea were collected. By software tracing of the anterior and posterior corneal boundaries in individual image frames, parameters describing the corneal deformation response were automatically generated by the instrument.

The CorVis ST measurements were performed three times by technician A and once by technician B. The measurement sequence between the technicians was randomized using a randomization table. A one-minute pause was taken between each measurement. Repeatability was evaluated by comparing the three consecutive measurements performed by technician A. Reproducibility was determined by comparing the first measurement by technician A with the measurement performed by technician B. Mean CorVis ST measured values obtained from the three measurements by technician A were used to compare the differences between the virgin and post-PRK eyes groups, as well as for the correlation analysis.

7.4 Data Analysis

7.4.1 Visual Acuity Analysis

Visual acuity was measured using a Snellen chart with a decimal scale and converted to logMAR for analysis. The mean CDVA and UDVA were converted back to Snellen for the calculation of efficacy and safety indexes. The efficacy index was the ratio between the mean postoperative UDVA at the end of the follow-up and the mean preoperative CDVA. The safety index was defined as the ratio between the mean postoperative CDVA and the mean preoperative CDVA. The predictability was evaluated by calculating the number of eyes having an achieved spherical equivalent refraction change or vector analysis indices within ±0.50 D and ±1.00 D of the attempted correction.

7.4.2 Vector Analysis
To determine the efficacy of astigmatic correction, vector analysis was performed using an established method of Alpins.\textsuperscript{534, 535} We calculated the target-induced astigmatism (TIA) and surgically induced astigmatism (SIA). The TIA represents the intended astigmatic change, which is the vectorial difference between the target postoperative cylinder vector, usually 0, and preoperative cylinder vector. The SIA is defined as the vectorial difference between the postoperative and preoperative astigmatism. The difference vector (DV) is the vectorial difference between the TIA and SIA, and represents the remaining uncorrected (difference) vector. Magnitude of error (ME) demonstrates the arithmetic difference between the magnitude of the SIA and TIA, whereas the angle of error (AE) is the angle difference between the SIA and TIA (positive or negative if the SIA is counter-clockwise or clockwise to the TIA, respectively). The correction index is the ratio of magnitude of SIA and TIA, and is preferably 1.0; values larger than 1.0 mean that an overcorrection occurred and values lower than 1.0 mean that an under-correction occurred. The index of success (IOS) is given as the ratio of DV to the TIA. In an ideal case, in which all preoperative refractive astigmatism is corrected, the DV or IOS should be zero, or analogously, the remaining uncorrected astigmatism should be zero, which is the goal of the treatment. Coefficient of adjustment (CA), calculated by dividing TIA by SIA, is the coefficient required to adjust future astigmatism treatment magnitudes (TIA). Its value is preferably 1.0 and is the inverse of the CI. The geometric mean correction index and CA were derived by taking the mean of the individual logarithmic values, followed by the antilog of this calculated mean value.

The ocular residual astigmatism (ORA) was determined as the vector difference between the preoperative refractive astigmatism at the corneal plane using a vertex distance of 12 mm and the anterior corneal topographic astigmatism (obtained by aconic fitting within central 3.5 mm, and use of corneal refractive index of 1.376) obtained by Precisio topo/tomographer.

### 7.4.3 Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics (IBM Corp., Armonk, NY). The Shapiro-Wilk test was used to test the normality of the data distribution. Independent sample \( t \)-test or Mann-Whitney \( U \) test was used to compare two groups, whereas paired \( t \)-test or Wilcoxon signed rank test was used to compare paired samples. In study 7, for the parameters that showed significant differences, univariate analysis of covariance (ANCOVA) was applied to adjust for selected covariates (age, CCT measured by the CorVis ST, and mean
simulated keratometry value measured by OPD Scan II) to control for potentially confounding factors. In study 2, a linear mixed model was employed to compare the measurements at different postoperative times. Pearson or Spearman correlation coefficient was applied to seek possible correlations between different parameters. In all analyses, $p < 0.05$ was considered statistically significant.

In study 7, MedCalc software 11.4.2 (MedCalc Software, Ostend, Belgium) was used to calculate the within-subject standard deviation ($S_w$), within-subject coefficient of variation (COV), and intraclass correlation coefficient (ICC) to assess the intraobserver repeatability and interobserver reproducibility.
8. SUMMARY OF RESULTS

8.1 Paper I

In paper I, we compared the surface ablation treatments with wavefront optimized and custom-Q ablations for myopia and astigmatism. Preoperative and 3-month postoperative Q-values, higher order aberrations, low contrast visual acuity, and classic outcome parameters were analyzed.

The wavefront optimized ablation group comprised of 46 eyes of 23 patients with a mean SE of -3.64 D (range: -1.15 to -8.25 D). Mean Q-value changed from -0.33 preoperatively to 0.06 postoperatively. The custom-Q ablation group comprised of 42 eyes of 21 patients with a mean SE of -3.24 D (range: -1.47 to -8.00 D). Their mean Q-value changed from -0.36 preoperatively to -0.03 postoperatively. A statistically significant difference in postoperative change in Q-values ($P = 0.049$) between the two groups was noted, but there was no such difference in higher order aberrations, low contrast visual acuity, or classic outcome parameters.

8.2 Paper II

In paper II, we retrospectively analyzed the postoperative corneal epithelial and stromal thickness profile changes in 46 left eyes of 46 patients treated with PRK for myopia. Corneal and epithelial thickness maps within the central 6 mm were obtained by anterior segment spectral-domain optical coherence tomography preoperatively and at 1, 3, and 6 months postoperatively. Stromal thickness was calculated by subtracting the epithelial thickness from the total corneal thickness. Correlations between postoperative thickness changes and the amount of correction, treatment zone, and preoperative epithelial thickness were analyzed.

Compared to preoperative values, the central 2 mm and the paracentral 2 to 5 mm zone epithelium was 5.20±3.43 and 5.72±3.30 µm thicker, respectively, at 3 months postoperatively ($p < 0.05$). No significant difference was detected between 3 and 6 months postoperatively. The stromal thickness did not change between 1 and 6 months postoperatively. The spherical equivalent (SE) changed from -2.82±1.54 D preoperatively to -0.06±0.42 D at 1 month postoperatively, and remained stable thereafter.

8.3 Paper III
In paper III, we evaluated corneal epithelial remodelling after transepithelial TGCA followed by CXL in the treatment of keratoconus. This study retrospectively analyzed the epithelial thickness distribution changes in 53 keratoconic eyes of 44 patients. Manifest refraction, maximum (K\text{max}) and minimum (K\text{min}) keratometry obtained by Placido topography, corneal irregularity index (IRI) measured by Scheimpflug topography, and the epithelial thickness profile over the central 5-mm zone obtained by anterior-segment spectral domain optical coherence tomography (SD-OCT) were evaluated preoperatively and at 1–3, 3–6, and >6 months postoperatively.

The epithelial thickness at the preoperatively thinnest area (Min\text{area}) was 48.8±4.4 µm, correlating negatively with K\text{max} (r=-0.310, \(p<0.05\)) and IRI (r=-0.362, \(p<0.05\)). Improvements in corrected distance visual acuity, decreased refractive astigmatism, K\text{max}, K\text{min}, and IRI were registered after the treatment (\(p<0.05\)). At > 6 months postoperatively, epithelial thickening of 5.9±5.1 µm was registered at the respective area. No statistically significant change in the epithelial thickness was detected in other sectors, leading to a decrease in epithelial thickness difference between Min\text{area} and the rest of the paracentre areas by 5.6±4.4 µm.

8.4 Paper IV

Paper IV investigated the transepithelial TGCA in the treatment of low to moderate myopic astigmatism using a 1 KHz excimer laser. In this study, 117 consecutive eyes available for evaluation 12 months after surgery were included. Pre- and post-operative visual and refractive data as well as post-operative pain and haze were analyzed.

The mean pre-operative SE and the mean cylinder were: –3.22 D ± 1.54 (range –0.63 to –7.25 D) and –0.77 D ± 0.65 (range 0 to –4.50 D), respectively. At 12 months after surgery: no eyes lost ≥ 2 lines of CDVA. Safety and efficacy indexes were 1.27 and 1.09, respectively. Uncorrected distant visual acuity was ≥ 20/20 in 96.6% of the eyes. Manifest refraction spherical equivalent was within ± 0.5 D of the desired refraction in 93.2% of the eyes. Average root mean square (RMS) wavefront error measured at central 6 mm, increased from 0.38 pre-operatively to 0.47 µm post-operatively. Refractive stability was achieved and sustained 1 month after surgery. No visually significant haze was registered during the observation period. Post-operative pain was reported in 4.5% of patients.

8.5 Paper V
Paper V analyzed the outcomes of treatment of astigmatism $\geq 2.0$ diopters (D) with transepithelial TGCA. In this study we retrospectively analyzed a series of 206 eyes, divided into two groups: myopic astigmatism (153 eyes) and mixed astigmatism (53 eyes). Efficacy, safety and predictability were evaluated, and vector analysis of cylindrical correction was performed.

The median preoperative spherical equivalent (SE) was -2.63D and -0.63D for the myopic and mixed astigmatism groups, respectively, with a median cylinder -2.50D. Postoperative uncorrected distance visual acuity (UDVA) was $\geq 20/20$ in 92% and 83% of eyes in the myopic and mixed astigmatism groups, respectively; the corresponding efficacy indices were 1.00 and 0.96 and residual astigmatism $\leq 0.50$D was present in 82.4% and 56.7% of myopic and mixed astigmatism eyes respectively. The arithmetic mean magnitude of the difference vector (DV) was 0.38D (myopic) and 0.65D (mixed). DV magnitude was positively correlated with the magnitude of target induced astigmatism (TIA) in both groups. The geometric mean coefficient of adjustment index was 1.04 and 1.19, representing under-correction of 4% and 19%, in the myopic and mixed astigmatism group, respectively.

8.6 Paper VI

Paper VI evaluated the efficacy and safety of transepithelial TGCA in the treatment of visually disturbing irregular astigmatism and/or scattering caused by LASIK-flap/interface complications. In this study, 17 eyes of 16 patients with LASIK flap/interface complications and central residual stromal thickness $\geq 300$ microns were treated with transepithelial TGCA. The laser regularized the irregular corneal surface and in the same continuous process ablated the flap/interface pathology that caused the irregularity and/or scattering. UDVA, CDVA, refraction, corneal irregularity, ocular HOAs, and visual symptoms were analyzed.

At 15.9±11.0 months postoperatively, mean UDVA increased from 20/87 to 20/25. Mean CDVA increased from 20/28 to 20/19 ($P<0.001$), with 64.7% of the eyes gaining two lines of CDVA or more. The mean corneal irregularity index decreased from 25.82 to 20.36 microns ($P=0.009$). The mean root-mean-square (RMS) of total HOAs decreased from 1.30 to 0.49 ($P=0.042$), while RMS of the odd-order (3rd and 5th) and even order (4th and 6th) HOAs decreased from 0.85 to 0.38 ($P=0.001$) and from 0.43 to 0.24 ($P=0.036$), respectively. All patients claimed their visual symptoms to be better (8 eyes) or cured (9 eyes). Corneal regularization and removal of the underlying flap/interface pathology by cTEN ablation appears to be a simple and effective treatment for LASIK-flap/interface complications.
associated with visually disturbing irregular astigmatism and/or scattering in cases with sufficient residual stromal thickness.

8.7 Paper VII

Paper VII investigated the measurement reliability of CorVis ST, a dynamic Scheimpflug analyser, in virgin and post-photorefractive keratectomy (PRK) eyes and compared the results between these two groups. Forty virgin eyes and 42 post-PRK eyes underwent CorVis ST measurements performed by two technicians. Repeatability was evaluated by comparing three consecutive measurements by technician A. Reproducibility was determined by comparing the first measurement by technician A with one performed by technician B. Intraobserver and interobserver intraclass correlation coefficients (ICCs) were calculated. Univariate analysis of covariance (ANCOVA) was used to compare measured parameters between virgin and post-PRK eyes.

The intraocular pressure IOP, CCT and 1st applanation time demonstrated good intraobserver repeatability and interobserver reproducibility (ICC≥0.90) in virgin and post-PRK eyes. The deformation amplitude showed a good or close to good repeatability and reproducibility in both groups (ICC≥0.88). The CCT correlated positively with 1st applanation time (r= 0.437 and 0.483, respectively, p<0.05) and negatively with deformation amplitude (r=-0.384 and -0.375, respectively, p<0.05) in both groups. Compared to post-PRK eyes, virgin eyes showed longer 1st applanation time (7.29±0.21 vs. 6.96±0.17 ms, p<0.05) and lower deformation amplitude (1.06±0.07 vs. 1.17±0.08 mm, p<0.05).

8.8 Paper VIII

Paper VIII evaluated the efficacy and safety of epithelium-on corneal collagen cross-linking (CXL) using a multifactorial approach to achieve proper stromal riboflavin saturation in 61 eyes with progressive keratoconus treated with epithelium-on CXL. Chemical epithelial penetration enhancement (benzalkonium chloride-containing local medication and hypotonic riboflavin solution), mechanical disruption of the superficial epithelium, and prolongation of the riboflavin-induction time until verification of stromal saturation were used prior to the UVA irradiation. Uncorrected and corrected distance visual acuity (UDVA, CDVA), refraction, corneal topography, and aberrometry were evaluated at baseline and at 1, 3, 6, and 12 months postoperative.
At 12-months, UDVA and CDVA improved significantly. None of the eyes lost lines of CDVA, while 27.4% of the eyes gained 2 or more lines. Mean spherical equivalent decreased by 0.74 D, and mean cylindrical reduction was 1.15 D. Irregularity index and asymmetry from Scheimpflug-based topography and Max-K at the location of cone from Placido-based topography showed a significant decrease. Higher-order-aberration data demonstrated a slight reduction in odd-order aberrations S3, 5, 7 ($P=0.04$). Postoperative pain without other complications was recorded.

8.9 Paper IX

Paper IX evaluated the combination of transepithelial TGCA and CXL in a single procedure for the treatment of keratectasia. Twelve eyes of 12 patients with keratectasia were treated with topography-guided custom ablation and CXL. Topography-guided custom ablation was performed using a transepithelial technique with the iVIS Suite 1 kHz flying spot excimer laser. Collagen cross-linking was performed immediately after topography-guided custom ablation, according to standard protocol. Postoperative follow-up examinations were performed at 1, 3, 6, and 12 months. Uncorrected visual acuity (UDVA), best spectacle-corrected visual acuity (CDVA), refractive change, corneal topography, and pachymetry were analyzed pre- and postoperatively.

Mean UDVA increased from 20/1000 preoperatively to 20/125 12 months postoperatively. Mean CDVA increased from 20/57 to 20/35, with no loss of lines of visual acuity. Mean astigmatism was reduced from 5.40±2.13 diopters (D) to 2.70±1.44 D, and keratometric asymmetry decreased from 6.38±1.02 D to 2.76±0.73 D. Only minor changes in posterior corneal surface elevation and stability of refraction were found, confirming that no progression of ectasia occurred during the observation time.
9. GENERAL DISCUSSION

9.1 Rationale for Transepithelial Topography-Guided Custom Ablation in Treatment of Irregular Astigmatism

9.1.1 Custom Ablation

Corneal excimer laser surgery for correction of refractive errors has advanced significantly since its introduction. It is reported that conventional excimer laser keratorefractive procedures induce an increase in spherical-like and coma-like HOAs, mainly due to the modification of corneal asphericity/inadequate optical zone size and optical decentration, respectively. The ablation profile can have a significant impact on postoperative outcome. Application of modern wavefront and topography technologies in corneal refractive surgery offers opportunities for better optical quality and visual performance.

9.1.1.1 Aspheric ablation profile

It is currently known that for most eyes a negative (prolate) corneal asphericity is required to balance the positive (oblate) asphericity of the crystalline lens to achieve minimal spherical aberration. However, the conventional (non-aspheric) treatments for myopia result in an oblate shift of the cornea, which disturbs this balance. Furthermore, this shift seems to be directly correlated with the amount of correction. Among the HOAs induced by corneal refractive surgery, spherical aberration caused by the change in corneal asphericity after laser ablation seems to be the most prevalent. Good control of spherical aberration and a physiological postoperative corneal shape may be achieved with aspheric laser ablation profile design. Modern refractive ablation design, with considerations to control asphericity, reduces this oblate shift, which typically become progressively less effective towards high degrees of myopic treatment. In study 1, we compared the wavefront optimized treatment and the custom-Q treatment employed by WaveLight ALLEGRO TO (Wave-Light AG, Erlangen, Germany) laser. The wavefront optimized ablation has an aspheric profile in which the amount of asphericity is not adjustable (and is the default treatment type on that platform). The custom-Q ablation is also an aspheric ablation; however, it allows the surgeon to define the intended Q-shift (postoperative Q-value minus preoperative Q-value) by specifying a desired asphericity target. The custom-Q treatment uses only preoperative mean corneal asphericity data in addition to refractive data. It does not attempt to achieve a given asphericity at all points within the optical zone because no preoperative local
asphericity values are taken into account in programming of the ablation; it aims only to
change the mean asphericity by symmetrically adjusting the number of mid-peripheral laser
pulses.

Our results showed almost universal oblate Q-shift in both groups. The shift was less
in the custom-Q group, especially for higher degrees of myopia. However, the difference
between groups was just marginally statistically significant ($P=0.049$) and did not result in
any significant difference in postoperative spherical aberration or low contrast visual acuity.
A combination of factors such as the decrease in effective radiant exposure to laser energy
from the corneal center towards the periphery (due to inclination of corneal surface);
reflections losses according to Fresnel’s law, and the corneal biomechanical response,
seem to be responsible for the oblate shift. The first two factors are addressed by
aspheric ablations that feature a correction matrix, which compensates for the reduced laser
ablation efficacy in the mid-peripheral cornea. Ablation profiles used in both groups in our
study are compensated for by such a correction matrix and, therefore, the residual oblate
shift that our results show is probably, or at least partially, due to a biomechanical response of
the cornea in agreement with study the by Koller et al.

Because we treated only virgin eyes with good preoperative contrast sensitivity and a
low preoperative level of HOAs, our aim was to eliminate the patient’s spherocylindrical error
without disturbing the remainder of the corneal optics significantly. Koller et al. used the
same laser platform and found a much larger increase in the 3rd order HOAs (from
0.181±0.072 µm before surgery to 0.296±0.115 µm after surgery) in eyes treated with
custom-Q ablation, compared to wavefront-guided treatments (from 0.182±0.094 µm before
surgery to 0.192±0.088 µm after surgery). Tran and Shah compared WaveLight wavefront
optimize treatments and LADARVision4000 (Alcon Laboratories Inc., Ft Worth, Tex)
wavefront-guided treatments and found significantly more induced 3rd HOAs with the
former. The 3rd order HOAs in both groups changed only insignificantly (from 0.163±0.081
µm before surgery to 0.191±0.092 µm after surgery in the wavefront optimized group and
from 0.182±0.092 µm before surgery to 0.193±0.085 µm after surgery in the custom-Q
group). Hence, our results in both groups were more comparable to Koller et al. and Tran
and Shah’s wavefront-guided treatments than with their wavefront optimized treatments.
The reason for this may be our centration of the ablation on the corneal vertex. Our total RMS
HOAs, spherical aberration, and the 3rd order HOAs increased very little and resulted in well-
preserved low contrast visual acuity under light and dark conditions, with and without glare (a
finding comparable to the study by Koller et al.), and a statistically significant correlation
between the postoperative Q-values and low contrast visual acuity has not been found. Tuan and Chernyak\textsuperscript{552} also did not find such a correlation and concluded that “an oblate cornea is as likely to produce high-quality vision as a prolate one.” Other explanations may be that low contrast visual acuity is a subjective measurement dependent on a number of interrelated factors and the low quality of postoperative asphericity data.

The non-significant difference in postoperative Q-value or spherical aberration or low contrast visual acuity between the two groups may be caused either by the insufficient measurement reliability of our asphericity and wavefront aberrometry, or by a small influence of the achieved Q-value changes on the spherical aberration and low contrast visual acuity. It is uncertain whether more prolate Q-targets would further diminish or eliminate the remaining oblate shift found in our custom-Q treatments. We are limited to what we can achieve by the Q-target adjustment, especially if we bear in mind that part of the oblate shift most likely occurs due to biomechanical response of the cornea, which may need to be counteracted by a different approach. However, with the custom-Q treatments we have a new possibility to better control the oblate shift. Hopefully, in the future, it will be possible to achieve specific Q-targets resulting in minimal spherical aberration.

9.1.1.2 Topography-guided custom ablation

Topography-guided custom ablation uses the patient’s corneal topography as the starting point. Corneal topography measures the corneal surface irregularities that represent the substrate for the vast majority of ocular aberrations; therefore, using this information for programming of the treatment is the most direct approach. The TGCA smoothens the anterior corneal surface irregularities and changes the anterior corneal curvature to treat the anterior corneal HOAs and the refractive error of the eye at the same time. In cases with highly aberrated corneal optics, as after PKP and where corneal irregularities and/or media opacity prevent reliable wavefront analysis, TGCA is the only refractive surgery choice.\textsuperscript{542, 553} In paper 4 and 5, we investigated the effect of TGCA in treatment of myopia and astigmatism in healthy eyes.

In study 4, all eyes were within $\pm 1.0$ D of emmetropia, 94% of eyes were within $\pm 0.5$ D of emmetropia and 97% of eyes had refractive astigmatism less than or equal to 0.5 D at 12 months post-operatively. Postoperative UDVA of at least 20/20 was achieved in 96.6% of the eyes. No eyes lost $\geq$ two lines of CDVA. In study 5, a postoperative UDVA of at least 20/20 was achieved in 92% and 83% of eyes treated for myopic and mixed astigmatism.
respectively. No change or a gain of up to two lines of CDVA was observed in 97% (myopic astigmatism) and 96% (mixed astigmatism) of eyes; no eye lost more than one line of CDVA. The safety, efficacy, and predictability in our results are comparable with the best outcomes of surface ablation in treatment of myopic astigmatism \(^{358, 359, 554-569}\) (Table 2) and corneal laser refractive surgery for moderate to high astigmatism \(^{402, 546, 570-579}\) published in recent years (Table 3).

The ablation profile can have a significant impact on postoperative outcome. Alpins\(^{580}\) recommended the use of vector planning to link preoperative topographic measurements into the treatment plan with the refractive values. The topography-guided custom ablation pattern used in this study was based on a resolution of the vectors of the manifest and the corneal astigmatism as measured by topography and is in consistent with Alpins’s recommendations. Vinciguerra et al.\(^{581}\) argued that creating a smooth transition (low dioptric gradient) between the treated and untreated cornea might improve the outcomes of surgery for astigmatism. We sought to achieve this by creating a customized transition that results in a continuously low dioptric gradient towards the untreated cornea. A smooth transition zone may be the key to low regression as it may prevent the counterproductive epithelial remodelling, which is induced by non-smooth transitions.

Misalignment of the axis in astigmatic treatment results in under correction.\(^{582}\) It is well known that the pupil centroid varies with pupil size, resulting in registration error that may significantly affect the quality of laser surgery outcomes.\(^{583, 584}\) It follows that accurate centration and control of the cyclotorsional movements of the eye are necessary to optimize visual and refractive outcomes and reduce the induction of optical aberrations.\(^{584}\) The system used in this study deals with the centroid shift by using an intra-operation laser illumination adjustment to control pupil size and using x,y pupillary tracking for accurate centration during the ablation procedure. The use of iris registration and dynamic cyclotorsional eye-tracking has also been shown to improve the accuracy of astigmatism treatment.\(^{582}\) We observed small absolute mean AEs (2.2 degrees and 3.7 degrees for myopic and mixed astigmatism groups respectively) which is consistent with the closeness of the aggregate vector mean TIA (0.83D x 172 and 0.89D x 173) and SIA (0.85D x 174 and 0.95D x 173) axes. Therefore, no significant systematic error due to misaligned treatment was evident. However, at the individual patient level, AE ranged from -14 degrees to 13 degrees in myopic astigmatic correction, and from -22 degrees to 13 degrees in mixed astigmatic correction, which may suggest variable factors at work, such as healing response.
Hyperopic and mixed astigmatism are technically demanding and difficult to correct.\textsuperscript{546, 577} We observed a slight under-correction of astigmatism in both our groups, but this was more evident in the mixed astigmatism group. Data indicating under-correction of 4\% (myopic astigmatism) and 19\% (mixed astigmatism) are corroborated by negative mean MEs of -0.08D and -0.34D, and arithmetic mean DVs of 0.38D and 0.65D for the myopic and mixed astigmatism groups, respectively. The DV is a useful vector measure of uncorrected astigmatism. In our sample, DV magnitude was positively correlated with TIA and the DV was larger in the mixed astigmatism group.

Furthermore, our studies showed minimal or no significant change in HOAs. Unlike the custom-Q treatment which uses only preoperative mean corneal asphericity data, the ablation procedure used in our TGCA minimizes the induction of spherical aberration by adjusting the target value for asphericity according to the preoperative anterior corneal curvature and the planned curvature change, as well as by compensating for the reduction in radial efficiency of the laser towards the corneal periphery. We did not observe an increase in coma-like HOAs in our study and this may also be attributed to the ablation design, which is based on the existing centering and symmetry of the corneal optics measured by topography. Some studies have demonstrated that visual outcomes of astigmatic correction are worse in eyes with high ORA,\textsuperscript{585-587} but the predictability of astigmatic correction was similar for eyes with high and low ORA in our study. Further investigation is warranted to determine whether the topography-guided design is advantageous in this respect.

The TGCA performed with the iVIS platform is a safe, effective and predictable treatment for myopia and for moderate to high astigmatism in healthy eyes. Outcomes could be further improved by a better understanding of postoperative wound healing, postoperative irregular epithelial thickening and corneal biomechanical changes.\textsuperscript{538, 588, 589}

### 9.1.1.3 Epithelial remodelling

The corneal epithelium protects the eye and plays an important role in maintaining corneal transparency and optical quality.\textsuperscript{590} It has a rapid cell turnover and is highly reactive to irregularities of the underlying stromal surface.\textsuperscript{196, 591, 592} The clinical application of corneal epithelial thickness profile measurements is becoming important in the diagnosis of keratoconus and especially so in corneal therapeutic refractive surgery.\textsuperscript{196, 593, 594} The epithelial thickness has been studied using Artemis very high-frequency digital ultrasound (ArcScan Inc., Morrison, Colo),\textsuperscript{595} confocal microscopy,\textsuperscript{596} optical coherence tomography,\textsuperscript{597}
ultrasound,\textsuperscript{598} and optical pachymetry.\textsuperscript{599} However, apart from the studies applying Artemis,\textsuperscript{595} the majority of other studies were solely based on central epithelial thickness measurements. One of the recent applications of SD-OCT allows non-contact, \textit{in vivo} three-dimensional mapping of the corneal epithelial thickness.\textsuperscript{190, 600, 601} In the current project, we used the RTVue 100 SD-OCT in evaluating epithelial thickness profile changes in healthy myopic eyes treated with PRK (study 2), as well as in keratoconic eyes treated with combined topography-guided transepithelial PRK and CXL (study 3).

The corneal epithelium in normal eyes is mostly evenly distributed, being only slightly thicker inferiorly and nasally than superiorly and temporally.\textsuperscript{40, 588, 591, 600, 602} In study 2, the preoperative average central epithelial thickness in myopic eyes was 54.79±3.71 \(\mu\)m, which is within the range of previously reported values (from 48.3 to 58.4 \(\mu\)m).\textsuperscript{40, 591, 603-606} Similar to the finding by Kanellopoulos et al.,\textsuperscript{600} we found the preoperative epithelium in male eyes to be thicker than in female eyes. The non-uniform preoperative epithelial thickness profile, characterized by thinner epithelium superiorly than inferiorly and temporally than nasally, was also in accordance with other studies.\textsuperscript{40, 591, 595, 600, 602} Our results generally matched the data obtained by SD-OCT,\textsuperscript{190, 591, 600, 602} while they differed from the measurements with Artemis very high-frequency ultrasound in the following way: 1) We did not detect a significant difference between central and paracentral epithelial thickness after annular averaging, whereas the measurements with Artemis demonstrated 2.3 \(\mu\)m thinner epithelium centrally (within 1.5 mm) than paracentrally (annulus between 3 and 3.4 mm);\textsuperscript{595} and 2) the difference of 1.7±2.1 \(\mu\)m between superior and inferior paracentral epithelium in the current study is considerably smaller than 5.3 to 5.9 \(\mu\)m, as reported by Reinstein et al.\textsuperscript{40, 595} The variations may be caused by the differences in technology and measurement technique (noncontact SD-OCT vs. saline immersion ultrasound). The SD-OCT measurements’ inability to discriminate the tear film, and the central specular hyper refractive reflex, may affect the detection of layer boundaries in the center due to the reduced signal to noise ratio. The patient’s demographics may also contribute to the discrepancies. For example, Yang et al.\textsuperscript{607} reported that except for the central 2-mm sector and the inferiortemporal sector in the paracentral area, corneal epithelial thickness was negatively correlated with age.

After the myopic treatment, the epithelial thickness in all the measured areas continued to increase between 1 and 3 months after the surgery, whereas refractive stability was achieved by 1 month. No correlation was found between the epithelial thickening and postoperative refraction change. The epithelium may affect refraction due to its shape at the air-tear film interface and because of its different refractive index compared to the stroma.\textsuperscript{608}
but a uniform epithelial thickening (or thinning) would not appreciably change the curvature of the corneal surface and refraction. The difference between central and paracentral epithelial thickening in the current study was within 1 µm; therefore, it did not affect the postoperative SimK_{mean}, nor the manifest refraction. In eyes with refractive regression, pronounced postoperative epithelial thickening or thinning occur, after myopic and hyperopic treatments, respectively, in addition to a large difference in the postoperative central and paracentral epithelial thickness.

In agreement with previous findings, the postoperative epithelial thickening in the current study correlated with the programmed SE correction and the treatment zone. Our findings concur with those of Gauthier and associates who demonstrated that epithelial hyperplasia was greater with smaller zone sizes. Interestingly, we found a significant negative correlation between the postoperative epithelial thickening and the preoperative epithelial thickness. Our data demonstrated that the preoperatively thinnest epithelium in the temporal superior region had the most pronounced postoperative thickening. The preoperative superior-inferior epithelial thickness asymmetry was explained by eyelid dynamics, and the effect of gravity upon tear film when measured with SD-OCT. However, the mechanism behind the negative correlation between epithelial thickening and the preoperative epithelial thickness is unclear. One may speculate that the corneal flattening due to myopic treatment results in a mismatch of anatomy between the eyelid and the cornea, allowing the epithelium to fill the gap.

In eyes with irregular stromal surface due to corneal pathology, injury, or corneal refractive surgery that results in non-physiologic stromal shape, the epithelium remolds, in an attempt to establish a smoother anterior corneal surface. This process results in thinning above the relatively elevated corneal area and thickening above the relatively depressed regions. It is hypothesized that the magnitude of epithelial compensation is determined by the curvature gradient. Keratoconus is characterized by conical ectasia, and the epithelial profile in keratoconus is reported to be doughnut-shaped with localized central thinning over the apex of the cone, surrounded by an annulus of thickened epithelium around the cone.

In study 3, we found thinning of the epithelium in the temporal inferior area in keratoconic eyes preoperatively, which is in accordance with other studies. Furthermore, we found that the epithelial thickness at Min_{area} correlated negatively with K_{max} and IRI, whereas the epithelial thickness difference between Min_{area} and Para_{Rest} areas correlated positively with these parameters. This means that in the keratoconic corneas, the
steeper and more irregular corneal shape is, the thinner the epithelium at the cone apex and larger its variation from the surroundings will be, confirming the previously described compensatory epithelial thickness changes.

After the combined treatment of transepithelial TGCA and CXL, the postoperative epithelial thickness increase was limited to the areas where the thinnest epithelium was located preoperatively, whereas the mean central and paracentral corneal thickness did not show a statistically significant change. This is different from the findings in study 2, where we demonstrated that 3 months after topography-guided PRK in treatment of myopia, the epithelial thickness was increased centrally by 5.20±3.43 µm and paracentrally by 5.72±3.30 µm. The difference in epithelial remodelling between our two studies may be attributed to the different starting points (regular vs. irregular), the additional CXL procedure performed after the topography guided PRK, or to the different epithelial healing pattern in keratoconic eyes. Kanellopoulos and Asimellis\textsuperscript{617} investigated the epithelial thickness profile changes after high myopic femtosecond laser LASIK with or without concurrent high-fluence CXL (UVA fluence of 30 mW/cm\textsuperscript{2} for a total of 80 seconds). The comparison of matched myopic correction subgroups treated for myopia over -7 D indicated significantly less thickening of the epithelium paracentrally in eyes treated with concurrent CXL, indicating that the application of CXL might play a role in preventing postoperative epithelial thickening which is commonly found in normal virgin eyes.\textsuperscript{190, 588}

In line with study 2, where preoperatively thinner epithelium correlated to more thickening postoperatively after topography-guided PRK in the treatment of myopia in normal eyes, our data in study 3 showed postoperative epithelial thickening in the areas with preoperatively thinned epithelium at the cone location. Hence, the thickening correlated negatively with the preoperative thickness values in both studies. Reinstein et al.\textsuperscript{612} advocated that the amount of epithelial thickening is determined by the rate of change of the curvature of the stromal surface. This is in agreement with our findings that the area with preoperatively thinner epithelium due to the local “bulging” of the anterior stroma increased in thickness after the smoothening of the “bulge”. This smoothening is achieved by the effect of the topography-guided ablation (as shown by decreased $K_{\text{max}}$ and IRI), which transfers to a decrease in the rate of change of the stromal curvature.

The limitations of the studies are the inability of the RTVue 100 to measure corneal and epithelial thickness outside the central 6-mm diameter of the cornea, and a lack of longer follow-up data. Limitations of the current SD-OCT technology (limited axial resolution and no ability for tear film discrimination) may also influence the validity of our analysis. Higher
axial resolution equipment with a better ability for discrimination of the tear film would be beneficial. Future studies with longer follow-up time, particularly pertaining to changes in epithelial thickness profile extending beyond the 6 mm diameter, are warranted. Still, the values presented in our results are in accordance with the earlier findings obtained by VHF ultrasound technology,\(^40\) which features higher resolution, as well as with the findings of the related research with OCT instrumentation.\(^190,600\)

**9.1.1.4 Transepithelial TGCA**

As described above, in eyes with irregular stromal surface, the epithelial remodelling compensates to some degree for the irregular astigmatism, meaning that the irregularity we see and measure on topography is only part of the stromal irregularity, implying a mismatch between the epithelial and the stromal surfaces. Therefore, a topography-guided ablation based on the preoperative measurements of the epithelial surface applied to the stroma after removal of the epithelium can only correct the irregularity that has not been compensated by the epithelium. It also means that as a consequence of epithelial remodelling, mechanical or alcohol epithelial removal used with traditional surface ablation techniques (ASA, LASEK, Epi-LASIK) will reveal an irregular stromal surface that does not match the preoperative corneal topography (Figure 11). This may represent a source of potentially significant ablation error when topography-guided surface ablation is used. To circumvent this issue, we use a transepithelial approach in which manual epithelial removal is replaced by adding a lamellar component to the refractive ablation.

The lamellar part is aimed at removal of the epithelium and the simultaneous preservation of its smoothening effect, achieved by its previous remodelling. With this approach, the epithelium and anterior stroma protruding into the lamellar ablation depth are uniformly ablated across the treatment diameter (Figure 13). The depth of the lamellar part can be decided by using the information from the preoperative epithelial thickness map obtained by OCT, while the ‘refractive’ ablation map is produced on the basis of Scheimpflug topography. The sum of the two has to be no less than the epithelial thickness at any point throughout the area of the treatment, to ensure that the post-ablation surface would be beneath the thickest point of the epithelium. As a result, no epithelium rested at the post-ablation surface, and the minimal use of stromal tissue will be achieved at the same time. Based on similar reasoning, Renstein et al.\(^193\) used transepithelial phototherapeutic keratectomy (PTK) to regularize the irregular corneal stromal surface.
In integrated transepithelial treatment, total ablation volume far exceeds stromal ablation volume, typically by a factor of 3 to 6; it is therefore necessary to use a high-frequency excimer laser to achieve short ablation times in order to prevent stromal dehydration effects. With the iRES 1KHz laser, full thickness epithelial removal can be performed in only 16 s, whilst a myopic ablation for 6 D (6.5 mm optical zone and 7.5 mm total ablation zone) requires 15 s.

With conventional flying-spot lasers, there is a linear increase in the number of pulses per mm² per sec (local frequency), as the ablation area decreases towards the end of the treatment, causing an increased thermal effect and increased plume production that would culminate in a decreased ablation effect. This is traditionally compensated for by empirical nomogram adjustments. In contrast, the laser used in this study maintains a local frequency of 4Hz, i.e. the laser beam will always hit the same spot in the treated area four times per second. Because it produces a low, constant and even delivery of energy across the ablation area, the unwanted thermal effects due to the high frequency are avoided. This is essential to achieving a smooth ablation surface, which is important for the prevention of postoperative haze.
To apply transepithelial concept to treat refractive errors with high precision, the compatibility of ablation rates between the epithelium and the stroma must be achieved. As the laser used in the cases reported here was specially designed for transepithelial ablation it optimizes the fluence, shot pattern and frequency to minimize the difference in ablation rate between the epithelium and the stroma. This approach is different from that used in another laser platform (Schwind Amaris, Schwind eye-tech-solutions GmbH and Co. KG), which attempts to compensate for the ablation rate difference by employing a non-modifiable nomogram of ablating approximately 55µm epithelium at the center and 65µm at the periphery (4.0mm radially from the center), presumably based on population measurements of epithelial thickness.620

Using transepithelial approach, the de-epithelialization area fits exactly the edge of the refractive part of the ablation, thus generates less corneal trauma compare to mechanical epithelial removal, potentially resulting in faster and less painful re-epithelialization. The idea of transepithelial laser treatment appeals to patients because it is much quicker and more comfortable than traditional excimer laser surgery. The main appeal of this procedure to the surgeon is its perceived lack of serious complications as well as ease and speed of performance.
<table>
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<th>Author, year</th>
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<th>Laser platform</th>
<th>Follow-up (months)</th>
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<th>SI ± 0.50 D</th>
<th>EI ± 1.0 D</th>
<th>MMC</th>
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<td>91%</td>
<td>97%</td>
<td>n/a</td>
</tr>
<tr>
<td>Gambato558, 2011</td>
<td>WF-optimized PRK</td>
<td>Allergretto Eye-Q</td>
<td>12</td>
<td>303</td>
<td>1.05</td>
<td>1.05</td>
<td>99%</td>
<td>n/a</td>
<td>Abl. dep. ≥ 80 µm</td>
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<tr>
<td>Mifflin565, 2012</td>
<td>Custom PRK</td>
<td>VIX Star S4</td>
<td>12</td>
<td>40</td>
<td>1.23</td>
<td>1.15</td>
<td>89%</td>
<td>94%</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>Standard PRK</td>
<td>VIX Star S4</td>
<td>12</td>
<td>40</td>
<td>1.19</td>
<td>1.06</td>
<td>86%</td>
<td>97%</td>
<td>n/a</td>
</tr>
<tr>
<td>Sia566, 2012</td>
<td>Standard PRK</td>
<td>LADAR Vision 6000</td>
<td>12</td>
<td>298</td>
<td>1.29</td>
<td>0.67</td>
<td>83%</td>
<td>100%</td>
<td>n/a</td>
</tr>
<tr>
<td>Aslanides555, 2012</td>
<td>WF-optimized PRK</td>
<td>Schwind Amaris</td>
<td>12</td>
<td>60</td>
<td>n/a</td>
<td>0.99</td>
<td>n/a</td>
<td>n/a</td>
<td>Abl. dep. ≥ 75 µm</td>
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<td>Schwind Amaris</td>
<td>12</td>
<td>84</td>
<td>1.00</td>
<td>1.00</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
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<td>Schwind Amaris</td>
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<td>29</td>
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<td>0.95</td>
<td>85.7%</td>
<td>89.3%</td>
<td>all eyes</td>
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<td>Hashemi555, 2015</td>
<td>WF-optimized PRK</td>
<td>Allegretto EX500</td>
<td>6</td>
<td>30</td>
<td>1.00</td>
<td>0.89</td>
<td>n/a</td>
<td>n/a</td>
<td>all eyes</td>
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<td>Schallhorn555, 2015</td>
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<td>6</td>
<td>662</td>
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<td>Abl. dep. ≥ 70 µm</td>
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<tr>
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<td>Standard PRK</td>
<td>Technolas 217z</td>
<td>6</td>
<td>25</td>
<td>With eye-tracking</td>
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<td>0.99</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>Standard PRK</td>
<td>Technolas 217z</td>
<td>6</td>
<td>25</td>
<td>Without eye-tracking</td>
<td>1.00</td>
<td>0.99</td>
<td>n/a</td>
<td>n/a</td>
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<td>Schwind</td>
<td>3</td>
<td>103</td>
<td>n/a</td>
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<td>100%</td>
<td>100%</td>
<td>all eyes</td>
</tr>
<tr>
<td>Year</td>
<td>Type</td>
<td>Platform</td>
<td>Mdl</td>
<td>Code</td>
<td>CDVA</td>
<td>SI</td>
<td>EI</td>
<td>Abl dep</td>
<td>Grade</td>
</tr>
<tr>
<td>------</td>
<td>------</td>
<td>----------</td>
<td>-----</td>
<td>------</td>
<td>------</td>
<td>----</td>
<td>----</td>
<td>---------</td>
<td>-------</td>
</tr>
<tr>
<td>2016</td>
<td>RPK</td>
<td>Standard PRK</td>
<td>Mel 80</td>
<td>3</td>
<td>347</td>
<td>1.09</td>
<td>0.88</td>
<td>96.5%</td>
<td>99.7%</td>
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<tr>
<td>2016</td>
<td>RPK</td>
<td>Standard PRK</td>
<td>Schwind Estiris</td>
<td>42.3</td>
<td>22</td>
<td>1.21</td>
<td>1.08</td>
<td>n/a</td>
<td>n/a</td>
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<tr>
<td>2016</td>
<td>WF-optimized PRK</td>
<td>Allegretto Eye-Q</td>
<td>60</td>
<td>145</td>
<td>1.04</td>
<td>1.02</td>
<td>89%</td>
<td>96%</td>
<td>all eyes</td>
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</table>

**Transepithelial PRK**

<table>
<thead>
<tr>
<th>Year</th>
<th>Type</th>
<th>Platform</th>
<th>Mdl</th>
<th>Code</th>
<th>CDVA</th>
<th>SI</th>
<th>EI</th>
<th>Abl dep</th>
<th>Grade</th>
<th>20% Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>T-PRK</td>
<td>Nidek EC-5000</td>
<td>8.9</td>
<td>37</td>
<td>Low to moderate myopia</td>
<td>n/a</td>
<td>0.93</td>
<td>95%</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>2009</td>
<td>T-PRK</td>
<td>Nidek EC-5000 CXIII</td>
<td>8.9</td>
<td>22</td>
<td>High myopia</td>
<td>n/a</td>
<td>0.84</td>
<td>95%</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>2011</td>
<td>T-PRK</td>
<td>Schwind Amaris</td>
<td>3</td>
<td>15</td>
<td>1.17</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>grade &lt;2</td>
</tr>
<tr>
<td>2012</td>
<td>WF-optimized T-PRK</td>
<td>Schwind Amaris</td>
<td>12</td>
<td>60</td>
<td>n/a</td>
<td>0.98</td>
<td>n/a</td>
<td>n/a</td>
<td>Abl dep.≥ 75 µm</td>
<td>not significant</td>
</tr>
<tr>
<td>2014</td>
<td>WF-optimized T-PRK</td>
<td>Schwind Amaris</td>
<td>12</td>
<td>84</td>
<td>1.04</td>
<td>1.03</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>grade=0.5-1 in 30% eyes</td>
</tr>
<tr>
<td>2015</td>
<td>WF-optimized T-PRK</td>
<td>Schwind Amaris</td>
<td>12</td>
<td>41</td>
<td>n/a</td>
<td>1.08</td>
<td>91.4%</td>
<td>97.1%</td>
<td>all eyes</td>
<td>grade&lt;1 in 1 eye</td>
</tr>
<tr>
<td>2013</td>
<td>Topo-guided T-PRK</td>
<td>WIS Suite</td>
<td>12</td>
<td>117</td>
<td>1.27</td>
<td>1.09</td>
<td>94%</td>
<td>100%</td>
<td>Abl dep. &gt; 100 µm</td>
<td>grade=0.5 in 11% eyes</td>
</tr>
</tbody>
</table>

WF: Wavefront; T-PRK: Transepithelial PRK; SI: Safety Index= preoperative CDVA/postoperative CDVA; EI: Efficacy Index = postoperative UDVA/preoperative CDVA; MMC: Mitomycin C; Abl. dep.= ablation depth.
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Surgery (platform)</th>
<th>Follow-up (months)</th>
<th>Eyes</th>
<th>Sphere (D)</th>
<th>Cylinder (D) Preoperative</th>
<th>Cylinder (D) Postoperative</th>
<th>UDVA≥ 20/40</th>
<th>UDVA≥ 20/20</th>
<th>Cylinder≤ 0.5 D</th>
<th>Cylinder≤ 1.0 D</th>
<th>SE within ±0.5 D</th>
<th>SE within ±1.0 D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arbelaez573, 2009</td>
<td>LASIK (Amaris)</td>
<td>6</td>
<td>50</td>
<td>3.54±0.85</td>
<td>(-4.75, -2.00)</td>
<td>(-3.54, -2.00)</td>
<td>100%</td>
<td>84%</td>
<td>76%</td>
<td>94%</td>
<td>78%</td>
<td></td>
</tr>
<tr>
<td>Abolhassani570, 2009</td>
<td>LASIK (NIDEK EC-5000)</td>
<td>30</td>
<td>34</td>
<td>-0.125±0.50</td>
<td>(-0.75, +0.75)</td>
<td>(-0.75, -0.25)</td>
<td>97.1%</td>
<td>23.5%</td>
<td>70.6%</td>
<td>94.1%</td>
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<tr>
<td>Igarashi576, 2012</td>
<td>LASIK (Technolas 217z)</td>
<td>12</td>
<td>48</td>
<td>-5.10±2.11</td>
<td>(-10.75, -1.50)</td>
<td>(-5.10, -0.25)</td>
<td>53.3%</td>
<td>83.3%</td>
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<tr>
<td>Hasegawa575, 2012</td>
<td>LASIK (MEL-80)</td>
<td>12</td>
<td>30</td>
<td>-2.26±2.39</td>
<td>(-10.75, -1.50)</td>
<td>(-2.26, -0.25)</td>
<td>85%</td>
<td>26.9%</td>
<td>39%</td>
<td>54%</td>
<td>86%</td>
<td></td>
</tr>
<tr>
<td>Katz578, 2013</td>
<td>LASIK (Allegretto 200/400)</td>
<td>2-6</td>
<td>57</td>
<td>-1.89±1.72</td>
<td>(-6.75, 0)</td>
<td>(-1.89, -0.25)</td>
<td>91.2%</td>
<td>12%</td>
<td>54%</td>
<td>67%</td>
<td>93%</td>
<td></td>
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<tr>
<td></td>
<td>PRK (Allegretto 200/400)</td>
<td>57</td>
<td>-2.82±2.06</td>
<td>3.81±0.63</td>
<td>(-6.75, 0)</td>
<td>(-2.82, -0.25)</td>
<td>77.2%</td>
<td>21%</td>
<td>39%</td>
<td>54%</td>
<td>86%</td>
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<tr>
<td>Alio571, 2013</td>
<td>LASIK (Amaris)</td>
<td>6</td>
<td>37</td>
<td>-2.72±1.93</td>
<td>(-8.00, -0.25)</td>
<td>(-2.72, -0.25)</td>
<td>94%</td>
<td>61%</td>
<td>67%</td>
<td>93%</td>
<td>87%</td>
<td>97%</td>
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<td>Alio571, 2013</td>
<td>LASIK (Amaris)</td>
<td>3</td>
<td>52</td>
<td>2.41±1.26</td>
<td>(0.25, 5.00)</td>
<td>(2.41, -0.25)</td>
<td>85%</td>
<td>26.9%</td>
<td>65.3%</td>
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<td>Ivarsen577, 2013</td>
<td>LASIK (MEL-80)</td>
<td>3</td>
<td>46</td>
<td>-3.10±2.60</td>
<td>(-10.00, -0.25)</td>
<td>(-3.10, -0.25)</td>
<td>94.4%</td>
<td>25%</td>
<td>87%</td>
<td>98%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>3.50±2.30</td>
<td>-4.42±1.10</td>
<td>(-0.00, 8.75)</td>
<td>(-3.50, -0.25)</td>
<td>(-4.42, -0.25)</td>
<td>86.4%</td>
<td>13.6%</td>
<td>71%</td>
<td>90%</td>
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<td>Procedure</td>
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<td>Mean ± SD</td>
<td>Worst ± SD</td>
<td>Best ± SD</td>
<td>1 SD</td>
<td>2 SD</td>
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<td>Bohac et al.</td>
<td>LASIK (Allegretto 400)</td>
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<td>-2.80±2.01</td>
<td>-3.30±1.00</td>
<td>-0.55±0.46</td>
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<tr>
<td></td>
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<td>61</td>
<td>2.72±1.79</td>
<td>-3.84±1.21</td>
<td>-0.85±0.41</td>
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<td>LASIK (Amaris 750S)</td>
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<td>119</td>
<td>-2.44±2.17</td>
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<td>-0.43±0.36</td>
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<td>(-7.50, 0.00)</td>
<td>(-6.50, -2.00)</td>
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<td>-4.30±2.66</td>
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<td>97%</td>
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<td>-3.98±2.28</td>
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<td>-0.27±0.41</td>
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<td>89%</td>
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<td>Schallhorn et</td>
<td>LASIK (Visx S4 IR)</td>
<td>3</td>
<td>-2.79±2.32</td>
<td>-2.76±0.81</td>
<td>-0.37±0.38</td>
<td>83.8%</td>
<td>95.7%</td>
<td>90.3%</td>
<td>99.2%</td>
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<td>611</td>
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<tr>
<td>Current study</td>
<td>Trans PRK (iVIS)</td>
<td>≥ 6</td>
<td>-1.58±1.42</td>
<td>-2.67±0.77</td>
<td>-0.39±0.32</td>
<td>99%</td>
<td>92%</td>
<td>82.4%</td>
<td>97.4%</td>
<td>83.7%</td>
<td>99.3%</td>
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<td>153</td>
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<td>(-5.75, -2.00)</td>
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<td></td>
<td></td>
<td>53</td>
<td>0.83±0.54</td>
<td>-2.82±0.79</td>
<td>-0.65±0.46</td>
<td>98%</td>
<td>83%</td>
<td>56.7%</td>
<td>84.9%</td>
<td>79.2%</td>
<td>94.3%</td>
<td></td>
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</table>
9.2 Effect of Transepithelial TGCA in the Treatment of Irregular Astigmatism

In study 6, we analyzed the transepithelial TGCA technique in 17 eyes of 16 patients with visually disturbing irregular astigmatism and/or scattering in LASIK flap/interface complications. The concept was: 1) to regularize the cornea and treat the irregular astigmatism and 2) to ablate the underlying anatomical substrate and remove a potential source for scattering.

In addition to the successful treatment of optical irregularities, our study generally showed good refractive outcomes as well, even though the treatment of regular sphere and cylinder was our secondary aim. Already in 2000, Alessio and colleagues\textsuperscript{621} published a study where basically the same concept as in ours was applied by using an early version of CIPTA software for the TGCA planning in the treatment of irregular astigmatism. The outcomes showed good safety, while the efficacy and predictability were below the level of the current results, which can be explained by far the more advanced hardware technology and continuous refinement of the software that occurred in the years between the two projects.

Two of the eyes in our study were not aimed for emetropia; one due to a lack of available corneal tissue, and the other in order to achieve isometropia. Five eyes still needed enhancement to treat the residual refractive error, which may be attributed to: First, error in planning of the spherocylindrical correction due to the influence of HOAs on the measured amount of the preoperative sphere and cylinder;\textsuperscript{622} and second, epithelial remodelling, which occurs after the current treatment, affecting the final refractive outcome. Thus, a more precise preoperative refraction measurement and better knowledge and control of the postoperative epithelial healing/remodelling process may improve the refractive outcome.

9.3 Combined Treatment of Transepithelial TGCA and CXL in Treatment of Keratoconus

9.3.1 Corneal Biomechanics in Virgin and Post-PRK Eyes

Corneal refractive laser ablation in virgin eyes weakens the cornea mechanically due to tissue removal, leading to a deterioration in corneal biomechanical strength.\textsuperscript{623} Biomechanical changes may also affect the refractive outcome.\textsuperscript{624} Moreover, biomechanical weakening after corneal refractive laser treatment may potentially induce iatrogenic keratectasia.\textsuperscript{625} Therefore,
knowledge of corneal biomechanical properties is important in predicting clinical outcomes and in identifying cases with high risk for postoperative keratectasia after corneal refractive surgery. In study 7, we tested the hypothesis that the CorVis ST performs reliable measurements in both virgin and post-refractive surgery eyes. The secondary purpose was to test the hypothesis that the measurements can reveal differences in biomechanical properties between these two groups.

Similar to the studies performed by Nemeth et al. and Hon et al., we found that the following parameters had the best repeatability in both groups: CCT, IOP, 1st applanation time, and deformation amplitude. Our study also presented good repeatability for 2nd applanation time. In addition, the ICCs in the current study were generally higher than in the mentioned studies for most of the parameters measured. The differences between the studies may be attributed to different patient populations and software versions. For example, in the study by Nemeth et al., the mean age was 61.24±15.72 years (95% CI: 57.62 to 64.86 years), while the population in the current study was much younger. In the study by Hon et al., the software did not offer values for the radius of curvature and peak distance. When comparing reproducibility, Hon et al. found a statistically significant difference in the CCT measurement between the two sessions. However, the intersession difference was calculated by comparing the examinations performed in the morning (9:00–10:99 am) and afternoon (3:99–5:99 pm) by the same observer. This time difference may have affected the reproducibility evaluation, as corneal thickness demonstrates diurnal variation. The other parameters measured with the CorVis ST did not show satisfactory reliability. The ICCs varied between the virgin and post-PRK eyes.

It is conceivable that the cornea would be more difficult to deform and would deform less in eyes with a greater CCT. In line with other studies, we revealed a negative correlation between CCT and deformation amplitude in both groups. In addition, the CCT correlated positively with 1st applanation time and radius of curvature in both virgin and post-PRK eyes. However, correlations between CCT and 1st applanation velocity, 2nd applanation time, length, and velocity were only found in post-PRK eyes. This may imply that CCT in normal virgin eyes does not introduce much variation to some of the CorVis ST parameters, while affecting those measurements in biomechanically compromised corneas. The MRSE in our virgin-eyes group demonstrated correlation with some of the parameters measured by CorVis ST. This may need to be taken into consideration if a database of “healthy corneas” is built for the purpose of identifying biomechanically weaker corneas.
The IOP measured with the CorVis ST was significantly lower in the post-PRK eye group compared to the virgin-eye group, while the historical preoperative data (IOP measured by Icare, CCT, and corneal curvature) of the post-PRK group showed no significant difference compared to the respective data in the virgin-eye group. The CorVis ST measurements in our post-PRK group were performed a minimum of two months postoperatively, by which time the patients had discontinued the use of local steroids for at least three weeks, to exclude a possible pharmacological effect on their IOP. Some studies have demonstrated that IOP measured with the CorVis ST is more reliable compared to Goldmann applanation tonometry (GAT) and Topcon noncontact tonometry in virgin eyes (Topcon CT-80A Computerized Tonometer; Topcon, Tokyo, Japan). Still, in the version of CorVis ST used in this study, IOP is calculated based on the timing of the 1st applanation event and is not adjusted for corneal biomechanical properties. Both CCT and corneal biomechanical properties can affect IOP measurements, with the latter suggested to be more influential. The difference in the CorVis ST measured IOP between the groups was most likely caused by changes in corneal biomechanical properties and CCT after PRK.

Interestingly, before being adjusted for age, CCT, and simK, the CorVis ST parameters that demonstrated differences between the virgin and post-PRK eyes (1st applanation time, 1st applanation velocity, 2nd applanation time, 2nd applanation velocity, deformation amplitude and radius of curvature) were the same parameters as those showing differences between normal eyes and keratoconus eyes in the study conducted by Ali et al. It seems that these parameters may be of value in evaluating corneal biomechanical properties.

The earlier start of the apex indentation (shorter 1st applanation time) and greater deformation amplitude in post-PRK eyes indicates a lower resistance to deformation due to a decrease in corneal stiffness. Shen et al. compared corneal deformation parameters after femtosecond laser small incision lenticule extraction (SMILE), laser-assisted sub-epithelial keratomileusis (LASEK), and femtosecond laser-assisted LASIK (FS-LASIK). They found greater deformation amplitude and shorter 1st applanation time in the FS-LASIK group compared to the LASEK group. However, those parameters did not differ significantly between the SMILE and LASEK groups, or between SMILE and FS-LASIK groups. This indicates that corneal refractive surgery alters the stiffness of the cornea to different degrees with respect to different surgical approaches.

In the current study, the CorVis ST measurements in virgin- and post-PRK eyes were taken from two groups of unrelated populations. Pre- and postoperative comparison of the
same population would have been better suited to evaluate changes in the biomechanical properties caused by the surgery. We attempted to compensate for this by applying age, CCT, and simK as covariates to adjust for potential confounding factors. For the sake of this discussion, we also introduced a separate group of 28 eyes of 16 patients who underwent PRK for myopic astigmatism (mean preoperative MRSE: -3.35±1.98 D, mean postoperative time 9.21±5.09 months) with both pre- and postoperative CorVis ST measurements. The pre- and postoperative CorVis ST measurements of CCT and IOP in that group [547.53±28.89 µm vs. 460.32±48.57 µm (p<0.05), and 15.00±1.48 mmHg vs. 13.48±1.24 mmHg (p<0.001), respectively] were similar to the differences found in the virgin and post-PRK eyes in the current study. Comparable similarity was also found for the 1st appplanation time [7.37±0.23 vs. 7.14±0.20 ms (p<0.001)], 2nd appplanation time (21.39±0.32 vs. 21.57±0.25 ms (p<0.05), radius of curvature [7.76±0.83 vs. 6.55±0.66 mm (p<0.001)] and deformation amplitude [1.03±0.08 vs. 1.10±0.08 mm, (p<0.05)]. Still, a separate study measuring pre- and post-PRK parameters with a larger population is warranted.

9.3.2 CXL in Treatment of Keratectasia

Corneal collagen crosslinking is a minimally invasive procedure for treating keratoconus and iatrogenic keratectasia by increasing the biomechanical stability of the corneal stroma.438-442 The corneal epithelium with its tight junctions and hydrophobic character is the most important barrier to the penetration of hydrophilic macromolecules like riboflavin.453 Thus, with the “standard protocol” (“epithelium-off” CXL), the removal of the corneal epithelium before the application of riboflavin is considered crucial to enable sufficient intrastromal diffusion of riboflavin.443, 454, 455 To avoid potential complications occurring due to epithelial removal, such as delayed re-epithelialization, keratitis, or severe pain, modifications of the technique with the epithelium kept intact (“epithelium-on” CXL) have been developed.459, 464, 472, 631 The safety and efficacy of different CXL protocols have been documented by various studies.472, 632-635 In study 8, a multifactorial approach was utilized to enhance the riboflavin penetration by employing: 1) BAC-containing local medication; 2) hypotonic riboflavin solution without dextran; 3) increased riboflavin solution concentration; 4) mechanical disruption of the superficial epithelium (micro-abrasions); and 5) prolongation of the riboflavin-induction time until objective verification of the stromal saturation is confirmed.

Epithelial permeability can be enhanced by the application of several tensioactive substances including BAC and gentamycin at concentrations normally used in industrial
preparations. Such pharmacological enhancements, which are commonly used in epithelium-on CXL, were included in our protocol. On the basis of previous studies reporting increased epithelial riboflavin permeability using hypotonic solution compared to isotonic solution, hypotonic solution was applied in our protocol. Furthermore, we avoided the use of riboflavin solution with dextran due to its high viscosity, which inhibits penetration through the epithelium. Hypotonic riboflavin was originally used with epithelium-off CXL protocol to induce significant edema in corneas thinner than 400 μm. However, the swelling of the corneas with intact epithelium seem to be of a considerably lower degree. In our study, only around 10 microns of swelling was recorded in a subgroup of 29 eyes, presenting respective mean pachymetry of 450.50±42.90 and 471.65±41.43 μm before and after the corneal saturation with the 0.5% hypotonic riboflavin solution. Even though the clinical safety of CXL with hypotonic riboflavin solution has been documented, issues with the corneal endothelial cell toxicity were considered because of the decreased UV-protective effect with hypotonic riboflavin due to the decrease of UV-absorption coefficient from ≈ 53 cm⁻¹ for 0.1 % isotonic Riboflavin solution, to ≈ 42 cm⁻¹ for 0.1 % hypotonic riboflavin solution. To compensate for this, the concentration of riboflavin in the hypoosmolar solution may be increased to enhance UVA absorption and hence decrease the UV-radiation at the endothelial level. In our study, a hypotonic riboflavin concentration of 0.5% was applied. A secondary benefit of the increased concentration was the presumably increased availability of the riboflavin molecules to penetrate the epithelium and saturate the stroma. In a subgroup analysis of 21 eyes by the use of pre- and postoperative specular microscopy with Konan CellCheck XL (Konan Medical, Irvine, CA), the endothelial count decreased insignificantly, from 2738±188 cells/mm² to 2608±311 cells/mm².

Our protocol also employed mechanical scarification of the epithelial surface by the creation of micro-abrasions caused by the movement of a merocel sponge over the corneal surface with patient blinking. The amount of such micro-abrasions could obviously not be standardized and varied between cases. This may be the reason for a relatively large variation in saturation time (mentioned in the next paragraph).

Finally, in addition to the chemical and mechanical enhancements, our protocol demanded a slit-lamp verification of the stromal saturation before the UVA-radiation (Figure 12). Our clinical observations showed that riboflavin saturation was achieved after 25-45 minutes, so that a commonly used set induction time of e.g. 30 minutes, would in many cases lead to insufficient riboflavin concentration in the stroma.
Our visual and refractive outcomes were comparable to other published CXL studies. In the current study, there were no cases with a loss of ≥ 2 lines of CDVA, endothelial cell count did not change significantly, and there were no infections or other types of keratitis.

Most of the patients reported pain peaking 4-6 hours after the treatment despite the mostly preserved epithelium. This may be explained by actinic-keratoconjunctivitis-like reaction caused by the UV-exposure during the treatment and by the micro-abrasions caused on purpose, to enhance riboflavin penetration.

Corneal topography change in curvature has often been used to evaluate the effect of CXL. In our study, the maximum-K value and DSI (differential sector index) decreased significantly on the OPD II, Placido-based topography, as did the posterior elevation, irregularity index and asymmetry on the Precisio, Scheimpflug-based topography. However, our mean-K did not decrease in contrast to most other studies. In most of our cases, in addition to the Max-K decrease, a moderate increase in steepness on the opposite side of the cone occurred, resulting in only a minor decrease of the mean-K, but more symmetrical corneal optics, decreased higher-order-aberrations, and improved vision. We hypothesize that this may be a consequence of a possibly heavier riboflavin load in the inferior corneal stroma due to the sitting position and blinking during the riboflavin induction, leading to a locally increased cross-linking effect. This theory warrants further study and may be a small step in the direction of “customized” CXL.

Detection of the demarcation line after CXL has been considered proof of the efficacy and the measure of the depth of the corneal cross-linking. Although the precise nature and significance of the demarcation line (increased optical density) in relation to the cross-linking process are uncertain, it may be consequent to the keratocyte apoptosis and their subsequent repopulation. Keratocyte apoptosis has to a lesser extent been demonstrated after epithelium-on CXL. Filipello’s epithelium-on CXL with 0.1% isotonic riboflavin solution showed that the demarcation line two weeks postoperatively was located approximately 100 μm from the corneal epithelium. In 24 eyes treated with the current protocol that could be evaluated by RTVue (Optovue Inc., Fremont, CA) AS-OCT (anterior segment optical coherence tomography), the mean demarcation line was located at the depth of 316.92±49.16 μm (range 260 to 367) from the surface, which is close to the observations after epithelium-off CXL.

Epithelial absorption/filtering of the UVA light that could potentially lead to lesser energy delivered to riboflavin-saturated stroma, has also been stated as an argument against the use of epithelium-on CXL. Different studies offer a variety of evaluation backgrounds of
this matter. A study performed by Baiocci et al.\textsuperscript{453} claimed that human corneal epithelium and the underlying basement membrane naturally absorbs 30\% to 33\% of UVA radiation (400 to 350 nm), while other studies showed that the epithelial UV absorption occurs only with wavelengths lower than 310 nm.\textsuperscript{501, 644, 645} We may assume that the UV-absorption of the riboflavin within the epithelium is probably low (since the epithelial cells are hydrophobic and do not absorb riboflavin) and that the epithelial interstitial space is of negligible volume to allow riboflavin to accumulate and contribute to UV-absorption. In addition, our protocol included washing off the riboflavin from the corneal surface before the UVA-radiation in order to minimize the UV-energy loss due to its possible absorption by riboflavin.

Our retrospective study showed that epithelium-on CXL using our novel protocol appeared to be effective and safe in treating progressive keratoconus. The improvements in visual, refractive and topographic parameters indicated that epithelium-on CXL had a sufficient effect to halt the progression of keratoconus and improve the corneal shape. However, CXL is an oxygen-dependent process\textsuperscript{646} and the intact epithelium might represent an additional barrier to oxygen molecules and could be the reason for lower efficacy in corneal flattening compared to the epithelium-on CXL. At present, the literature on transepithelial CXL reports an evident morphologic impact of epi-on that is approximately a third of that of epi-off \textit{in vitro} and \textit{in vivo}, without uniformity of protocols. The epi-on CXL was around 70-80\% less effective in terms of biomechanical strength than standard epi-off CXL.\textsuperscript{88, 466} A randomized controlled trial is warranted to verify that the effect of the current approach is comparable with the standard epithelium-off CXL. Furthermore, the combination of different enhancers, osmolality and concentration of riboflavin should be explored to further improve the procedure.

9.3.3 Combination of Transepithelial TGCA and CXL in Treatment of Keratectasia

A study with ten-year results demonstrated that CXL was effective in treating progressive keratoconus, achieving long-term stabilization of the condition.\textsuperscript{634} However, the CXL does not directly address the patient’s refractive error. The sphero-cylindrical change that it achieves is limited. Our study 6 has shown that the topography-guided custom ablation is a suitable treatment for regularizing visually disturbing irregular astigmatism in non-ectatic corneas with sufficient thickness. However, laser ablation is commonly considered contraindicated in unstable corneas with keratectasia because of the danger of worsening of
the corneal structural stability and its consequences, caused by tissue removal. Topography-guided custom ablation and CXL combines the benefits of both treatments with the potential for creating a safe and stable optical improvement of the irregular keratectatic cornea in a less invasive fashion. The concept was first introduced by Kanelloloulos and Binder. In that study, the laser ablation was performed 12 months after CXL. Ablation performed after CXL may however cause a reduction in corneal strength in the ablation area by removal of the superficial (supposedly the most strengthened) part of the CXL-treated tissue. In our studies 3 and 9, we performed the procedure in a reverse order by applying CXL immediately after topography-guided custom ablation to strengthen the stroma, before the ectatic process had a chance to progress.

Our studies showed relatively good safety, efficacy, and predictability of the combined treatment, which was confirmed by improvement in CDVA and corneal regularity. The advantages of performing cTEN and CXL within the same surgical session may include the fact that laser ablation does not interfere with the cornea where the CXL takes place, while CXL of the ablated stroma depletes keratocytes of the anterior cornea, thus reducing the possibility of postoperative haze. Similar results have been reported by other studies. Generally, patients undergoing this dual procedure experience an increase in uncorrected visual acuity and improved corneal irregularity and resistance. However, the procedure is not free from complications. Guell et al. recently reported a late onset, persistent, deep stromal haze alteration in contact with the endothelium after the combined procedure. The focal haze improved with a corticoid-based treatment; however, the haze clearly affected visual acuity. The authors therefore advocated that a longer follow-up study that includes data from more cases was necessary to determine whether this combination should be a standard approach. Alessio and colleagues performed a long-term confocal microscopy evaluation of corneas up to 48 months after the treatment by the combined procedure. They showed permanently decreased keratocyte density in the anterior stroma, while the other corneal structures mostly reached their preoperative characteristics six months after treatment. A prospective clinical study by the same authors compared two eyes of 17 patients, with progressive keratoconus, where the best eye was treated by only CXL, and the fellow eye received CXL treatment after previous corneal regularization by transepithelial TGCA. They showed that the combined procedure increased vision, reduced corneal aberrations and stabilized corneas, while CXL alone stabilized and flattened corneas with no significant visual impact. A recently published study by Kontadakis and colleagues came to a similar conclusion in a long-term comparative study. Their simultaneous transepithelial PRK followed by CXL offered
significantly improved vision to treated patients with keratoconus in comparison with CXL alone, while similar results regarding postoperative stability were achieved.

9.4 Ethics
All studies were performed in accordance with the Declaration of Helsinki. All examinations mentioned in the current project are part of the clinic’s standard routine examinations. The approvals from The Regional Ethics Committee (REC) were obtained. The use of data for retrospective studies was reported to the Norwegian Data Protection Authority. Informed consent for the anonymous use of data for analysis and publication was obtained from study subjects.

10. FUTURE PERSPECTIVES
10.1 Corneal Biomechanical Property Measurement with the CorVis ST
The CorVis ST’s direct view of corneal deformation may offer information that promises to yield clinically relevant parameters correlated with corneal biomechanical properties. However, more work needs to be done to explore the optimal parameters representing the corneal biomechanical strength.

10.2 Stromal Surface Topography-Guided Custom Ablation
Our study shows that transepithelial TGCA is effective in the treatment of corneal irregular astigmatism. Stromal surface topography-guided ablation by incorporating the epithelial thickness profile into the corneal topography might offer advantages over the current transepithelial TGCA.

11. CONCLUSIONS
11.1 General Conclusion
Topography-guided customized corneal ablation is safe and effective in optical regularization in eyes with irregular astigmatism, while CXL is the first causative therapy that achieves biomechanical stabilization in keratoconus. The combination of topography-guided treatment
with CXL is a promising therapy for keratoconus and corneal keratectasia to achieve both goals and decrease the need for corneal transplantation.

11.2 Conclusions of the individual papers

Paper I
We concluded that Custom-Q ablation resulted in a mean postoperative asphericity that was closer to preoperative compared to wavefront optimized ablation, whereas the other outcome parameters showed no statistically significant differences.

Paper II
We conclude there was a trend towards greater epithelial thickening with a larger amount of programmed SE correction, smaller treatment zone, and thinner preoperative epithelium. No correlation between epithelial thickness change and postoperative change in refraction was detected.

Paper III
We concluded that a significant epithelial thickness profile change occurred after treatment, with an increase in thickness in the preoperatively thinnest area. The thickness remained largely unchanged in the other areas, resulting in a more even epithelial thickness distribution, which may be attributed to regularized postoperative corneal stromal shape.

Paper IV
We concluded that one-step transepithelial topography-guided treatment for low to moderate myopia and astigmatism performed with a 1 KHz laser provided safe, effective, predictable and stable results with low pain and no visually significant haze.

Paper V
We concluded that transepithelial TGCA appeared to be a safe, effective, and predictable treatment for moderate to high astigmatism.

Paper VI
We concluded that corneal regularization and removal of the underlying flap or interface pathology by transepithelial TGCA appeared to be an effective treatment for LASIK flap or interface complications associated with visually
disturbing irregular astigmatism and light scattering in cases with sufficient residual stromal thickness.

Paper VII  We concluded that CorVis ST demonstrated reliable measurements for CCT, IOP, and $1_{st}$ applanation time, as well as relatively reliable measurement for deformation amplitude in both virgin and post-PRK eyes. There were differences in $1_{st}$ applanation time and deformation amplitude between virgin and post-PRK eyes, which may reflect corneal biomechanical changes occurring after surgery in the latter.

Paper VIII  We concluded that epithelium-on CXL with our novel protocol appeared to be safe and effective in the treatment of progressive keratoconus.

Paper IX  We concluded that the combination of topography-guided custom ablation and CXL improved patients’ visual, refractive, and topography outcomes and halted the progression of keratectasia within the observation period of 12 months. This method may postpone or eliminate the need for corneal transplantation in suitable cases with keratectasia.
12. ERRATA

Page 4, line 29: Combination with
Page 4, line 31: .. the Study
Page 4, line 32: Aims of the
Page 6, line 6: with the
Page 10, line 4: laser-assisted
Page 14, line 32: high astigmatism
Page 22, line 32: Gullstrand’s eye model
Page 24, line 2: Gullstrand’s eye model
Page 104, line 14: deleted “Figure 6”
Page 104, line 30, deleted “Figure 7”
13. REFERENCES

64. Hamada R, Giraud JP, Graf B, Pouliquen Y. [Analytical and statistical study of the
lamellae, keratocytes and collagen fibrils of the central region of the normal human
cornea. (Light and electron microscopy)]. Arch Ophtalmol Rev Gen Ophtalmol
1972;32:563-570.
collagen organization using high-resolution nonlinear optical microscopy. Eye
Contact Lens 2010;36:260-264.
corneal collagen organization and axial biomechanics. Invest Ophthalmol Vis Sci
2011;52:8818-8827.
organisation as a function of depth in the human cornea and limbus. J Struct Biol
2010;169:424-430.
69. Muller LJ, Pels E, Vrensen GF. The specific architecture of the anterior stroma
70. Petsche SJ, Chernyak D, Martiz J, Levenston ME, Pinsky PM. Depth-dependent
transverse shear properties of the human corneal stroma. Invest Ophthalmol Vis Sci
2012;53:873-880.
71. Randleman JB, Dawson DG, Grossniklaus HE, McCarey BE, Edelhauser HF. Depth-
dependent cohesive tensile strength in human donor corneas: implications for
72. Boote C, Dennis S, Huang Y, Quantock AJ, Meek KM. Lamellar orientation in human
73. Daxer A, Fratzl P. Collagen fibril orientation in the human corneal stroma and its
74. Boote C, Dennis S, Newton RH, Puri H, Meek KM. Collagen fibrils appear more
closely packed in the prepupillary cornea: optical and biomechanical implications.
75. Jester JV, Moller-Pedersen T, Huang J, et al. The cellular basis of corneal
76. Bourne WM, Nelson LR, Hodge DO. Central corneal endothelial cell changes over a
77. Tanaka E, Aoyama J, Tanaka M, et al. The proteoglycan contents of the
temporomandibular joint disc influence its dynamic viscoelastic properties. J Biomed
78. Glass DH, Roberts CJ, Litsky AS, Weber PA. A viscoelastic biomechanical model of
the cornea describing the effect of viscosity and elasticity on hysteresis. Invest
80. Richoz O, Kling S, Zandi S, Hammer A, Spoerl E, Hafezi F. A constant-force
technique to measure corneal biomechanical changes after collagen cross-linking.
82. Torricelli AA, Ford MR, Singh V, Santhiago MR, Dupps WJ, Jr., Wilson SE. BAC-
EDTA transepithelial riboflavin-UVA crosslinking has greater biomechanical


110. Carlson N. Optometry: Daniel Kurtz; 1983.


122. Yanai R, Ueda K, Nishida T. Retrospective analysis of vision correction and lens
tolerance in keratoconus patients prescribed a contact lens with dual aspherical curves.
_Eye Contact Lens_ 2010;36:86-89.
124. Durrie DS, Schumer DJ, Cavanaugh TB. Holmium:YAG laser thermokeratoplasty for
125. Thompson VM, Seiler T, Durrie DS, Cavanaugh TB. Holmium:YAG laser
thermokeratoplasty for hyperopia and astigmatism: an overview. _Refract Corneal Surg_
1993;9:S134-137.
127. Hennekes R. Holmium:YAG laser thermokeratoplasty for correction of astigmatism. _J
128. Asbell PA, Maloney RK, Davidorf J, Hersh P, McDonald M, Manche E. Conductive
keratoplasty for the correction of hyperopia. _Trans Am Ophthalmol Soc_ 2001;99:79-
84; discussion 84-77.
keratoplasty: treatment for advanced keratoconus. _Am J Ophthalmol_ 2010;150:481-
489.e481.
procedure followed by accelerated cross-linking for the treatment of keratoconus: a
132. Schanzlin DJ, Asbell PA, Burris TE, Durrie DS. The intrastromal corneal ring
133. Burris TE. Intrastromal corneal ring technology: results and indications. _Curr Opin
laser tunnel creation for intrastromal corneal ring segment implantation in
137. Sogutlu E, Pinero DP, Kubaloglu A, Alio JL, Cinar Y. Elevation changes of central
posterior corneal surface after intracorneal ring segment implantation in keratoconus.
intracorneal ring segments for keratoconus: mechanical versus femtosecond-assisted
139. Rabinowitz YS, Li X, Ignacio TS, Maguen E. INTACS inserts using the femtosecond
laser compared to the mechanical spreader in the treatment of keratoconus. _J Refract
140. Ertan A, Colin J. Intracorneal rings for keratoconus and keratectasia. _J Cataract


159. Drews RC. DEPTH OF FIELD IN SLIT LAMP PHOTOGRAPHY. AN OPTICAL SOLUTION USING THE SCHEIMPFLUG PRINCIPLE. *Ophthalmologica* 1964;148:143-150.


405. Reinstein DZ, Archer TJ, Randleman JB. Mathematical model to compare the relative tensile strength of the cornea after PRK, LASIK, and small incision lenticule extraction. *J Refract Surg* 2013;29:454-460.


139
552. Tuan KM, Chernyak D. Corneal asphericity and visual function after wavefront-guided LASIK. *Optom Vis Sci* 2006;83:605-610.


14. PAPERS
One-Step Transepithelial Topography-Guided Ablation in the Treatment of Myopic Astigmatism

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Abstract

Purpose: To evaluate one-step topography-guided transepithelial ablation in the treatment of low to moderate myopic astigmatism using a 1KHz excimer laser.

Methods: Retrospective study of 117 consecutive eyes available for evaluation 12 months after surgery. Pre- and post-operative visual and refractive data as well as post-operative pain and haze were analyzed. A novel technique integrating custom refractive- and epithelial- ablation in a single uninterrupted procedure was used.

Results: The mean pre-operative spherical equivalent (SE) and the mean cylinder were: –3.22 diopters (D) ± 1.54 (SD) (range –0.63 to –7.25 D) and –0.77 D ± 0.65 (range 0 to –4.50 D), respectively. At 12 months after surgery, no eyes lost ≥2 lines of corrected distant visual acuity (CDVA). Safety and efficacy indexes were 1.27 and 1.09, respectively. Uncorrected distant visual acuity (UDVA) was ≥20/20 in 96.6% of the eyes. Manifest refraction spherical equivalent was within ±0.5 D of the desired refraction in 93.2% of the eyes. Average root mean square (RMS) wavefront error measured at central 6 mm, increased from 0.38 pre-operatively to 0.47 μm post-operatively. Refractive stability was achieved and sustained 1 month after surgery. No visually significant haze was registered during the observation period. Post-operative pain was reported in 4.5% of patients.

Conclusions: One-step transepithelial topography-guided treatment for low to moderate myopia and astigmatism performed with a 1 KHz laser, provided safe, effective, predictable and stable results with low pain and no visually significant haze.

Introduction

Due to its lower impact on corneal biomechanical stability and lower risk of dry eye[1,2] compared to LASIK, surface ablation is often used in cases with thin corneas, recurrent erosion, predisposition for trauma, or in patients who are anatomically or psychologically unsuitable for use of microkeratome.[3] Post-operative haze,[4–6] slow visual recovery,[7,8] and post-operative discomfort,[9,10] all inherent to the traditional photorefractive keratectomy, are now better controlled with modern surface ablation protocols. Smooth ablation achieved by small-Gaussian-beam, high-frequency lasers, pre-operative use of mitomycin-C, post-operative protection from UV-radiation and dietary supplementation with vitamin-C have previously reduced the incidence and severity of post-operative haze after surface ablation.[11–14] ENREF_10

Refinement of de-epithelialization techniques and pre- and post-operative medication continues to decrease the severity of post-operative discomfort[15] and to increase the speed of visual recovery.[16,17] The current transepithelial topography-guided ablation technique has been reported earlier for treatment of irregular astigmatism.[18–20] To our knowledge, not only has the combination of one-step transepithelial ablation, topography-guided custom ablation and 1000 Hz-laser technology never been published before, but there are few reports concerning any of the mentioned technologies; transepithelial ablation,[21–23] topography-guided ablation,[24–30] or use of 1000 Hz-laser[31,32] in routine treatments of low to moderate myopia in virgin eyes.

Patients and Methods

The present retrospective study comprises 117 consecutive eyes of 61 patients (30 female, 31 male) who were available for evaluation at ≥12 months after treatment for myopic astigmatism, at SynsLaser Clinic in Tromsø, Norway. The mean age was 32.71±9.7 years (range 18 to 61). Patients provided written,...
informed consent and the study was approved by the regional ethics committee “Regional komité for medisinsk og helsefaglig forskningsetikk, Nord-Norge (REK Nord)” - P REK NORD 55/2009 Sikkerhet et effekt av refraktiv kirurgi med iVIS lasersystem: en retrospektiv analyse av kliniske data. Inclusion criteria for treatment were: age ≥18 years; no soft contact lens wear for 1 week (hard contact lens for 4 weeks) before the baseline examination; SE between -0.5 and -10.00 diopters (D) with ≤5.00 D of refractive astigmatism; stable refractive error (change of SE ≤0.50 D) for ≥2 years; and CDVA of 20/25 or better. Exclusion criteria were: eye pathology, including keratoconus or keratoconus suspect (detected by corneal topo-/tomography); previous eye surgery; glaucoma; diabetes; and systemic diseases that could affect corneal wound healing (e.g. collagen vascular diseases).

The refraction, corneal anterior elevation and pachymetry maps of patients, as well as their dynamic pachymetry data were imported to “Corneal Interactive Programmed Topographic Ablation” (CIPTA, iVIS Technology, Taranto, Italy) software to generate a custom ablation plan within a treatment zone suggested by the dynamic pupillometry. The pupillometer measures pupil size and the speed of the pupillary reactions under various lighting conditions adjusted for the patient’s “life-style”. The result of the examination produces so-called “ideal entrance pupil” size, to target the optical zone diameter that can be overwritten by the user. The CIPTA software uses the first 2 weeks, and then replaced by a low potency steroid Rimexolone 1% (Vexol, Alcon, Fort Worth, Texas) eye drops in tapering doses for another 2–4 weeks. Refrigerated 0.2% hyaluronic acid (Ocyal, SantenPharma, Solna, Sweden) was used for lubrication and lavage purposes every 10 minutes the rest of the day after the surgery and later as needed. Additionally, the patients were supplied with “comfort drops”, a single container with 0.5 ml, 1% Tetracaine hydroxide (Tetrakain mimins, Chauvin, Kingston, England) in case of pain. All patients were questioned regarding postoperative pain and the need for use of “comfort drops”.

Bandage contact lens was removed from the cornea of patients between post-operative days 3 to 7 and patients were observed at 1, 3, 6, and 12 months after surgery. Post-operative examinations were similar to pre-operative examinations. Haze was defined as: grade 0 - clear; grade 0.5 - trace; grade 1 - trace easily seen using a slit-lamp microscope; grade 2 - dense patches affecting the corneal stroma, and the pupil eye-tracker-registration, the treatment was performed with a 0.6-mm dual-flying-spot 1 kHz (2×500 Hz) excimer laser (iRES, iVIS Technology, Taranto, Italy) using the one-step custom transepithelial “no-touch” (cTEN, iVIS Technology, Taranto, Italy) ablation technique.

At the end 1–2 non-preserved Chloramphenicol (Novarit, Basel, Switzerland) eye drops were applied, followed by a bandage contact lens (Acuvue Oasis, Johnson & Johnson, USA). Dexamethasone with Chloramphenicol mixture (Spersadex and Kloramfenikol, Novarit, Basel, Switzerland) eye drops QID were used the first 2 weeks, and then replaced by a low potency steroid Rimexolone 1% (Vexol, Alcon, Fort Worth, Texas) eye drops in tapering doses for another 2–4 weeks. Refrigerated 0.2% hyaluronic acid (Ocyal, SantenPharma, Solna, Sweden) was used for lubrication and lavage purposes every 10 minutes the rest of the day after the surgery and later as needed. Additionally, the patients were supplied with “comfort drops”, a single container with 0.5 ml, 1% Tetracaine hydroxide (Tetrakain mimins, Chauvin, Kingston, England) in case of pain. All patients were questioned regarding postoperative pain and the need for use of “comfort drops”.

Bandage contact lens was removed from the cornea of patients between post-operative days 3 to 7 and patients were observed at 1, 3, 6, and 12 months after surgery. Post-operative examinations were similar to pre-operative examinations. Haze was defined as: grade 0.5 - trace easily seen using a slit-lamp microscope; grade 1 – trace that does not affect vision; grade 2 - dense patches affecting the corneal stroma, and the pupil eye-tracker-registration, the treatment was performed with a 0.6-mm dual-flying-spot 1 kHz (2×500 Hz) excimer laser (iRES, iVIS Technology, Taranto, Italy) using the one-step custom transepithelial “no-touch” (cTEN, iVIS Technology, Taranto, Italy) ablation technique.
vision; grade 3 - dense partially obscuring iris details; grade 4 - dense haze completely obscuring iris details.

Statistical analysis was performed using SPSS 13.0. The initial Snellen visual acuity was recorded using logMAR steps. Calculation of mean visual acuity was achieved via conversion from Snellen to logMAR and back to Snellen. The outcomes were reported according to the Standardized graphs and terms for refractive surgery results.[34].

Results

Accountability
Among the 165 eyes of 86 patients treated for primary myopia from September 21 to December 19, 2009, 117 eyes of 61 patients (71%) were available for evaluation ≥12 months after surgery. Among those, 104 (89%), 110 (94%), and 66 (56%) eyes were also available for evaluation at 1, 3 and 6 months post-operatively, respectively.

Baseline data
The mean pre-operative UDVA and CDVA were 20/160 (range 20/1000 to 20/25) and 20/18.2 (range 20/20 to 20/13.3), respectively. The mean pre-operative SE was –3.22±1.54 D (range –0.63 to –7.25 D) and the mean cylinder was –0.77±0.65 D (range 0 to –4.50 D).

Efficacy
Cumulative UDVA at 6 and 12 months after surgery compared to pre-operative CDVA are shown on Figure 2. Efficacy index was 0.91, 1.00, 1.09 and 1.09, at 1, 3, 6 and 12 months post-operatively, respectively.

Predictability
The attempted versus achieved SE at 6 and 12 months post-operatively are shown in Figure 3. All eyes were within ±1.0 D of emmetropia, 94% of eyes were within ±0.5 D of emmetropia at 12 months and 97% of eyes had refractive astigmatism less or equal to 0.5 D at 12 months post-operatively.

Safety
Loss and gain of lines of CDVA are shown in Figure 4. The safety index was 1.27 for both 6 and 12 months.

Stability of refraction
The stability of refraction is shown on Figure 5. Post-operative spherical equivalent refraction stability was reached at 1 month, with no statistically significant difference between each two follow-up points thereafter. Seven (10.6%) out of 66 eyes available for a 6 month follow-up control, regressed more than -0.50 D between 6 and 12 months post-operatively.

Higher order aberrations
Pre-operative and 12-month post-operative average RMS of total HOAs, coma-type- (S3+S5+7) and spherical- (S4+S6+S8) aberrations are shown in table 1. RMS of total HOAs and coma-type aberrations increased, while the spherical aberration showed no statistically significant change.
Pain
On post-operative day one, 4.5% of patients reported pain, as assessed by need for use of “comfort eye drops”. No complaints of post-operative pain were registered thereafter.

Complications
No sight-threatening complications (decentration of ablation, infection, persistent epithelial defect, recurrent erosion, scarring or keratectasia) were observed. Thirteen eyes (11.1%) developed trace haze (grade ≤0.5) within 6 months after surgery, but corneas regained their pre-operative transparency without treatment within the next 6 months. No visual symptoms or loss of acuity that could be attributable to haze were found. No consistency with respect to the amount of treatment or any other preoperative parameters was found in the cases where the haze was identified.

Discussion
The appealing idea of photorefractive keratectomy (PRK) combined with excimer laser epithelial removal instead of mechanical or alcohol debridement has been explored during the last 15–20 years in various forms, with different lasers.[35–38] Lamellar (non-refractive) excimer laser ablation in the form of phototherapeutic keratectomy (PTK), has been used for laser epithelial removal preceding PRK. Using this approach, some studies have demonstrated minimal keratocyte apoptosis[39,40] and less haze,[41] as well as better visual outcomes[23] compared to mechanical epithelial debridement. Other studies, however, either did not achieve better outcomes compared to mechanical techniques,[42–45] or less pain compared to ethanol-assisted epithelial debridement.[46] Hence, transepithelial surface ablation has previously been limited to the treatment of highly irregular corneas.[18,22,47,48] To ensure the necessary predictability of outcome that is required for routine use of the transepithelial technique in treatment of refractive errors in virgin eyes, the following advancements in excimer laser technology were necessary: 1) sufficient ablation speed to avoid corneal hydration issues; 2) even radial thickness of each ablation layer for refractive-neutral epithelial ablation, and 3) a smooth ablation surface regardless of the high total ablation depth. With the iRES 1 KHz laser, a full
thickness epithelial removal can be performed in only 16 s, whilst a myopic ablation for 6 D (6.5 mm optical zone and 7.5 mm total ablation zone) requires 15 s. In conventional flying-spot lasers there is typically a linear increase in the number of pulses per mm² per s (local frequency), as the ablation area decreases (towards the end of the treatment). This leads to an increased thermal effect and plume production, culminating in a lower ablation effect, which is traditionally compensated for by empirical nomogram adjustments. Meanwhile, the laser used in the current study keeps a constant local frequency of 4 Hz, i.e. the laser beam will hit the same spot of the treated area 4 times per s. Hence, the entire ablation retains a consistent local delivery of energy across the ablation area, producing a constant and even thermal effect. This is crucial for achievement of the constant thickness of each ablation layer as well as a smooth ablation surface after the ablations of high volume of tissue (when both the epithelium and the stroma are ablated within one uninterrupted treatment). Linking refractive ablation with epithelial removal by performing the corneal reshaping on the epithelium and then translating the new shape to the stroma (Figure 1), assumes compatibility of ablation rates between the two corneal layers. Published data for one laser platform show a relatively small difference in the ablation rate between epithelium and stroma (0.55 ± 0.1 vs. 0.68 ± 0.15 μm per pulse),[49] but the difference in ablation rate between epithelium and stroma may differ among the lasers depending on their energy fluence, shot pattern and frequency. The laser used in the present study optimizes these three parameters to minimize differences in ablation rates between the epithelium and stroma. A former study by our research group demonstrated that transepithelial surface ablation using the iRES laser speeded reepithelialization and reduced post-operative pain compared to traditional PRK with Allegretto 400 Hz laser using Amoils brush for deepithelialization.[50].

The Scheimpflug-based Precisio topographer used in the current system provides true primary elevation information acquired by triangulation. Elevation data exported from Precisio are referenced to the center of the pupil. It is well known, however, that the pupil centroid will shift with different pupil size, resulting in a registration error that may significantly affect the quality of the outcome.[51] The current system addresses this issue by employing an intra-operative laser illumination adjustment, which automatically modulates the intensity of light until the same pupil size, as registered during the Precisio data acquisition, is achieved. The “constant pupil size” during the ablation contributes to a robust registration along with the systems iris/scleral vessel dynamic cyclotorsional tracking as well as its synchronized x, y-pupil-tracking.

Using topography-guided custom ablation in treatment of virgin eyes[52] implies correction of HOAs originating only from the corneal surface. This seems to be a reasonable approach as the
corneal surface is responsible for the majority of light refraction in the eye. Furthermore the corneal HOAs are static and hence more appropriate as a target for treatment than the dynamic HOAs of crystalline lens. In addition to correcting the corneal surface HOAs, the current topography-guided ablation creates a customized transition zone that keeps a constant dioptic gradient towards the untreated cornea, instead of employing commonly used fixed transition zone diameter. This may lead to lower regression by preventing any counterproductive epithelial remodeling.

Peer-reviewed publications from the recent five years reporting the outcomes of PRK in treatment of myopic astigmatism in virgin eyes are listed in table 2. The table shows that the safety, efficacy and predictability in our study are comparable with the best outcomes of the other studies, independent on the mode of epithelial removal or the excimer laser used. Moreover, the table shows only trace haze (grade ≤0.3). This also compares very favorably with the other studies. There is only one publication[21] reporting the outcomes using a similar approach to the current one, with the epithelial removal integrated within a single ablation. However, the treatments from that report were non-customized and only the initial 3-month results in 50 eyes were analyzed.

One shortcoming of the transepithelial technique used in this study is the epithelial thickness estimation (estimated to 65 μm by default), instead of using the real measurement. This estimation may lead to too deep ablation and waste of corneal tissue if the real epithelial thickness is lower, or to shallower ablation and a smaller optical/treatment zone than intended if the real epithelial thickness is higher. A high-resolution corneal imaging technology, that could be expected in the near future, is likely to provide the necessary precision for measurement of epithelial thickness. This may provide an epithelial thickness map usable for custom ablation planning. In that event, an ablation plan consisting of an epithelial and a stromal component would allow for accurate compensation for any difference in laser ablation rates between these two corneal tissues, further increasing the precision of the procedure. This would address the issues of slightly irregular ablation in eyes with non-uniform epithelial thickness,[33] as described in our previous work.[33] Precise epithelial thickness mapping would also be of great value in studying the influence of different epithelialization profiles on the refractive outcomes and would supposedly be of great help in refining the ablation design.

The idea of one-step “no-touch” laser treatment appeals to the patients because it is much quicker and more comfortable than traditional excimer laser surgery. The main appeal of this procedure to the surgeon is its perceived lack of serious complications as well as ease and speed of performance.

In conclusion, the outcomes of this study suggest that transepithelial topography-guided surface ablation, which integrates epithelial debridement and refractive error correction into a single custom ablation, is safe, effective, and predictable in treatment of low to moderate myopic astigmatism.

Author Contributions
Conceived and designed the experiments: AS SC XC FS JZ TZ. Performed the experiments: AS SC XC FS JZ TZ. Analyzed the data: AS SC XC FS JZ TZ. Contributed reagents/materials/analysis tools: AS SC XC FS JZ TZ. Wrote the paper: AS SC XC FS JZ TZ.

References


Reliability of Corneal Dynamic Scheimpflug Analyser Measurements in Virgin and Post-PRK Eyes

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Abstract

Purpose: To determine the measurement reliability of CorVis ST, a dynamic Scheimpflug analyser, in virgin and post-photorerefractive keratectomy (PRK) eyes and compare the results between these two groups.

Methods: Forty virgin eyes and 42 post-PRK eyes underwent CorVis ST measurements performed by two technicians. Repeatability was evaluated by comparing three consecutive measurements by technician A. Reproducibility was determined by comparing the first measurement by technician A with one performed by technician B. Intraobserver and interobserver intraclass correlation coefficients (ICCs) were calculated. Univariate analysis of covariance (ANCOVA) was used to compare measured parameters between virgin and post-PRK eyes.

Results: The intraocular pressure (IOP), central corneal thickness (CCT) and 1st applanation time demonstrated good intraobserver repeatability and interobserver reproducibility (ICC ≥ 0.90) in virgin and post-PRK eyes. The deformation amplitude showed a good or close to good repeatability and reproducibility in both groups (ICC ≥ 0.88). The CCT correlated positively with 1st applanation time (r = 0.437 and 0.483, respectively, p < 0.05) and negatively with deformation amplitude (r = −0.384 and −0.375, respectively, p < 0.05) in both groups. Compared to post-PRK eyes, virgin eyes showed longer 1st applanation time (7.29 ± 0.21 vs. 6.96 ± 0.17 ms, p < 0.05) and lower deformation amplitude (1.06 ± 0.07 vs. 1.17 ± 0.08 mm, p < 0.05).

Conclusions: CorVis ST demonstrated reliable measurements for CCT, IOP, and 1st applanation time, as well as relatively reliable measurement for deformation amplitude in both virgin and post-PRK eyes. There were differences in 1st applanation time and deformation amplitude between virgin and post-PRK eyes, which may reflect corneal biomechanical changes occurring after the surgery in the latter.

Introduction

The cornea is a viscoelastic structure with quantifiable biomechanical properties [1]. These properties are related to corneal thickness, age, intraocular pressure (IOP), hydration, and various pathologies [2–5]. The cornea’s biomechanical behaviour is mostly dictated by the stroma, which encompasses 90% of the total corneal thickness and has a greater mechanical stiffness than the other corneal layers [6].

Corneal biomechanical failure is the basis of keratectatic diseases [7] such as keratoconus and pellucid marginal degeneration. The ability to quantify corneal biomechanical failure represents an important step towards better understanding and treatment of keratectatic diseases. In addition, corneal refractive laser ablation in virgin eyes weakens the cornea mechanically due to tissue removal, leading to deterioration in corneal biomechanical strength [8]. Biomechanical changes may also affect the refractive outcome [9]. Moreover, biomechanical weakening after corneal refractive laser treatment may potentially induce iatrogenic keratectasia [10]. Therefore, knowledge of corneal biomechanical properties is important in predicting clinical outcomes [11] and in identifying cases with high risk for postoperative keratectasia after corneal refractive surgery. Most of the earlier studies concerning corneal biomechanical properties were performed in vitro [12–14]. The Ocular Response Analyser (ORA, Reichert Inc., Depew, NY) was the first device available to evaluate in vivo corneal biomechanical response to an air-puff [1]. It employs a quantitative electro-optical system to monitor the pressures at which the cornea flattens inward and outward by registering the corneal reflex of infrared light. The
recently introduced ultra-high-speed Scheimpflug video-imaging device (CorVis ST; Oculus, Wetzlar, Germany) is the first instrument allowing visualization and measurement of corneal deformation in response to a standardized air-puff pressure. Data evaluating the intraobserver repeatability and interobserver reproducibility of measurements with this relatively new device are scarce [15,16]. Furthermore, such studies as are available concern only healthy virgin eyes. The main goal of the present study was to test the hypothesis that the CorVis ST performs reliable measurements in both virgin and post-refractive surgery eyes. To our knowledge, this is the first study to evaluate the repeatability and reproducibility of CorVis ST measurements in post-refractive surgery eyes. The secondary purpose was to test the hypothesis that the measurements can reveal differences in biomechanical properties between these two groups.

**CorVis ST**

The CorVis ST utilizes an ultraviolet free blue (455 nm wavelength) light emitting diode (LED) and an ultra-high-speed (4330 frames per second) Scheimpflug camera to record the corneal deformation response to a high intensity air impulse. The air impulse originates from a metered, symmetrical, and fixed maximal internal pump generating a pressure of 25 kilopascal [16]. When the eye is aligned and the Scheimpflug image is in focus, the air puff gets released automatically and the cornea is imaged during the deformation event. The air pulse (lasting approximately 20 ms) forces the cornea inwards through application until it achieves its highest concavity (concavity phase). On its way back, the cornea undergoes a second application before achieving its natural shape. A total of approximately 140 images of the cornea’s two-dimensional cross-section are collected. By software tracing of the anterior and posterior corneal boundaries in individual image frames, parameters describing the corneal deformation response are automatically generated by the instrument. The CorVis ST software version 1.00r30 rev. 771 was used in the current study.

With the Corvis ST the biomechanical response of the cornea is characterized by three phases: 1a, application, highest concavity, and 2a, application. In addition to intraocular pressure (IOP) and central corneal thickness (CCT) values, time (time to reach application) and velocity (the velocity of the corneal apex movement during application) at the moment of both the 1a and 2a application events are recorded. The following characteristics at the point of highest concavity are also presented: the highest concavity time, parameters describing the corneal deformation response for the correlation analysis.

### Results

**Patient Demographics**

Forty candidates for laser refractive surgery (virgin-eye group: 28 males and 12 females) and 42 subjects treated for myopia and astigmatism with photorefractive keratectomy (PRK) earlier (post-PRK group: 23 males and 19 females) were recruited. The PRK treatments were performed using topography-guided transepithelial surface ablation with the iRES system (iRES, IVIS Technology, Taranto, Italy) at SynLaser Clinic in Tromsø, Norway, 12.69±10.08 months (range: 2 to 48) prior to the current examination. All participants received an extensive ophthalmic examination including Placido-based topography (Nidek OPD Scan II, Nidek Co. Ltd., Aichi, Japan), Scheimpflug topography (Priccio, IVIS Technology, Taranto, Italy), slit-lamp biomicroscopy and tonometry (Icare tonometer, Revenio Group Corporation, Helsinki, Finland) to exclude corneal and other ocular pathologies. The Regional Committee for Medical and Health Research Ethics in Norway approved the study entitled "2013/762 - Biomechanical cornea measurements by means of CorVis ST". The research complied with the tenets of the Declaration of Helsinki and written informed consent was obtained from each participant before examination. Only the data from the right eye of each participant was used for the present study.

The CorVis ST measurements were performed three times by technician A and one time by technician B. The measurement sequence between the technicians was randomized using a randomization table. A one-minute pause was given between each measurement. Repeatability was evaluated by comparing the three consecutive measurements performed by technician A. Reproducibility was determined by comparing the first measurement by technician A with the one performed by technician B. Mean CorVis ST measured values obtained from the three measurements by technician A were used to compare the differences between the virgin and post-PRK eyes groups, as well as for the correlation analysis.

### Statistical Analysis

MedCalc software 11.4.2 (MedCalc Software, Ostend, Belgium) and SPSS for Mac software (version 19, SPSS, Inc) were used for statistical analysis. A p-value of less than 0.05 was considered statistically significant. Descriptive statistical results were expressed as mean ± standard deviation (SD). The within-subject standard deviation (SD), within-subject coefficient of variation (Cov), and intraclass correlation coefficient (ICC) were determined to assess the intraobserver repeatability. Interobserver Sd, Cov, and ICC were calculated to assess interobserver reproducibility. Independent sample t-test was used to compare the CorVis ST measured parameters in virgin and post-PRK eyes groups. For the parameters that showed significant differences, univariate analysis of covariance (ANCOVA) was then applied to adjust for selected covariates (age, CCT measured by the CorVis ST, and mean simulated keratometry (simK) value measured by OPD Scan II) to control for potentially confounding factors. Pearson or Spearman correlations were applied to examine the relationship between CCT, manifest refractive spherical equivalent (MRSE) and the deformation parameters.

### Reproducibility

**Intraobserver Repeatability and Interobserver Reproducibility**

Tables 2 and 3 present the intraobserver repeatability of the CorVis ST measurements. In the virgin-eye group, the IOP, CCT, 1a application time, and 2a application time demonstrated good
repeatability (ICC ≥ 0.92), followed by deformation amplitude (ICC: 0.88), Radius of Curvature (ICC: 0.70), 2nd applanation velocity (ICC: 0.65), and highest concavity time (ICC: 0.64). The other parameters showed poor repeatability with large COVs and low ICCs. In the post-PRK group, the IOP, CCT, 1st applanation time, and deformation amplitude demonstrated good repeatability (ICC ≥ 0.90), followed by 2nd applanation time (ICC: 0.89), 2nd applanation velocity (ICC: 0.79), highest concavity time (ICC: 0.66), and radius of curvature (ICC: 0.63). The other parameters showed poor repeatability with large COVs and low ICCs.

When comparing the interobserver reproducibility of the CorVis ST parameters, the IOP, CCT, 1st applanation time, and 2nd applanation time demonstrated good reproducibility (ICC ≥ 0.91), followed by deformation amplitude (ICC: 0.88), radius of curvature (ICC: 0.64) and 2nd applanation velocity (ICC: 0.59) in the virgin-eye group. In the post-PRK group, the IOP, CCT, and 1st applanation time demonstrated good reproducibility (ICC ≥ 0.90), followed by deformation amplitude (ICC: 0.88), radius of curvature (ICC: 0.83), 2nd applanation time (ICC: 0.79), highest concavity time (ICC: 0.63), 2nd applanation velocity (ICC:

Figure 1. The CorVis ST utilizes the Scheimpflug camera to record the dynamic procedure of the corneal response to an air puff. A) The 1st applanation is achieved. B) The cornea reaches its highest concavity. C) The 2nd applanation is achieved when the cornea rebounds to its original position from the highest concavity.

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Table 1. Demographic Data of Participants.

<table>
<thead>
<tr>
<th></th>
<th>Virgin eyes (n = 40)</th>
<th>Post-PRK eyes (n = 42)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>27.9 ± 9.0 (18, 48)</td>
<td>31.8 ± 6.9 (20, 48)</td>
<td>0.03</td>
</tr>
<tr>
<td>CCT (Precisio), μm</td>
<td>547.82 ± 26.78</td>
<td>preop 542.02 ± 30.68 postop 485.00 ± 40.10</td>
<td>0.30* 0.000*</td>
</tr>
<tr>
<td>IOP (Icare), mmHg</td>
<td>15.20 ± 2.57</td>
<td>preop 15.81 ± 3.29 postop 12.71 ± 2.77</td>
<td>0.46* 0.000*</td>
</tr>
<tr>
<td>MRSE, D</td>
<td>−2.15 ± 2.28</td>
<td>preop −3.52 ± 1.93 postop 0.01 ± 0.48</td>
<td>0.03* 0.000*</td>
</tr>
<tr>
<td>Mean simK (OPD Scan III), D</td>
<td>43.47 ± 1.38</td>
<td>preop 43.81 ± 1.58 postop 40.87 ± 1.63</td>
<td>0.18* 0.000*</td>
</tr>
</tbody>
</table>

CCT = central corneal thickness; IOP = intraocular pressure; MRSE = manifest refraction spherical equivalent; simK = simulated keratometry.

*p values were adjusted for age-difference.

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Corneal Dynamic Scheimpflug Analyzer in Virgin and Post-PRK Eyes

Table 2. Intraobserver Repeatability of Parameters Obtained by Corvis in Virgin-Eye Group (n = 40).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± SD</th>
<th>Sw</th>
<th>2.77Sw</th>
<th>COV (%)</th>
<th>ICC (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IOP (mmHg)</td>
<td>14.46±1.33</td>
<td>0.59</td>
<td>1.62</td>
<td>3.59</td>
<td>0.93 (0.89–0.96)</td>
</tr>
<tr>
<td>CCT (µm)</td>
<td>543.32±25.08</td>
<td>5.34</td>
<td>12.56</td>
<td>0.69</td>
<td>0.99 (0.98–0.99)</td>
</tr>
<tr>
<td>1st appl. time (ms)</td>
<td>5.17±0.21</td>
<td>0.09</td>
<td>0.24</td>
<td>1.09</td>
<td>0.94 (0.90–0.97)</td>
</tr>
<tr>
<td>1st appl. length (mm)</td>
<td>1.83±0.18</td>
<td>0.29</td>
<td>0.81</td>
<td>13.94</td>
<td>0.10 (–0.52–0.49)</td>
</tr>
<tr>
<td>1st appl. velocity (m/s)</td>
<td>0.14±0.02</td>
<td>0.03</td>
<td>0.08</td>
<td>18.82</td>
<td>0.25 (–0.26–0.57)</td>
</tr>
<tr>
<td>2nd appl. time (ms)</td>
<td>21.65±0.34</td>
<td>0.17</td>
<td>0.48</td>
<td>0.71</td>
<td>0.92 (0.87–0.95)</td>
</tr>
<tr>
<td>2nd appl. length (mm)</td>
<td>2.03±0.29</td>
<td>0.45</td>
<td>1.24</td>
<td>21.53</td>
<td>0.17 (–0.39–0.53)</td>
</tr>
<tr>
<td>2nd appl. velocity (m/s)</td>
<td>–0.34±0.04</td>
<td>0.04</td>
<td>0.10</td>
<td>–9.77</td>
<td>0.65 (0.42–0.80)</td>
</tr>
<tr>
<td>Highest concavity time (ms)</td>
<td>16.40±0.37</td>
<td>0.37</td>
<td>1.02</td>
<td>2.02</td>
<td>0.64 (0.40–0.80)</td>
</tr>
<tr>
<td>Peak distance (mm)</td>
<td>4.36±0.66</td>
<td>1.17</td>
<td>3.23</td>
<td>21.80</td>
<td>–0.04 (~0.74–0.41)</td>
</tr>
<tr>
<td>Radius of curvature (mm)</td>
<td>7.49±0.60</td>
<td>0.55</td>
<td>1.51</td>
<td>6.31</td>
<td>0.70 (0.49–0.83)</td>
</tr>
<tr>
<td>Deformation amplitude (mm)</td>
<td>1.06±0.07</td>
<td>0.04</td>
<td>0.11</td>
<td>3.34</td>
<td>0.88 (0.81–0.93)</td>
</tr>
</tbody>
</table>

SD = standard deviation, ICC = intraclass correlation coefficient, CI = confidence interval, Sw = within-subject standard deviation, COV = within-subject coefficient of variation, IOP = intraocular pressure, CCT = central corneal thickness.

doi:10.1371/journal.pone.0109577.t002

Table 3. Intraobserver Repeatability of Parameters Obtained by Corvis in Post-PRK Group (n = 42).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± SD</th>
<th>Sw</th>
<th>2.77Sw</th>
<th>COV (%)</th>
<th>ICC (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IOP (mmHg)</td>
<td>14.46±1.33</td>
<td>0.59</td>
<td>1.62</td>
<td>3.59</td>
<td>0.93 (0.89–0.96)</td>
</tr>
<tr>
<td>CCT (µm)</td>
<td>543.32±25.08</td>
<td>5.34</td>
<td>12.56</td>
<td>0.69</td>
<td>0.99 (0.98–0.99)</td>
</tr>
<tr>
<td>1st appl. time (ms)</td>
<td>5.17±0.21</td>
<td>0.09</td>
<td>0.24</td>
<td>1.09</td>
<td>0.94 (0.90–0.97)</td>
</tr>
<tr>
<td>1st appl. length (mm)</td>
<td>1.83±0.18</td>
<td>0.29</td>
<td>0.81</td>
<td>13.94</td>
<td>0.10 (–0.52–0.49)</td>
</tr>
<tr>
<td>1st appl. velocity (m/s)</td>
<td>0.14±0.02</td>
<td>0.03</td>
<td>0.08</td>
<td>18.82</td>
<td>0.25 (–0.26–0.57)</td>
</tr>
<tr>
<td>2nd appl. time (ms)</td>
<td>21.65±0.34</td>
<td>0.17</td>
<td>0.48</td>
<td>0.71</td>
<td>0.92 (0.87–0.95)</td>
</tr>
<tr>
<td>2nd appl. length (mm)</td>
<td>2.03±0.29</td>
<td>0.45</td>
<td>1.24</td>
<td>21.53</td>
<td>0.17 (–0.39–0.53)</td>
</tr>
<tr>
<td>2nd appl. velocity (m/s)</td>
<td>–0.34±0.04</td>
<td>0.04</td>
<td>0.10</td>
<td>–9.77</td>
<td>0.65 (0.42–0.80)</td>
</tr>
<tr>
<td>Highest concavity time (ms)</td>
<td>16.40±0.37</td>
<td>0.37</td>
<td>1.02</td>
<td>2.02</td>
<td>0.64 (0.40–0.80)</td>
</tr>
<tr>
<td>Peak distance (mm)</td>
<td>4.36±0.66</td>
<td>1.17</td>
<td>3.23</td>
<td>21.80</td>
<td>–0.04 (~0.74–0.41)</td>
</tr>
<tr>
<td>Radius of curvature (mm)</td>
<td>7.49±0.60</td>
<td>0.55</td>
<td>1.51</td>
<td>6.31</td>
<td>0.70 (0.49–0.83)</td>
</tr>
<tr>
<td>Deformation amplitude (mm)</td>
<td>1.06±0.07</td>
<td>0.04</td>
<td>0.11</td>
<td>3.34</td>
<td>0.88 (0.81–0.93)</td>
</tr>
</tbody>
</table>

SD = standard deviation, ICC = intraclass correlation coefficient, CI = confidence interval, Sw = within-subject standard deviation, COV = within-subject coefficient of variation, IOP = intraocular pressure, CCT = central corneal thickness.

doi:10.1371/journal.pone.0109577.t003

0.60), and 2nd applanation length (ICC: 0.52), (Table 4 and 5). The other parameters showed poor reproducibility.

The IOP, CCT, and 1st applanation time demonstrated good intraobserver repeatability and interobserver reproducibility in both groups. The 2nd applanation time had good repeatability and intraobserver repeatability and interobserver reproducibility in the virgin eyes, with close to good repeatability but not good reproducibility in post-PRK eyes. The deformation amplitude showed a good or close to good repeatability and reproducibility in both groups.

Comparison of the Measurements between Virgin-Eye and Post-PRK Groups

Differences in the CorVis ST measured parameters between the virgin and post-PRK eyes are listed in Table 6. After adjustment for age, CCT, and mean simK, the differences in the mean values of IOP, 1st applanation time, 2nd applanation time, radius of curvature, and deformation amplitude remained significant.

Compared to the virgin-eye group, the post-PRK group demonstrated a shorter 1st applanation time, longer 2nd applanation time, smaller radius of curvature, and larger deformation amplitude. The CCT demonstrated a confounding effect in the above-mentioned parameters (p < 0.05 in all analyses), while age and simK did not show statistically significant confounding effects (p > 0.05 in all analyses).

Central corneal thickness measured with the CorVis ST correlated to IOP, 1st applanation time, radius of curvature, and deformation amplitude (r = 0.439, 0.437, 0.357, and –0.384, respectively, p < 0.05), without significant correlation to other parameters in the virgin-eye group. In the post-PRK group, it correlated to IOP, 1st applanation time, 1st applanation velocity, 2nd applanation length, 2nd applanation velocity, radius of curvature, and deformation amplitude (r = 0.492, 0.483, 0.401, 0.440, 0.395, 0.303, –0.373, respectively, p < 0.05). The MRSE correlated to IOP, 1st applanation time, and 2nd applanation time, without significant correlation to other parameters in the virgin-eye group.
(r = −0.485, −0.492, and 0.420, respectively, \(p<0.05\)). The postoperative MRSE was found to correlate only to radius of curvature in the post-PRK group (r = 0.583, \(p<0.05\)).

**Discussion**

*In vitro* experiments [12,13] as well as theoretical mathematical models [17,18] have demonstrated that the cornea exhibits both elastic and viscoelastic properties. When loaded, the cornea shows instantaneous deformation (purely elastic behaviour) followed by a time-dependent deformation response (viscoelastic behaviour) [19]. The ideal device for measuring corneal biomechanical properties in *in vivo* should be accurate, provide repeatable and reproducible results, and be minimally invasive. In the current study, the intraobserver repeatability and interobserver reproducibility of CorVis ST measurements in virgin eyes and post-PRK eyes were investigated.

**Table 4.** Interobserver Reproducibility of Parameters Obtained by Corvis in Virgin-Eye Group (n = 40).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean Difference ± SD</th>
<th>Sw</th>
<th>2.775Sw</th>
<th>COV (%)</th>
<th>ICC (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IOP (mmHg)</td>
<td>0.01 ± 0.26</td>
<td>0.58</td>
<td>1.60</td>
<td>3.25</td>
<td>0.92 (0.86–0.96)</td>
</tr>
<tr>
<td>CCT (µm)</td>
<td>−1.49 ± 6.76</td>
<td>4.78</td>
<td>13.24</td>
<td>0.72</td>
<td>0.98 (0.97–0.99)</td>
</tr>
<tr>
<td>1st appl. time (ms)</td>
<td>0.01 ± 0.12</td>
<td>0.08</td>
<td>0.23</td>
<td>0.96</td>
<td>0.93 (0.88–0.96)</td>
</tr>
<tr>
<td>1st appl. length (mm)</td>
<td>−0.02 ± 0.38</td>
<td>0.27</td>
<td>0.73</td>
<td>11.67</td>
<td>0.29 (–0.35(0.63)</td>
</tr>
<tr>
<td>1st appl. velocity (m/s)</td>
<td>(0.005 ± 0.05)</td>
<td>0.03</td>
<td>0.09</td>
<td>19.10</td>
<td>0.08 (0.75(0.51)</td>
</tr>
<tr>
<td>2nd appl. time (ms)</td>
<td>(0.01 ± 0.24)</td>
<td>0.17</td>
<td>0.47</td>
<td>0.61</td>
<td>0.91 (0.82–0.95)</td>
</tr>
<tr>
<td>2nd appl. length (mm)</td>
<td>(0.10 ± 0.58)</td>
<td>0.44</td>
<td>1.14</td>
<td>17.37</td>
<td>0.12 (0.65–0.53)</td>
</tr>
<tr>
<td>2nd appl. velocity (m/s)</td>
<td>(0.01 ± 0.06)</td>
<td>0.04</td>
<td>0.11</td>
<td>(9.63)</td>
<td>0.59 (0.25–0.78)</td>
</tr>
<tr>
<td>Highest concavity time (ms)</td>
<td>(0.02 ± 0.54)</td>
<td>0.38</td>
<td>1.06</td>
<td>1.81</td>
<td>0.47 (0.00–0.72)</td>
</tr>
<tr>
<td>Peak distance (mm)</td>
<td>0.11 ± 1.47</td>
<td>1.04</td>
<td>2.89</td>
<td>13.08</td>
<td>0.06 (1.45–0.63)</td>
</tr>
<tr>
<td>Radius of curvature (mm)</td>
<td>0.01 ± 0.81</td>
<td>0.57</td>
<td>1.58</td>
<td>5.01</td>
<td>0.64 (0.31–0.81)</td>
</tr>
<tr>
<td>Deformation amplitude (mm)</td>
<td>−0.002 ± 0.06</td>
<td>0.04</td>
<td>0.11</td>
<td>1.95</td>
<td>0.88 (0.78–0.94)</td>
</tr>
</tbody>
</table>

SD = standard deviation, ICC = intraclass correlation coefficient, CI = confidence interval, Sw = within-subject standard deviation, COV = within-subject coefficient of variation, IOP = intraocular pressure, CCT = central corneal thickness.

doi:10.1371/journal.pone.0109577.t004

Similar to the studies performed by Nemeth *et al.* [16] and Hon *et al.* [15], we found that the following parameters had the best repeatability in both groups: CCT, IOP, 1st applanation time, and deformation amplitude. The current study also presented good repeatability for 2nd applanation time. In addition, the ICCs in the current study were generally higher than in the mentioned studies for most of the parameters measured. The differences between the studies may be attributed to different patient populations and software versions. For example, in the study by Nemeth *et al.*, the mean age was 61.24±15.72 years (95% CI: 57.62 to 64.86 years), while the population in the current study was much younger. In the study by Hon *et al.*, the software did not offer values for radius of curvature and peak distance. When comparing reproducibility, Hon *et al.* found a statistically significant difference in the CCT measurement between the two sessions. However, the intersession difference was calculated by comparing the examinations performed in the morning (9:00–10:99 am) and afternoon (3:99–5:99 pm) by the same observer. This time difference may have

**Table 5.** Interobserver Reproducibility of Parameters Obtained by Corvis in Post-PRK Group (n = 42).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean Difference ± SD</th>
<th>Sw</th>
<th>2.775Sw</th>
<th>COV (%)</th>
<th>ICC (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IOP (mmHg)</td>
<td>−0.17 ± 0.70</td>
<td>0.50</td>
<td>1.38</td>
<td>2.81</td>
<td>0.90 (0.81–0.95)</td>
</tr>
<tr>
<td>CCT (µm)</td>
<td>0.43 ± 5.05</td>
<td>3.57</td>
<td>9.89</td>
<td>0.58</td>
<td>1.00 (0.99–1.00)</td>
</tr>
<tr>
<td>1st appl. time (ms)</td>
<td>−0.02 ± 0.11</td>
<td>0.08</td>
<td>0.23</td>
<td>0.84</td>
<td>0.90 (0.82–0.95)</td>
</tr>
<tr>
<td>1st appl. length (mm)</td>
<td>0.08 ± 0.46</td>
<td>0.32</td>
<td>0.90</td>
<td>14.45</td>
<td>0.27 (–0.36–0.60)</td>
</tr>
<tr>
<td>1st appl. velocity (m/s)</td>
<td>−0.01 ± 0.04</td>
<td>0.03</td>
<td>0.09</td>
<td>17.54</td>
<td>0.45 (–0.03–0.70)</td>
</tr>
<tr>
<td>2nd appl. time (ms)</td>
<td>0.04 ± 0.28</td>
<td>0.20</td>
<td>0.55</td>
<td>0.64</td>
<td>0.79 (0.61–0.89)</td>
</tr>
<tr>
<td>2nd appl. length (mm)</td>
<td>−0.11 ± 0.64</td>
<td>0.45</td>
<td>1.25</td>
<td>20.23</td>
<td>0.52 (0.12–0.74)</td>
</tr>
<tr>
<td>2nd appl. velocity (m/s)</td>
<td>0.01 ± 0.07</td>
<td>0.05</td>
<td>0.15</td>
<td>−11.03</td>
<td>0.60 (0.26–0.78)</td>
</tr>
<tr>
<td>Highest concavity time (ms)</td>
<td>0.05 ± 0.49</td>
<td>0.35</td>
<td>0.97</td>
<td>1.54</td>
<td>0.63 (0.31–0.80)</td>
</tr>
<tr>
<td>Peak distance (mm)</td>
<td>0.001 ± 1.57</td>
<td>1.11</td>
<td>3.08</td>
<td>15.23</td>
<td>0.26 (–0.39–0.61)</td>
</tr>
<tr>
<td>Radius of curvature (mm)</td>
<td>−0.06 ± 0.49</td>
<td>0.35</td>
<td>0.97</td>
<td>4.27</td>
<td>0.83 (0.68–0.91)</td>
</tr>
<tr>
<td>Deformation amplitude (mm)</td>
<td>−0.02 ± 0.15</td>
<td>0.04</td>
<td>0.12</td>
<td>3.89</td>
<td>0.88 (0.78–0.94)</td>
</tr>
</tbody>
</table>

SD = standard deviation, ICC = intraclass correlation coefficient, CI = confidence interval, Sw = within-subject standard deviation, COV = within-subject coefficient of variation, IOP = intraocular pressure, CCT = central corneal thickness.

doi:10.1371/journal.pone.0109577.t005
affected the reproducibility evaluation, as corneal thickness demonstrates diurnal variation [20]. The other parameters measured with the CorVis ST did not show satisfactory reliability. The ICCs varied between the virgin and post-PRK eyes.

It is conceivable that the cornea would be more difficult to deform and would deform less in eyes with a greater CCT. In line with other studies [15,21], we revealed a negative correlation between CCT and deformation amplitude in both groups. In addition, the CCT correlated positively with 1st applanation time and radius of curvature in both virgin and post-PRK eyes. However, correlations between CCT and 1st applanation velocity, 2nd applanation time, length, and velocity were only found in post-PRK eyes. This may imply that CCT in normal virgin eyes does not introduce much variation to some of the CorVis ST biomechanical properties. Both CCT and corneal biomechanical properties can affect IOP measurements, with the latter suggested to be more influential [18]. The difference in the CorVis ST measured IOP between the groups was most likely caused by changes in corneal biomechanical properties and CCT after PRK.

Interestingly, before being adjusted for age, CCT, and simK, the CorVis ST parameters that demonstrated differences between the virgin and post-PRK eyes (1\text{st} applanation time, 1\text{st} applanation velocity, 2\text{nd} applanation time, 2\text{nd} applanation velocity, deformation amplitude and radius of curvature) were the same parameters as those showing differences between normal eyes and keratoconus eyes in the study conducted by Ali et al. [23]. It seems that these parameters may be of value in evaluating corneal biomechanical properties.

The earlier start of the apex indentation (shorter 1\text{st} applanation time) and greater deformation amplitude in post-PRK eyes indicates a lower resistance to deformation due to a decrease in corneal stiffness [24,25]. Shen et al. [26] compared corneal deformation parameters after femtosecond laser small incision lenticule extraction (SMILE), laser-assisted sub-epithelial keratomileusis (LASEK), and femtosecond laser-assisted LASIK (FS-LASIK). They found greater deformation amplitude and shorter 1\text{st} applanation time in the FS-LASIK group compared to the LASEK group. However, those parameters did not differ significantly between the SMILE and LASEK groups, or between SMILE and FS-LASIK groups. This indicates that corneal refractive surgery alters the stiffness of the cornea to different degrees with respect to different surgical approaches.

In the current study the CorVis ST measurements in virgin- and post-PRK eyes were taken from two groups of unrelated populations. Pre- and postoperative comparison of the same population would have been better suited to evaluate the changes in biomechanical properties caused by the surgery. We attempted to compensate for this by applying age, CCT, and simK as covariates to adjust for potential confounding factors. For the sake of this discussion we also introduced a separate group of 28 eyes of
16 patients who underwent PRK for myopic astigmatism (mean preoperative MRSE: \( -3.35 \pm 1.98 \) D, mean postoperative time \( 9.21 \pm 5.09 \) months) with both pre- and postoperative CorVis ST measurements. The pre- and postoperative CorVis ST measurements of CCT and IOP in that group \([547.53 \pm 28.89 \, \mu m \, vs. \, 460.32 \pm 48.37 \, \mu m \, (p < 0.05), \) and \( 13.00 \pm 1.48 \, \mathrm{mmHg} \, vs. \, 13.48 \pm 2.14 \, \mathrm{mmHg} \, (p < 0.001), \) respectively) were similar to the differences found in the virgin and post-PRK eyes in the current study. Comparable similarity was also found for the \( 1_s \) applanation time \((7.37 \pm 0.23 \, \mathrm{ms} \, vs. \, 7.14 \pm 0.20 \, \mathrm{ms} \, (p < 0.001)), \) \( 2_s \) applanation time \((21.39 \pm 0.32 \, \mathrm{ms} \, vs. \, 21.57 \pm 0.25 \, \mathrm{ms} \, (p < 0.05)), \) radius of curvature \((7.76 \pm 0.83 \, \mathrm{mm} \, vs. \, 6.55 \pm 0.66 \, \mathrm{mm} \, (p < 0.001))), \) and deformation amplitude \((1.03 \pm 0.08 \, \mathrm{mm} \, vs. \, 1.10 \pm 0.08 \, \mathrm{mm} \, (p < 0.05))). \) Still, a separate study measuring pre- and post-PRK parameters with a larger population is warranted.

**Author Contributions**

Conceived and designed the experiments: XC AS TPU. Performed the experiments: DH JW. Analyzed the data: XC YH JRE. Contributed reagents/materials/analysis tools: XC AS YH JRE. Wrote the paper: XC AS YH JRE DH JW TPU.

**References**

Research Article

Safety and Efficacy of Epithelium-On Corneal Collagen Cross-Linking Using a Multifactorial Approach to Achieve Proper Stromal Riboflavin Saturation

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Purpose. To evaluate the efficacy and safety of epithelium-on corneal collagen cross-linking (CXL) using a multifactorial approach to achieve proper stromal riboflavin saturation. Methods. This non-randomized retrospective study comprised 61 eyes with progressive keratoconus treated with epithelium-on CXL. Chemical epithelial penetration enhancement (benzalkonium chloride-containing local medication and hypotonic riboflavin solution), mechanical disruption of the superficial epithelium, and prolongation of the riboflavin-induction time until verification of stromal saturation were used before the UV A irradiation. Uncorrected and corrected distance visual acuity (UDVA, CDVA), refraction, corneal topography, and aberrometry were evaluated at baseline and at 1, 3, 6, and 12 months postoperative. Results. At 12-month, UDVA and CDVA improved significantly. None of the eyes lost lines of CDVA, while 27.4% of the eyes gained 2 or more lines. Mean spherical equivalent decreased by 0.74 D, and mean cylindrical reduction was 1.15 D. Irregularity index and asymmetry from Scheimpflug-based topography and Max-K at the location of cone from Placido-based topography showed a significant decrease. Higher-order-aberration data demonstrated a slight reduction in odd-order aberrations S, 5, 7 (P = 0.04). Postoperative pain without other complications was recorded. Conclusion. Epithelium-on CXL with our novel protocol appeared to be safe and effective in the treatment of progressive keratoconus.

1. Introduction

Corneal collagen cross-linking (CXL) is a low-invasive treatment aimed to improve biomechanical stability in eyes with keratoconus [1–3]. A photodynamic reaction induced by photosensitizing riboflavin and ultraviolet A (UVA) light causes an increase of the number of intra- and interfibrillar covalent bonds and the corneal collagen resistance against enzymatic degradation [4–6]. Increased stromal biomechanical strength and lamellar compaction lead to stabilized corneal shape and better corneal symmetry, potentially causing an improvement in visual function [2, 7–9].

In CXL, riboflavin has a dual function acting both as a photosensitizer inducing the physical collagen cross-linking and as an absorber of the UVA irradiation, preventing damage to deeper ocular structures [10, 11]. Proper corneal stromal saturation with riboflavin is therefore essential in CXL, and without its presence the UVA radiation may cause the collagen fibers to degrade rather than to facilitate cross-linking [12].
The “standard CXL protocol” described by Wollensak and colleagues includes removal of the corneal epithelium in a diameter of 9 mm, followed by saturation of the corneal stroma using 0.1% isotonic riboflavin solution in 20% dextran [13]. This procedure is proved to be effective in increasing corneal stiffness [13], stabilization of keratoconus, and in some cases in improving the refractive and topographic features [14, 15]. Even so, the epithelial removal may lead to serious complications that include infection [16, 17], stromal haze [18], and corneal melting [19] in addition to severe pain and decrease in vision occurring during the first days after the treatment. To avoid such complications, Boxer Wachler et al. suggested a modification of the technique by keeping the epithelium intact (epithelium-on or transepithelial CXL) [20]. However, finding appropriate means of increasing corneal epithelial permeability prior to riboflavin application was warranted as riboflavin has a molecular weight of 338 Da, whereas the corneal epithelium is impermeable to compounds with a molecular weight greater than 100 Da [21]. Accordingly, various approaches have been tried clinically and in the laboratory to enhance the epithelial permeability before the riboflavin application. Chemical enhancers such as benzalkonium chloride (BAC), ethylenediaminetetraacetic acid (EDTA), gentamycin, tetracaine, and 20% ethanol [22–25] were used, as well as partial grid-like pattern deep epithelialization [22], excimer laser superficial epithelial removal [26], and the replacement of the isotonic by hypotonic riboflavin solution [24, 27]. The results varied between the studies, but the majority of the aforementioned methods lead to increased epithelial permeability for riboflavin.

In the current study, a multifactorial approach was utilized to enhance the riboflavin penetration by employing: (1) BAC-containing local medication; (2) hypotonic riboflavin solution without dextran; (3) increased riboflavin solution concentration; (4) mechanical disruption of the superficial epithelium (microabrasions); (5) prolongation of the riboflavin-induction time until objective verification of the stromal saturation is confirmed. By such an approach, this nonrandomized retrospective study aimed to evaluate the efficacy and safety of the epithelium-on CXL in treatment of progressive keratoconus.

2. Patients and Methods

In this retrospective, interventional case series, we reviewed the medical records of all patients with advanced progressive keratoconus who had 12-month observation time after the epithelium-on CXL treatment using our multifactorial approach. The treatment was performed at The Eye Department of the University Hospital North Norway, Tromsø, Norway, between September 15, 2009 and September 15, 2010. This study was approved by the regional ethics committee and adhered to the official ethical regulations for clinical research and the Tenets of the Declaration of Helsinki. Inclusion criteria included (1) documented progression of keratoconus during the last 12 months before treatment (increase of astigmatism or myopia by 1.00 D or increase in average SimK by 1.50 D); (2) minimum corneal thickness of no less than 400 μm at the thinnest point measured by ultrasound pachymetry; (3) age ranging from 18 to 45 years; (4) Amsler-Krumeich keratoconus classification stage II to III. Exclusion criteria were: (1) history of herpes virus keratitis; (2) severe dry eye; (3) concurrent corneal infections; (4) previous ocular surgery; (5) hard contact lens wear ≤4 weeks before the baseline examination.

Pre- and postoperative assessments consisted of slit lamp biomicroscopy, Scheimpflug-based corneal topography/tomography (Precisio, iVIS Technology, Taranto, Italy), Placido disk-based topography and wavefront aberrometry (ODP-Scan II, Nidek. Co., Ltd. Aichi, Japan), uncorrected (UDVA) and corrected (CDVA) distance visual acuities (Nidek RT 2100 system, Nidek Co. Ltd., Aichi, Japan), ultrasound pachymetry (Cornea-Gage Plus, Sonogage Inc., Cleveland, Ohio), tonometry (Icare tonometer, Revenio Group Corporation, Helsinki, Finland), and patients’ subjective evaluation of postoperative pain. The patients were examined at 1, 3, 6, and 12 months postoperative.

2.1. Surgical Technique. To reduce the risk for UV exposure of retroiridal eye structures, miosis was induced by applying two drops of pilocarpine 2% (Pilokarpin, Ophtha AS, Norway). It was followed by the application of two drops of local anesthetic proparacaine 0.5% (Alcaine, Alcon Norway AS), two drops of local antibiotic gentamycin 0.3% (Garamycin, Schering-Plough AS, Norway) followed by proparacaine again, one drop every minute for five minutes. All the drops were preserved by BAC (0.001% for Pilokarpin, 0.005% for Garamycin and 0.01% for Alcaine), aiming to increase the epithelial permeability by chemically disrupting the tight junction proteins. A round Merocel sponge (Medtronic, Inc., Minneapolis, MN) of 5 mm in diameter was inserted into the conjunctival sac to provide a depot of riboflavin, and to produce microabrasions of the superficial epithelial layers caused by friction upon patient’s blinking. Thereafter, two drops of proparacaine and two drops of 0.5% aqueous riboflavin solution without dextran (Vitamin B2; Streuli, Uznach, Switzerland) were applied alternating every 30 seconds, until the riboflavin saturation was verified by the slit-lamp inspection of the cornea and by the determination of the presence of riboflavin flare in the anterior chamber (Figure 1). Under the same examination the staining of the epithelial microabrasions was verified. The initial slit-lamp saturation evaluation was performed 25 minutes after the first application of riboflavin and repeated every five minutes until the saturation was confirmed. During the premedication and riboflavin induction time the patient was instructed to blink normally between eye drop instillation and to remain in a comfortable sitting position. The Merocel sponge was then removed and corneal thickness measured with ultrasound pachymetry, at which point the patient was placed in supine position. Irrigation with isotonic balanced salt solution (BSS) was performed before the UVA irradiation in order to avoid the shielding effect of riboflavin covering the epithelium. An eyelid speculum was then inserted, and a ring-shaped Merocel shield k20-5021 (Katena Products, Inc.
Figure 1: Slit lamp verification of the stromal riboflavin saturation before the UV A irradiation.

Denville, NJ) was applied to protect the limbal region and its stem cells from UV A radiation.

The cornea was subjected to UV A radiation for 30 minutes with a wavelength of 365 nm at a working distance of 5 cm. The UV-X lamp (IROC AG, Zürich, Switzerland) provided an irradiance of 3 mW/cm² within a circular diameter of 9 mm. During the irradiation, BSS was applied every three minutes, and proparacaine drops were added as needed.

After the UV A irradiation, two drops of atropine 1% (Atropin minims, Chauvin, England) and 2 drops of gentamycin were applied. The cornea was protected with a soft bandage contact lens for 12–18 hours. Instructions were given to apply a mixture of 0.1% dexamethasone and 0.5% chloromycetin (Spersadex med Kloramfenikol, Novartis, Norway) eye drops four times daily for seven days, as well as to use artificial tears as needed.

2.2. Statistical Analysis. All visual acuity values were recorded as Snellen values, converted to LogMAR for statistical analyses and then changed back to Snellen values for presentation purposes. Pre- and postoperative topography was analyzed using Precisio’s irregularity index (IRI) as well as by measuring the central 5 mm using OPD indices. Statistical analysis was performed to compare the postoperative data with the preoperative data using the paired t-test with IBM SPSS Statistics v19.0 (IBM, Armonk, NY). P < 0.05 was considered statistically significant.

3. Results

Sixty-one eyes of 53 patients fulfilled the inclusion and exclusion criteria. The mean age of the patients was 32 ± 10 years (range, 15–52 years). 85% of the eyes (52 eyes) were from male patients.

3.1. Visual Acuity. Figures 2, 3, 4, and 5 and Table 1 show the visual acuity and refraction measurements pre- and postoperatively. The UDVA and CDVA improved significantly (P < 0.05). At 12-month followup, none of the eyes lost lines of CDVA, while 27.4% of the eyes gained 2 or more lines and the safety index was 1.14. At the same time point, mean spherical equivalent refraction decreased by 0.74 D
3.2. Corneal Topography and Wavefront Aberrometry. Table 2 shows the postoperative changes of topography and aberrometry. Data from the Precisio showed reduction in posterior elevation ($P = 0.01$), irregularity index ($P = 0.01$), and asymmetry ($P = 0.01$). The $K$-value from OPD did not significantly alter regarding Mean-$K$, while the Max-$K$ showed a significant decrease ($P = 0.02$) at the location of the cone. Figure 6 shows an example of the topographic changes in one of the treated eyes.

Aberrometry data showed a reduction in odd-order S 3.5,7 ($P = 0.04$) and total higher-order-aberrations ($P = 0.05$).

3.3. Pachymetry. Precisio-measured pachymetry in Table 1 shows decrease in thickness at 1-month followup ($P = 0.00$) and thereafter a gradual increase to preoperative level at 12 months after the treatment ($P = 0.15$).

3.4. Pain Evaluation. Patients reported moderate to severe postoperative pain during the first 4–12 hours, peaking at 4–6 hours after surgery.

3.5. Complications. Discreet superficial epithelial layer damage could be observed on slit-lamp examination upon the verification of riboflavin saturation and after the CXL treatment. No serious complications were recorded during the follow-up period.

4. Discussion

Previous studies report conflicting results on the effects of epithelium-on CXL. While Pinelli and colleagues reported no significant difference in the analyzed parameters between epithelium-on and standard CXL [28], Wollensak and Iomdina found that the corneal biomechanical stiffening after epithelium-on CXL was about one-fifth compared to the epithelium-off CXL in an animal model [4]. Other clinical and laboratory studies have reported weaker or no effect of CXL using the epithelium-on method [22, 23, 25, 29–31]. Collectively, the studies suggested that the significantly weaker biomechanical effect of epithelium-on CXL was due to the insufficient and inhomogeneous transepithelial riboflavin diffusion into the corneal stroma. However, a limitation of most of the studies that procured the low cross-linking effect or low stromal saturation of riboflavin with the epithelium-on CXL includes the use of the standard—or only slightly modified—Wollensak/Seiler protocol on nondeepithelialized eyes. Moreover, the authors did not attempt to enhance the riboflavin penetration, effectively only showing that the epithelium-on CXL does not work with the standard epithelium-off protocol. Intriguingly, epithelial permeability can be enhanced by application of several tensioactive substances including BAC and gentamicin at concentrations normally used in industrial preparations [32]. Such pharmacological enhancements, which are commonly used in epithelium-on CXL [20, 33], were included in the current protocol.

On the basis of previous studies reporting increased epithelial riboflavin permeability using hypotonic solution compared to isotonic solution [24, 27, 34], hypotonic solution was applied in the current protocol. Furthermore, we avoided the use of riboflavin solution with dextran due to its high viscosity, which inhibits the penetration through the epithelium [35]. Hypotonic riboflavin was originally used with epithelium-off CXL protocol to induce significant edema in corneas thinner than 400 $\mu$m [27]. However, the swelling of the corneas with intact epithelium seems to be of a considerably lower degree. In the present study, only around 10 microns of swelling were recorded in a subgroup of 29 eyes, presenting respective mean pachymetry of 450.50 ± 42.90 and 471.65 ± 41.43 $\mu$m before and after the corneal saturation with the 0.5% hypotonic riboflavin solution. Even though the clinical safety of CXL with hypotonic riboflavin solution has been documented [36], issues of the corneal endothelial cell toxicity were considered because of the decreased UV-protective effect with
hypotonic riboflavin due to the decrease of UV absorption coefficient from $\approx 53 \text{ cm}^{-1}$ for 0.1\% isotonic Riboflavin solution to $\approx 42 \text{ cm}^{-1}$ for 0.1\% hypotonic riboflavin solution [36]. To compensate for this, increasing the concentration of riboflavin in the hypoosmolar solution may enhance UV absorption [37] and hence decrease the UV-radiation at the endothelial level. The current study addresses the endothelial safety as hypotonic riboflavin concentration of 0.5\% was applied. A secondary benefit of the increased concentration is the presumably increased availability of the riboflavin molecules to penetrate the epithelium and saturate the stroma. In a subgroup analysis of 21 eyes performing pre- and postoperative specular microscopy using Konan CellCheck XL (Konan Medical, Irvine, CA), the endothelial count decreased insignificantly ($P = 0.09$) from 2738 ± 188 cells/mm$^2$ to 2608 ± 311 cells/mm$^2$.

The current protocol also employed mechanical scarification of the epithelial surface by creation of microabrasions caused by movement of a Merocel sponge over the corneal surface with patient’s blinking. The amount of such microabrasions could obviously not be standardized, and it varied between cases. This may be the reason for a relatively large variation in saturation time (mentioned in the next paragraph).

Finally, in addition to the chemical and mechanical enhancements, the current protocol demanded a slit-lamp verification of the stromal saturation before the UVA radiation (Figure 1). Our clinical observations showed that riboflavin saturation was achieved after 25–45 minutes, so that a commonly used set induction time of, for example, 30 minutes would in many cases lead to insufficient riboflavin concentration in the stroma.

Our visual and refractive outcomes were comparable to other published CXL studies. In the current study there were no cases with a loss of ≥2 lines of CDVA, endothelial cell count did not change significantly, and there were no infections or other types of keratitis.

Most of the patients reported pain peaking 4–6 hours after the treatment despite the mostly preserved epithelium. This may be explained by actinic-keratoconjunctivitis-like reaction caused by the UV exposure during the treatment.
Corneal topography change in curvature has often been used to evaluate the efficacy of CXL [23, 38, 39]. In our study, the maximum-$K$ value and DSI (differential sector index) decreased significantly on the OPD II, Placido-based topography, as did the posterior elevation, irregularity index and asymmetry on the Precisio, Scheimpflug-based topography. However, our mean-$K$ did not decrease in contrast to most other studies. In most of our cases, in addition to the Max-$K$ decrease, a moderate increase in steepness on the opposite side of the cone occurred (Figure 6), resulting in only minor decrease of the mean-$K$ but more symmetrical corneal optics, decreased higher-order-aberrations, and improved vision. We hypothesize that this may be a consequence of a possibly heavier riboflavin load in the inferior corneal stroma due to the sitting position and blinking during the riboflavin induction, leading to a locally increased cross-linking effect. This theory warrants a further study and may be a small step in the direction of “customized” CXL.

Detection of the demarcation line [40] after CXL has been considered the proof of the efficacy and the measure of the depth of the corneal cross-linking. Although the precise nature and significance of the demarcation line (increased optical density) in relation to the cross-linking process are uncertain, it may be consequent to the keratocyte apoptosis and their subsequent repopulation [41]. Keratocyte apoptosis has to a lesser extent been demonstrated after epithelium-on CXL [4]. Filippello et al. epithelium-on CXL with 0.1% isotonic riboflavin solution showed that the demarcation line two weeks postoperatively was located approximately 100 μm from the corneal epithelium [23]. In 24 eyes treated with the current protocol that could be evaluated by RTVue (Optovue Inc., Fremont, CA) AS-OCT (anterior segment optical coherence tomography), the mean demarcation line was located at the depth of 316.92 ± 49.16 μm (range 260 to 367) from the surface (Figure 7), which is close to the observations after epithelium-off CXL.

Epithelial absorption/filtering of the UV A light that could potentially lead to lesser energy delivered to riboflavin-saturated stroma has also been stated as an argument against the use of epithelium-on CXL. Different studies offer a variety of evaluation backgrounds of this matter. A study performed by Baiocci et al. [21] claimed that human corneal epithelium and the underlying basement membrane naturally absorb 30% to 33% of UVA radiation (400 to 350 nm), while other studies showed that the epithelial UV absorption occurs only with wavelengths lower than 310 nm [42–44]. We may assume that the UV-absorption of the riboflavin within the epithelium is probably low (since the epithelial cells are hydrophobic and do not absorb...
riboflavin) and that the epithelial interstitial space is of negligible volume. The current protocol includes washing off the riboflavin from the corneal surface before the UVA-radiation in order to minimize the UV-energy loss due to its possible absorption by the riboflavin.

Finally, even if the epithelium-on CXL leads to a shallower cross-linking compared to the epithelium-off, the density of collagen fibers in corneal stroma is much higher in the anterior portion where most of the collagen cross-links occur [40, 45].

5. Conclusion

This retrospective study showed that epithelium-on CXL using our novel protocol appeared to be effective and safe in treating progressive keratoconus. The improvements in the visual, refractive, and topographic parameters in our patients indicate that epithelium-on CXL had sufficient effect to halt the progression of keratoconus and improve the corneal shape. A randomized controlled trial is warranted to verify that the effect of the current approach is comparable with the standard epithelium-off CXL. Furthermore, the combination of different enhancers, osmolality, and concentration of riboflavin should be explored to further improve the procedure.

Conflict of Interests

None of the authors has a commercial, proprietary, or financial interest in any material or procedure mentioned.

References


