Optical Coherence Tomography and Densitometry in Assessing the Effect of Corneal Cross-Linking Upon Photorefractive Ablation with Riboflavin

Abstract

**Purpose:** To assess the effect of corneal cross-linking upon photorefractive ablation with riboflavin according to optical coherence tomography and densitometry of the cornea.

**Methods:** Transepithelial photorefractive keratectomy, laser in situ keratomeluses, and femtosecond laser in situ keratomeluses were performed on 402 eyes in 201 patients with myopia and myopic astigmatism of varying degrees. Photorefractive ablation was performed after corneal stroma saturation with 0.25% isotonic riboflavin. Follow-up varied from 1 month to 4 years. The effects of corneal cross-linking were assessed by spectral-domain optical coherence tomography and corneal densitometry.

**Results:** The effect of crosslinking upon photorefractive ablation with riboflavin was accompanied by a damped effect of increased optical density in the layers of the stroma adjacent to the ablation zone. Corneal densitometry and anterior segment optical coherence tomography demonstrate that corneal cross-linking increases optical density of the residual stroma. Thin membrane-like structure on the ablation surface as well as higher optical density of the corneal stroma adjacent to the ablation zone are revealed after transepithelial photorefractive keratectomy with riboflavin. In addition to the higher optical density of the corneal stroma, demarcation line of varied intensity (which resolves later) is revealed after laser in situ keratomeluses, and femtosecond laser in situ keratomeluses. This improves and compensates impaired mechanical and photoprotective functions of the ablated cornea. Corneal cross-linking induced by photorefractive ablation with riboflavin is realized through the series of consequent reactions of riboflavin activation. Activation of riboflavin with the secondary radiation induced by corneal ablation is considered as a trigger. Broad spectrum of the secondary radiation covers all four peaks of maximum riboflavin absorption.

**Conclusion:** The formation of a membrane-like structure on the ablation surface, the increased optical density and the demarcation line in the corneal stroma layers are the objective criteria for the cross-linking effect upon photorefractive ablation with riboflavin according to optical coherence tomography and corneal densitometry.

**Keywords**

Laser-Induced Cross-Linking; Photorefractive Ablation; Riboflavin; Secondary Excimer Laser Radiation
Introduction

Corneal thinning affects not only biomechanics of the cornea but also its photoprotective function which has an important role in intraocular protection against external Ultraviolet (UV) irradiation [1,2]. The outcomes of modern corneal photorefractive surgeries demonstrate that most of these procedures result in corneal thinning which can cause corneal ectasia. This complication occurs more frequently after laser in situ keratomileusis (LASIK) or femtosecond laser in situ keratomeluses (FemtoLASIK). Thus, flap creation reduces corneal biomechanical strength by 15-35% and this range results from individual characteristics of flap size and thickness. Photorefractive ablation as a second step of LASIK provides even more profound decrease in corneal strength. The higher is ametropia degree, the greater the decrease in corneal strength is [3-5].

Various corneal cross-linking (CXL) protocols were described to prevent corneal ectasia after refractive surgery [6,7]. Thus, classical and accelerated epithelium-off (epi-off) or transepithelial CXL totals an energy output of 5.4 J/cm². This cumulative dose of UV light will inevitably results in extra generation of peroxyl radicals which contribute to the development of oxidative stress induced by photorefractive ablation. That is why fibroplasia of various degree with the development of stromal corneal opacities, excessive corneal flattening, and reduced accuracy of refractive outcomes are observed in some patients after LASIK and CXL. Considering this, ophthalmologists initiated the debates on the usefulness of standard or accelerated CXL during photorefractive procedures. In recent years, several studies were published which consider the exposure of half-dose UV as well as significantly reduced exposure time to riboflavin and irradiation time [7]. Some patients (in particular, those with thin cornea and high myopia) require an intervention to stiffen the cornea during PRK. However, CXL during Photorefractive Keratectomy (PRK) increases oxidative stress in the cornea and the risk of subepithelial fibroplasia regardless of myopia degree. Considering this, it is undoubtedly important to develop minimally invasive treatment protocol of CXL.

Purpose

To assess the effect of corneal cross-linking upon photorefractive ablation with riboflavin according to optical coherence tomography and densitometry of the cornea.

Materials and Methods

Various studies on corneal collagen cross-linking and secondary ablation-induced radiation originating from the interaction of 193-nm excimer laser radiation with the cornea were reviewed. Ablation with riboflavin was performed in 201 patients (402 eyes) with myopia and myopic astigmatism aged 18-55 (mean age 24.4 ± 4.6 years). The study was approved by the Institutional Ethics Committee of the NI Pirogov National Medical Surgical Center. The study and data collection were performed in compliance with the Declaration of Helsinki and local laws. Written informed consents for the surgery and the study were obtained from all patients. Excimer laser ablation was performed after aerosol or drop-by-drop corneal saturation with isotonic 0.25% riboflavin for 3 min. Transepithelial PRK (TransPRK) has gained special attention. In the course of TransPRK, riboflavin aerosol was applied to the corneal surface using ultrasonic nebulizer after corneal epithelial debridement. In the course of LASIK and FemtoLASIK, corneal stroma was saturated with 0.25% riboflavin for 3 min after flap creation. Follow-up varied from 1 month to 4 years. Photorefractive procedures were performed with the MEL-80 Excimer Laser (Carl Zeiss Meditec, Jena, Germany), WaveLight® EX500 Excimer Laser (Alcon, Fort Worth, USA), WaveLight® Allegretto Wave® 200 Hz (Alcon, Fort Worth, USA), and Excimer Microscan Visum 500® (Optosystems, Troitsk, Russia). Femto-LASIK hinged corneal flap was created with the VisuMax® Femtosecond Laser (Carl Zeiss Meditec, Jena, Germany), WaveLight® FS200 Femtosecond Laser (Alcon, Fort Worth, USA), and Femto LDV-Z8 (Ziemer Ophthalmic Systems, Port, Switzerland). Corneal topography, aberrometry, and corneal densitometry were performed with the Pentacam® HR (Oculus, Wetzlar, Germany), WaveLight® Analyzer (Alcon, Fort Worth, USA), WaveLight® Topolyzer™ VARIO (Alcon, Fort Worth, USA), WaveLight® Oculyzer™ (Alcon, Fort Worth, USA), and Topographic Modeling System TMS-5 (Tomey Corporation, Nagoya, Japan). High-resolution spectral-domain anterior segment OCT was performed with the Cirrus™ HD-OCT 5000 (Carl Zeiss Meditec, Jena, Germany), RTVue 100 and RTVue XR100 (Optovue, Fremont, USA).

Results

The summary table presents the main characteristics of the clinical material with various technologies of photorefractive ablation with riboflavin (Table 1). According to densitometry and OCT of the cornea during photo refraction ablation with riboflavin regardless of the type of operation, in all cases an increase of optical density was observed in the cornea layers adjacent to the ablation zone. Depending on the type of operation and the degree of myopia, a different frequency of the appearance of the demarcation line after photorefractive ablation with riboflavin was noted (Table 2).
Table 1: Characteristics of clinical material with various technologies of photorefractive ablation with riboflavin (402 eyes, 201 patients).

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Weak myopia</th>
<th>Moderate myopia</th>
<th>High myopia</th>
<th>Myopia of all degrees</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of patients</td>
<td>114</td>
<td>51</td>
<td>36</td>
<td>201</td>
</tr>
<tr>
<td>Men</td>
<td>58</td>
<td>26</td>
<td>18</td>
<td>102</td>
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<tr>
<td>Women</td>
<td>56</td>
<td>25</td>
<td>18</td>
<td>99</td>
</tr>
<tr>
<td>Number of eyes</td>
<td>228</td>
<td>102</td>
<td>72</td>
<td>402</td>
</tr>
<tr>
<td>TransPRK with riboflavin</td>
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<td>70</td>
<td>42</td>
<td>312</td>
</tr>
<tr>
<td>LASIK with riboflavin</td>
<td>10</td>
<td>8</td>
<td>10</td>
<td>28</td>
</tr>
<tr>
<td>FemtoLASIK with riboflavin</td>
<td>18</td>
<td>24</td>
<td>20</td>
<td>62</td>
</tr>
</tbody>
</table>

Table 2: The frequency of appearance of the demarcation line in the corneal stroma after various technologies of photorefractive ablation with riboflavin, taking into account the initial degree of myopia.

The refusal of the external UV and the use of the secondary (ablation-induced) radiation to activate riboflavin in the corneal stroma is the key factor of the novel approach to the preventive CXL for corneal photorefractive surgery. Clinical observations on the outcomes of TransPRK after stromal saturation with riboflavin demonstrated reduced levels of aseptic inflammatory response, more rapid epithelial healing, and earlier stabilization of optometric and visual outcomes. Epithelial healing was complete within two days (or even within a day) after TransPRK with riboflavin. This phenomenon can be accounted for by less significant oxidative stress and aseptic inflammatory response as well as by improved ablation surface. Corneal densitometry revealed higher optical density of the corneal stroma adjacent to the ablation zone. Occasionally, anterior segment OCT revealed thin membrane-like structure beneath the epithelium just after epithelial healing was complete (Figure 1,2).

Figure 1: Spectral-domain OCT of the cornea. Thin membrane-like structure is revealed under the epithelium two days after TransPRK with riboflavin.
Membrane-like structure was revealed when its thickness was more than 5 microns (which is equal to OCT resolution). Occasionally, a very fine reversible demarcation line was revealed after 2 to 4 weeks (Figure 3). The outcomes of clinical studies on TransPRK with riboflavin have been described in detail in our previous papers [2,8-12] are not reviewed here.

**Figure 2:** Spectral-domain OCT of the cornea. Membrane-like structure is revealed under the epithelium on seven days after TransPRK with riboflavin.

**Figure 3:** Spectral-domain OCT cross line of the cornea. Demarcation line is revealed one month after TransPRK with riboflavin.
Clinical observations on the outcomes of photorefractive ablation (LASIK, FemtoLASIK) with riboflavin were particularly illustrative (Figure 4, 5). Postoperative OCT demonstrated a more significant increase in the optical density of corneal stroma beneath the flap. Demarcation line with a wide range of thickness was revealed 2 to 4 weeks after the operation. The formation of the demarcation line at various depths was in good agreement with the features of the different distribution of irradiation along the ablation field and the damped nature of its absorption [13]. These findings provided a basis for further development of the technology of laser in situ keratomileusis with laser-induced cross-linking. The results of these studies will be published later due to patent pending of this technology.

**Figure 4:** Spectral-domain OCT cross line of the cornea. The increase of optical density of the cornea is one week after LASIK with riboflavin.

**Figure 5:** Spectral-domain OCT cross line of the cornea. Demarcation line is one month after LASIK with riboflavin.
In cases of suspicion of initial keratoectasia or subclinical keratoconus (Figure 6), we used special parameters of excimer laser radiation (patent pending) that extended the possibilities of laser modification of the ablation surface with the formation on it of a membrane-like structure with the effect of cross-linking in the adjacent layers of the stroma (Figure 7).

Figure 6: The case of suspicion of initial keratoectasia (subclinical keratoconus).

Figure 7: The case of laser modification of the ablation surface with the formation of a membrane-like structure on it with the effect of cross-linking in the adjacent layers of the stroma.
Discussion

The data available in the literature indicate the possibility of evaluating the effect of cross-linking according to OCT and densitometric studies. The advantage of such studies is the possibility of their wide application in the clinic and obtaining objective indicators of optical density. In addition, many researchers evaluate corneal cross-linking by the presence of the demarcation line and the depth of its location in the stroma. However, even with standard corneal cross-linking technology, the demarcation line was not observed in all cases. Moreover, the depth and severity of the demarcation line varied in a wide range. In our laser-induced cross-linking technology, the cross-linking effect is significantly weaker. This may explain the significantly lower frequency of detection of the demarcation line during photorefractive ablation with riboflavin. Nevertheless, only the results of tensiometric studies in the experiment make it possible to evaluate the biomechanical effect of corneal cross-linking. Our previous experimental studies confirmed the presence of the cross-linking effect upon photorefractive ablation with riboflavin. Biomechanical testing of corneal samples showed an increase in tensile strength by 35-47% without significant changes in the Young’s modulus [8]. Biomechanical effect of laser-induced CXL was lower than of classical and accelerated cross-linking. However, according to the safety criterion, there was no doubt in the advantage of the use in photorefractive surgery ablation-induced secondary radiation. This novel method of photorefractive ablation can be considered as a pathogenesis-targeted one [13] since corneal stromal layers saturated with riboflavin absorb secondary radiation induced by photorefractive ablation. Absorption was of a damping nature. Maximum effect was observed in the stromal layers closely adjacent to the ablation zone. All these factors reduced overall adverse effect of the secondary radiation. This is due to the fact that dynamic prolonged scanning was performed with a small-spot laser beam. Moreover, deoxygenation of corneal stroma was better when the scanning was performed with a small-spot laser beam.

Short-term action of the secondary (ablation-induced) radiation unwittingly doubts its adequacy to initiate cross-linking. Published data demonstrate the potential for different cross-linking effect through the reduced exposure time to UV light and significant variations in UV light intensity [21-24]. Theoretical basis for the potential cross-linking effect under the short exposure time to UV light was provided by Semchishen et al. [25]. The authors have demonstrated that it is possible to reduce UV exposure time significantly (to 70-80 sec).

We believe that corneal cross-linking in vivo will inevitably result in the violation of the Bunsen-Roscoe law which states that the effect of radiation is always the same regardless of the variation in the intensity or in the time of radiation. This is due to the fact that dynamic prolonged secondary reactions occur in vivo in response to the corneal oxidative stress and aseptic inflammation. Meanwhile, peroxyl radicals, nitrogen oxide accumulated in the corneal stroma provide an additional effect on riboflavin thus amplifying the effect of cross-linking.

According to our CXL technique for corneal photorefractive surgery, exposure time to the secondary (ablation-induced) radiation is determined by the extent of keratectomy and is less than 60 sec. However, experimental studies on biomechanical testing have demonstrated that this time was quite enough to initiate cross-linking effect in the corneal stroma saturated with 0.25% isotonic riboflavin. Absorption, transformation, and scattering of excimer laser radiation in the cornea provoked secondary radiation with collagen protein fluorescence.
The mechanism of corneal collagen cross-linking during photorefractive ablation with riboflavin is realized through the secondary (ablation-induced) radiation as well as peroxyl radicals of the oxidative stress and aseptic inflammation without any additional UV radiation. The above theses clarify the mechanism of laser-induced cross-linking for photorefractive keratectomy with riboflavin. Secondary ablative radiation is an important triggering mechanism of laser-induced cross-linking.

Conclusion

The formation of a membrane-like structure on the ablation surface, the increase in optical density and the demarcation line in the corneal stroma layers are objective criteria for the cross-linking effect upon photorefractive ablation with riboflavin according to optical coherence tomography and corneal densitometry.

References


