

Corneal Cross-Linking and Safety Issues

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Abstract: *Purpose:* To compile the safety aspects of the corneal collagen cross-linking (CXL) by means of the riboflavin/UVA (370 nm) approach.

Materials and Methodology: Analysis of the current treatment protocol with respect to safety during CXL.

Results: The currently used UVA dose density of 5.4 J/cm² and the corresponding irradiance of 3 mW/cm² are below the known damage thresholds of UVA for the corneal endothelium, lens, and retina. Regarding the photochemical damages due to the free radicals the damage threshold for endothelial cells is 0.35 mW/cm². In a 400µm thick corneal stroma saturated with riboflavin, the irradiance at the endothelial level is about 0.18 mW/cm², which is a factor of 2 smaller than the damage threshold.

Conclusion: As long as the corneal stroma treated has a minimal thickness of 400 microns (as recommended), neither corneal endothelium nor deeper structures such as lens and retina will suffer any damages. The light source should provide a homogenous irradiance avoiding hot spots.

Keywords: Keratoconus, biomechanics, cornea, ultraviolet light, cross-linking.

INTRODUCTION

From the beginning of the CXL research the safety of the treatment stood in the center of attention because this procedure was not applied in tissue engineering of isolated collagen structures. The aim was to develop a clinically applicable method for eyes *in vivo* to create additional chemical bonds inside the corneal stroma by means of a highly localized photopolymerization while minimizing exposure to the surrounding structures of the eye [1,2]. There were some points to taken under consideration:

- The duration of the treatment should not be too long.
- The transparency of the cornea should not be changed.
- The cross-linking effect should only include the cornea.

(Cross linking and damaging of adjacent tissue should be avoided).

It was a challenge in the optimizing of the treatment parameters to reach a sufficient biomechanical effect in the cornea and avoid damage of the endothelium, lens or retina. The treatment parameters should fulfill two requirements: the biomechanical effect and the safety. Many investigations were necessary for the fine-tuning of these treatment parameters [3-8].

1. The Choosing of the Photochemical Cross-Linking

There are two different collagen cross-linking methods: the chemical cross-linking uses solutions like glutaraldehyde,

transglutaminase, genepin and nitroalcohol, and the photooxidative (also called physical) cross-linking uses light especially UV-light [2]. The application of liquid cross-linker to the curved cornea is not easy and the diffusion of the liquid cannot be controlled during application. Thus, we decided to use the photooxidative cross-linking for a safer application [3, 9].

2. The Choosing of the Treatment Parameters with Respect to Safety

Singlet oxygen is necessary for the photooxidative cross-linking. Riboflavin is one of the most potent producers of these oxygen radicals. Riboflavin (vitamin B2) is not toxic and it is also used as a food dye. However, riboflavin is not only a photosensitizer - it acts also as a UV absorber. Since the UV light is effective only in the absorbed areas, it is desirable that the irradiation is absorbed in about 400 µm thick corneal stroma tissue [10, 11].

This high absorption is achieved by the selection of the wavelength of the used UV light at 370 nm, a wavelength that corresponds to one of the riboflavin's absorption maxima. For the absorption of ultraviolet light in the cornea the concentration of the superficially applied riboflavin solution of 0.1% and the time course of the diffusion process is relevant. Applied riboflavin must diffuse into the corneal stroma and this process requires certain time [11]. The intact epithelium acts as a barrier that inhibits the diffusion of riboflavin (molecular weight 376.37 g/mol) into the cornea [3, 12, 13]. For that reason the epithelium must be debrided from the intended treatment area because this simple procedure removes a diffusion barrier for the riboflavin molecule and speeds saturation of the corneal stromal tissue [3]. Then riboflavin diffuses through the cornea and a concentration gradient is formed. Though the highest concentration of riboflavin is reached in the anterior stroma

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[14], however after 20-30 min a sufficient concentration is reached also in the posterior stroma [11]. As a result only a small part of the UV penetrates through the cornea and thus deeper ocular structures like lens or retina are protected from the potential hazards of free radical formation. After the riboflavin has traversed the cornea, it enters into the anterior chamber. The aqueous humour without riboflavin does not have any relevant absorption at 370 nm but clinically it starts to stain after about 5 min of surface exposure to riboflavin. By means of slit lamp inspection using blue light the surgeon has to assure that riboflavin has appeared in the anterior chamber before the UV-irradiation has started [15]. The yellow staining of the anterior chamber serves as a safety feature, indicating that the riboflavin has penetrated the cornea and the cornea is thoroughly saturated. Only a circular area of 8 mm in diameter is then exposed to UV-light with a wavelength of 370 ± 5 nm and an irradiance of 3 mW/cm^2 for a total time of 30 min. This corresponds to a total dose of 3.4 J ($J = \text{Joule}$) or a total dose density of 5.4 J/cm^2 to the cornea. Lambert-Beer's law yields a reduction of the irradiance due to absorption in a $400 \mu\text{m}$ -thick stroma saturated with riboflavin (Fig. 1). Therefore, due to the additional riboflavin shielding all structures behind the corneal stroma including the cornea endothelium, anterior chamber, iris, lens, and retina are theoretically exposed to a residual UV-dose density that is less than 1 J/cm^2 as recommended by the UV-guidelines [11, 16].

The aim in the biomechanical effect is to reach a stiffness of the keratoconic cornea similar to the normal one. The stiffness of the keratoconic cornea is about 70% of a healthy cornea [17]. It was not our aim to harden the cornea extremely and to convert it in a non-physiological range. For that reason we choose a low irradiance of 3 mW/cm^2 for 30 minutes. Thus, we increase the stability of the cornea and the cross-linked collagen network allowing keratocyte repopulation [18, 19]. The strong cross-linked collagen inhibits the repopulation of cells [20].

3. Design of a Light Source with Respect to Safety

All the above safety considerations are based on an irradiance that is homogenous within the field of UVA-application. However, if optical inhomogenities such as hot spots are present the damage thresholds may be exceeded locally leading to localized endothelial damage although the average irradiance may be less than 3 mW/cm^2 . Therefore, clinically used light sources must guarantee a perfect homogeneity of the irradiance across the beamed area.

The UV illumination system was designed based on ultraviolet LEDs with a peak emittance at 365 nm. A beam path according to Koehler [21] is focused through a variable diaphragm onto the corneal surface with a special beam homogenizing micro structure [22] in order to ensure homogeneity of illumination on the cornea. In the optical design according to Koehler the UV light diaphragm is imaged onto the corneal front surface and as a consequence is the UV light that is focused on the front surface of the cornea strongly scattered in the ocular media behind the cornea. Thus the safety considerations are fully applied and the real radiant exposures in the eye are probably even lower. In order to obtain an estimate of the real intraocular illumination intensities during treatments using the presented homogenizing UV light source, the radiant exposure was experimentally measured in the retinal plane of porcines eyes [23].

4. Prevention of the Corneal Endothelium Damage

The photopolymerization process inducing additional cross-links in the corneal stroma is carried out by free radicals mediated by the riboflavin irradiated with UV-light. Such radicals can create cell damages that may be tolerable in keratocytes population but not in the corneal endothelium. The cytotoxicity of the riboflavin/UVA treatment on keratocytes and endothelium cells was studied by Wollensak and coworkers and the cytotoxic threshold of the UVA/Riboflavin for endothelial cells and keratocytes was

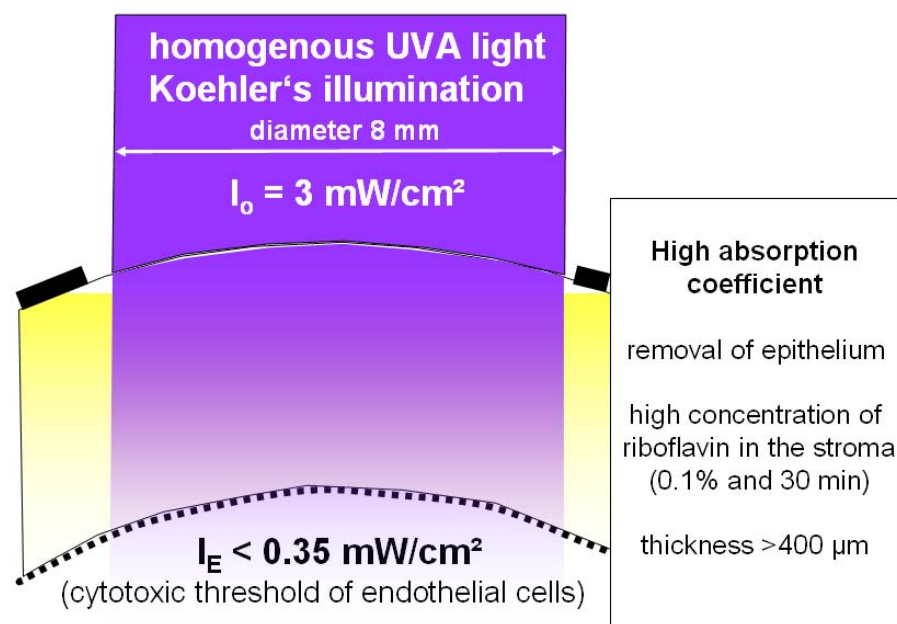


Fig. (1). Safety aspects of corneal cross-linking with riboflavin and UVA.

determined [4-7]. The cell population that is the most critical to suffer from damages either from UV-light directly or from the free radicals is the corneal endothelium because it is immediately adjacent to the corneal stroma and these cells in the normal human cornea have low regenerative capacity. With the above mentioned treatment parameters the cytotoxic threshold of the endothelial cells is not reached. Clinical studies provide the best evidence. By means of confocal microscopy in keratoconus eyes treated with CXL no pathologic signs of the endothelial damage were detected [24, 25]. In other clinical studies using the recommended treatment parameters there was no change in the endothelial cell count observed after CXL [9, 26, 27].

CONCLUSION

In summary, regarding the safety issues, procedure should respect the following criteria: (1) to facilitate diffusion of riboflavin the epithelium should be removed; (2) a 0.1% riboflavin solution must be applied for 30 minutes before UV-exposure, during this treatment riboflavin does not only serve as photosensitizer but also as a UV-blocker; (3) the UV-irradiance of 3 mW/cm² must be homogenous and must have a wavelength of 370 nm; and (4) the minimal thickness of corneal stroma to be cross-linked must at least 400 micrometer. Fulfilling these criteria a damage of the endothelium, the lens or the retina should not be expected.

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