Collagen Cross-linking with Riboflavin in a Hypotonic Solution, with UV light, on Corneas Less Than 400 microns thick: an exploratory study

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Corneal Collagen Cross-linking with Riboflavin and UV light is a physico-chemical treatment for keratoconus and corneal ectasia (and possibly also for the treatment of corneal melting and of corneal oedema). The cornea is saturated with riboflavin, and illuminated with UV at a frequency of 370nm – a wavelength which is strongly absorbed by the riboflavin. The riboflavin has a dual action of producing free radicals which cause cross-linking of the stromal collagen, so strengthening the cornea, as well as acting as a shield to prevent significant levels of UV from penetrating into the eye.

Background

Corneal collagen becomes progressively cross-linked with age, and this can be regarded as a natural physiological ageing process.

Increased cross-linking is associated with increasing rigidity, and this explains why the progressive ectasia seen in keratoconus occurs primarily in younger patients, and progression is not generally seen in later years. Diabetic patients seem to be protected against progressive keratoconus, and this may be related to increased collagen cross-linking associated with hyperglycaemia, the so called 'Maillard reaction'.

Cross-linking of the cornea can be achieved by a number of different chemical and physical processes, and the effect of UV light is particularly enhanced by the presence of riboflavin (vitamin B$_2$) [1].

Spoerl / Seiler: Techniques for stiffening the cornea. *J Refract Surg* 1999; 15: 711-713
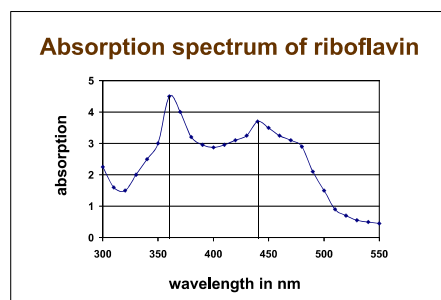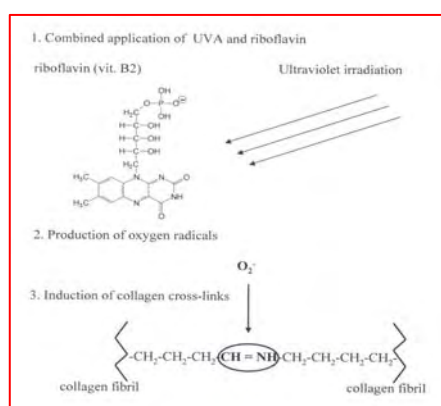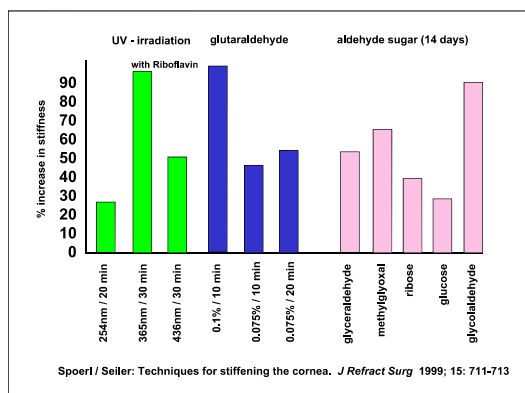

Riboflavin acts as a photo-sensitizer, undergoing fluorescent stimulation, and producing free-radicals which enhance the cross-linking process.

The osmolarity of corneal stroma is 380-420 mosmol/l, and the standard riboflavin / dextran solution is 400 mosmol/l, so application to the de-epithelialised surface does not lead to swelling of the cornea.

Riboflavin has peaks in its absorption spectrum at 270, 366, and 445nm. The effects on tissues of UV light at these frequencies varies: at 270nm there is high absorption in DNA, and tissue damage producing photo-conjunctivitis, and photo-keratitis. At 445nm

there is potential for photo-chemical damage to the retina – blue light hazard. At 366nm there is absorption by pigmented tissues, but relative high transmission of DNA, so this frequency is the optimal one for cross-linking the cornea.

Whilst light at this frequency can potentially be produced from a variety of sources such as incandescent or fluorescent lamps, or lasers, there are UV Light Emitting Diodes (LEDs) that have an output frequency of 365-370mn which are cheap and readily available.
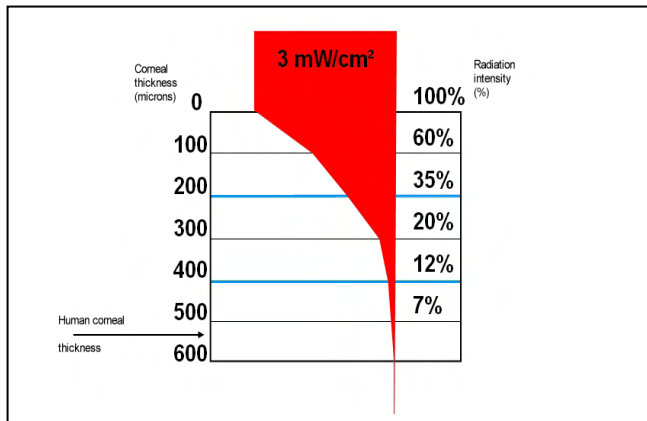

In addition to its action as a photo-sensitizer, the riboflavin has an important action as a shield.

More than 90% of the UV-light is absorbed in the cornea and in addition the anterior chamber riboflavin reduces the UV-intensity to a level that is a factor of 1000 smaller than the official safety level.
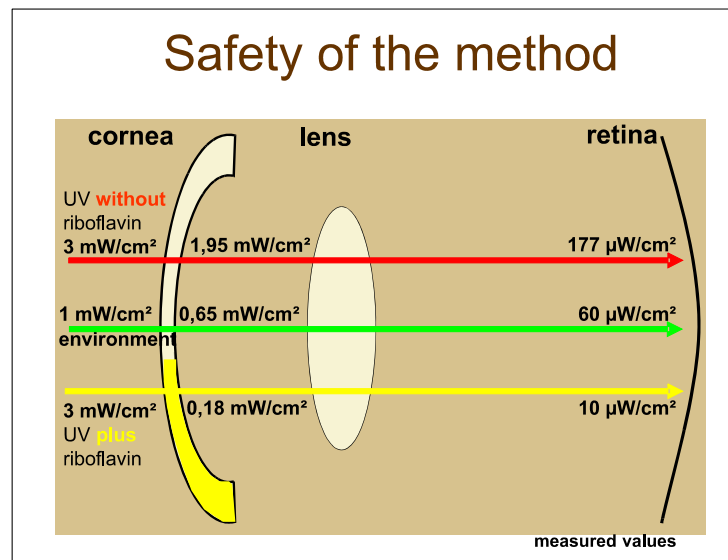
In vitro studies

In 1997, Theo Seiler's research group reported the results of *in vitro* cross-linking work on porcine corneas. They used riboflavin 0.5% in 20% dextran T500 applied to the cornea and irradiated with UVA at 365nm at an irradiance of 20W/m^2 for 60 min. This produced a significant increase in rigidity of the cornea [1,2].
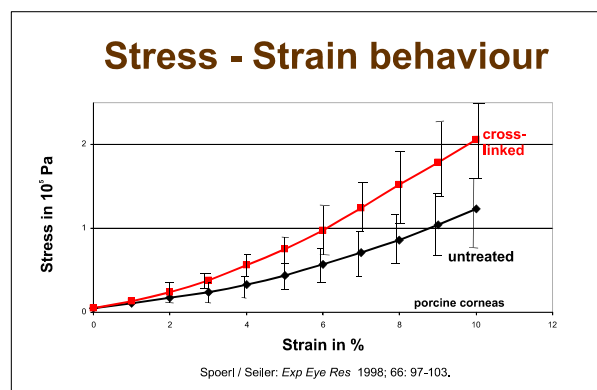
Subsequent *in vitro* work comparing porcine to human corneas undergoing cross-linking showed an even greater increase in rigidity in the human corneas, of more than 300%, and an increase of Young's modulus by 4.5 times [3]. Since the collagen cross-linking is maximal in the anterior 300μ of the cornea, the greater stiffening effect in human corneas was explained by the relatively larger portion of the cornea being cross-linked in the overall thinner human cornea.

Animal studies

Work in rabbits, followed for 1 – 3 months post-operatively, showed that following cross-linking treatment the corneas re-epithelialised and remained clear [4].

Studies were carried out in rabbits to determine the effect of cross-linking on the corneal endothelium [5]. Thirty four eyes were treated with various endothelial doses of UVA ranging from $0.16 – 0.9$ J/cm^2 ($0.09 – 0.5$ mW/cm^2, 370nm). In rabbit corneas of thickness less than 400μ, the endothelial UVA dose reached a cytotoxic level of ≥ 0.65 J/cm^2 (0.36 mW/cm^2) using the standard surface UVA dose of 5.4 J/cm^2 (3 mW/cm^2), but there was no endothelial damage at levels below this. Extrapolating these findings to the human cornea implies that the endothelium should be safe with a surface treatment of 5.4 J/cm^2 (3 mW/cm^2) provided that the cornea is at least 400μ thick.

Similar studies were also performed in rabbits to assess the effect of cross-linking on the survival of keratocytes [6]. Thirty four eyes were treated with 0.1% riboflavin and had surface irradiances from $1.35 – 7.2$ J/cm^2 ($0.75 – 4$ mW/cm^2) for 30 minutes. In the control eyes with corneal epithelial debridement only, apoptotic keratocytes were found in the anterior 50μ of the corneal stroma 4 hours post-operatively. However, the cross-linking-induced apoptosis was only visible in the rabbit eyes enucleated 24 hours post-operatively. In these eyes, apoptosis of keratocytes was seen down to a variable stromal depth, depending on the applied irradiance. A cytotoxic irradiance for keratocytes of $0.5 – 0.7$ mW/cm^2 could be deduced. From this data, dose-dependent keratocyte damage in human corneas can be expected down to a depth of 300μ when using a surface irradiance of 5.4 J/cm^2 (3 mW/cm^2).


Courtesy of Spoerl an Wollensac
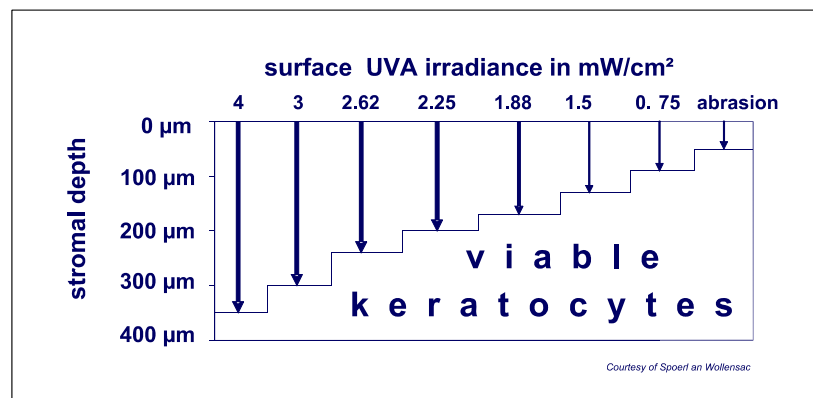
A further study looked at the effect of UVA alone or in combination with riboflavin on the survival of porcine keratocytes grown in culture [7]. An abrupt cytotoxic irradiance level was found at 0.5 mW/cm^2 for keratocytes exposed to UVA and riboflavin, which was 10-fold lower than the cytotoxic irradiance of 5 mW/cm^2 after UVA-irradiance alone.

Again, extrapolating this into the human setting would give a cytotoxic effect down to a depth of 300µ when using the standard surface irradiance of 3 mW/cm^2.

In another study of the effect of cross-linking on the morphology of the collagen fibrils, rabbit corneas were examined by electron microscopy [8]. Using the standard cross-linking treatment protocol, increases of fibril diameter of 12.2% in the anterior stroma, and 4.6% in the posterior stroma were found, compared to control fellow eyes. These findings correlate with the age-related increase of collagen fibril diameter seen in human corneas, and also demonstrate the progressive diminution of the effect of cross-linking in the deeper layers of the cornea.

Clinical studies of cross-linking
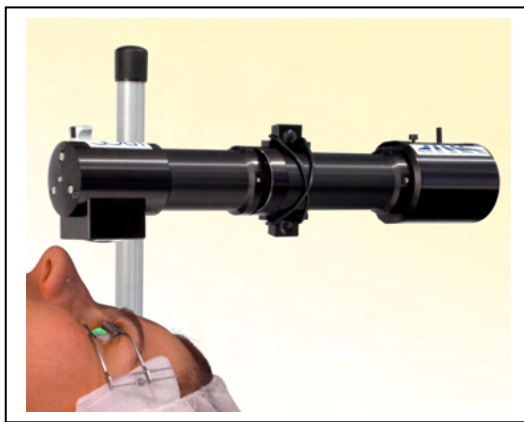
Patient undergoing cross-linking treatment with Peschke Meditade GmbH UV-X cross-linking system www.**peschke**med.ch.

The first clinical report of cross-linking was as a treatment for melting corneal ulcers. Four patients with melting ulcers of various origins were treated with cross-linking at 2.5mW/cm^2 for 30 minutes. In three out of the four patients, the melting process was arrested, allowing healing of the ulcer [9].

The first case of corneal collagen cross-linking for corneal ectasia was treated in 1998. The first report from Dresden of a series of 23 eyes followed for up to 48 months was published in 2003 [10]. Progression of keratoconus was arrested in all cases. In 16 eyes (70%) regression with a reduction of the maximal keratometry readings by 2.01 dioptres and of the refractive error by 1.14 dioptres was found. Visual acuity improved slightly in 15 eyes (65%).

In 2006 Caporossi's group in Siena published results of their independent series of ten cross-linked eyes of keratoconus patients [11]. They reported no progression in the treated eyes, against documented progression in 37% of untreated fellow eyes. The findings of confocal microscopic examination of the same cohort were reported in a separate publication [12]. This demonstrated depletion of keratocytes in the anterior and intermediate stroma after treatment, with a gradual repopulation by new keratocytes over a period of about six months. There were no changes observed in the endothelium.

In 2005 the Dresden group reported a single case of post-LASIK ectasia treated by cross-linking with stabilisation of the ectasia [13].

In 2006 Kanellopoulos from Athens reported a single case of post-LASIK ectasia treated by initial cross-linking followed by custom topography-guided surface ablation, with restoration of vision and stabilisation of the ectasia [14].

In 2007 Kanellopolous and Binder reported a case of keratoconus treated by cross-linking followed by topography guided surface excimer ablation. The treated eye was stabilised, with improved vision, whereas the untreated contra-lateral eye progressed [15].

So in summary the general clinical findings are of arrest of progression of ectasia, some reduction in corneal steepness, and no significant side effects observed.

In a recent review[16] of the safety of UVA-riboflavin cross-linking of the cornea, it was noted that the rate of riboflavin diffusion into the de-epithelialised corneal stroma was slow, and so an application regime of one drop every 3 minutes for 30 minutes was recommended before commencing the UV application, with additional applications at 5 minute intervals during treatment.

Cross-linking without epithelial removal

Despite the fact that there is no published scientific literature demonstrating the safety and efficacy of attempting to carry out cross-linking whilst the corneal epithelium is intact, there are some reports of such treatment being performed in humans.

In 2007, Boxer-Wachler et al. reported a mixed case series of keratoconus patients where some had just inferior segment Intacs, and some had inferior segment Intacs combined with epithelium-intact cross-inking [17]. The latter group had better outcomes, and they concluded that the two treatments could be beneficially combined.

An on-line non-peer-reviewed publication by Pinelli compared cross-linking in keratoconus between a group of five eyes treated with epithelial removal and a group without epithelial removal [18]. Unfortunately the article is not very clear as to the exact protocol, but the intact-epithelial-group appeared to have proparacaine 0.5%, 2 drops every five minutes for 25 minutes, in conjunction with riboflavin. As such one might expect a significant degree of epithelial toxicity, which might have allowed better penetration of the riboflavin. The two groups fared similarly in terms of refractive and keratometric outcome, but the epithelium-intact group had little post-operative pain or inflammation, and no apparent keratocyte depletion.

Riboflavin in Hypotonic Solution.

All the published studies of collagen cross-linking so far have been with riboflavin in a solution with high molecular weight dextran T500, used to prevent corneal swelling during the administration of the drops and the UV treatment, and have been on corneas with minimal pachymetry >400 μ.

Seiler's group have also treated treatment of patients with minimal pachymetry of < 400μ by using riboflavin 0.1% made up in water for injection (data presented at satellite meeting ESCRS, Stockholm 2007). The effect of these hypotonic drops when applied to the denuded stromal surface is to cause transient corneal oedema and corneal thickening.

In this way the stroma can be made to swell such that the minimal stromal pachymetry reaches or exceeds 350μ, and in this way it appears that the endothelium can remain protected from damage from the UV, whilst the beneficial effects of the cross-linking can still potentially be obtained. Corneas as thin as 240μ have been swollen sufficiently to undertake treatment. However, longer follow-up is needed to determine whether cross-linking in this way will achieve the same benefits as cross-linking with the isosmotic riboflavin/dextran preparation in thicker corneas.

Methodology

This initial exploratory study of five eyes is intended to gain experience and results of the technique in fully mentally competent adults.

Patient selection

Patients with known keratoconus will be invited to join the study.

Exclusion criteria:
Age < 16 years, > 60 years.
Minimal pachymetry <250μ or > 400μ.
Minimal endothelial cell density 2400 cells/mm^2
Evidence of other corneal disease in the eye to be treated (e.g. Herpes simplex keratitis).
Women who are pregnant or nursing at the time of the initial treatment.
Presence of significant central corneal opacity.

Patients will typically be only having one eye entered into the study, but will be allowed to have their second eye treated after their first eye reaches the 3 month post-operative gate, should they wish.

Anaesthesia

Topical anaesthesia will be employed.

Dose Rationale

The strength of the riboflavin in dextran drops will be 0.1% as has been used in previous clinical studies, as this has been found to provide the optimal degree of cross-linking whilst still protecting the deep stroma and endothelium from cellular damage.

When the corneal epithelium has been removed, frequent application of the hypotonic riboflavin drops will be commenced to cause the stroma to swell. The saturation of the anterior chamber can be checked before starting treatment by examination of the anterior segment on the slit-lamp bio-microscope. Once the riboflavin level has equilibrated in the anterior segment, and the cornea has swollen to >350μ, the UV irradiation will be commenced. Additional drops will be applied at five minute intervals to ensure that the

corneal surface does not dry out (with associated compaction, thinning, and risk of endothelial damage).

Surgery

An eye lid speculum will be placed. The central 9mm of corneal epithelium will be mechanically debrided. Topical application of hypotonic riboflavin 0.1% will be commenced at frequent intervals and the pachymetry monitored by ultrasound. When the cornea has swollen to >350μ, and an adequate saturation of the anterior chamber with riboflavin has been achieved, cross-linking treatment will proceed. Pachymetry will be monitored at 10 minute intervals during treatment.

Output from the UV light generating equipment will be measured with a UV light meter and set at 3mW/cm².

Patients will have 30 minutes of treatment interrupted at five minute intervals by the application of more topical riboflavin drops.

Finally the eye will have a bandage soft contact lens placed, with preservative free topical antibiotic, steroid, and cycloplegic drop application.

Post-operative topical treatment will consist of Minims chloramphenicol and Minims dexamethasone commencing hourly, on a tapering dose, until re-epithelialisation is achieved. At this point the bandage contact lens will be removed. Further treatment will be tapering G FML, with G Celluvisc prn, Oc Lacrilube at night for 1 month.

Follow-up

Patients will be seen at around 3-5 days post-operatively according to when full re-epithelialisation is anticipated, at 1 and 3 months post-operatively, and at other times if required.

Automated and subjective refraction, keratometry, and topography and endothelial examinations will be performed pre-operatively, and at 3 months post-operatively.

Confocal corneal microscopy, and Ocular Response Analyser (ORA) to measure corneal hysteresis, will be carried out pre-operatively and at 3 months post-operatively on selected patients according to patient and examiner availability.

Adverse Event Reporting:

The investigators will report all adverse events immediately to the Research Governance Committee. There will be a detailed written report of the adverse event. These reports will be reviewed and kept by the Research Governance Committee.
The Research Governance Committee will ensure that all relevant information about suspected serious unexpected adverse reactions that are fatal or life-threatening is recorded and reported within seven days of being alerted to the event to the appropriate authorities (Ethics Committee).

All other suspected serious unexpected adverse events will be reported to the appropriate authorities (Ethics Committee) as soon as possible but within a maximum of fifteen days of first knowledge by the trial steering committee.

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