**Intra- and postoperative corneal thickness changing after collagen cross-linking therapy**

**Short title: Corneal thickness after collagen cross-linking**

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**Abstract**

**Purpose:** To assess intra- and postoperative changes in corneal thickness subsequent to riboflavin-UV-A (collagen cross-linking, CXL) treatment.

**Patients and methods:** Forty one eyes of 41 patients (mean age: 27.97±6.97 years) were treated with CXL technique. During treatment isotonic riboflavin was instilled and corneal thickness measurements were obtained at the cornea apex, the thinnest point and the pupil centre at 15 and 30 minutes, then three days, one week, one, three, six and 12 months after surgery using Pentacam HR and an ultrasound pachymeter.

**Results:** A decrease in corneal thickness was detected intraoperatively 15 minutes with a value of 108.95±48.6 µm, and 112.35±47.3 µm at 30 minutes ($p<0.001$). Three days after the operation no deviation was found between the initial values ($p=0.17$). No further changes were detected during the follow-up period.

**Conclusion:** Isotonic riboflavin solution used during collagen cross-linking treatment resulted in a significant decrease in corneal thickness, however its effect had completely ceased by postoperative day 3.

**Keywords:** collagen cross-linking, corneal thickness, isotonic riboflavin, Pentacam HR

## Introduction

Collagen cross-linking is a promising treatment applied to delay and stop the progression of keratoconus (1-4). On one hand, riboflavin used during treatment acts as a photosensitizer; on the other hand, it protects the corneal endothelium from UV-A radiation (5,6). During and subsequent to this treatment, a significant change in corneal thickness can be detected (7-10), thus the protective effect of riboflavin is likely to fail in some cases. Our goal was to assess the changes in the thickness of corneas treated with isotonic riboflavin during and one year after surgery.

## Patients and methods

Inclusion criteria for collagen cross-linking were the following: keratoconus diagnosed on the basis of the known criteria and the Collaborative Longitudinal Evaluation of Keratoconus Study (11,12); age 18 to 45 years; cornea thicker than 400 micrometers at the thinnest point; significant decline in visual acuity and/or keratometric values and corneal pachymetry in the past three to six months. Further inclusion criteria were: optically clear cornea and a lack of Vogt-striae or anterior stromal scarring. Exclusion criteria were dry eye syndrome distorting the images captured by Pentacam HR and eye pathology other than keratoconus.

Prior to operation the following data were recorded: age, gender, corneal thickness measured at the apex, the pupil centre and the thinnest point of the cornea using Pentacam HR (Pentacam HR, Oculus Optikgeräte GmbH, Wetzlar, Germany, software version 1.17r139). Pentacam HR is the high-resolution version of the former Pentacam. Using a monochromatic light source (475 nm) and rotating around the optical axis, the camera captures 25

Scheimpflug images in two seconds while also correcting for small eye movements. For each examination, three of these complete images were captured of every eye and only automatically captured images with no distortion (blinking etc.) were included. Additionally, ultrasound measurements were carried out at the centre of the cornea at each visit, using an ultrasound pachymeter (PachPen; Accutome Inc, Malvern, PA).

*Surgical technique*

Topical anaesthesia was performed by administering tetracaine hydrochloride eye drops 15 minutes before the operation and every five minutes until the end of the operation. The patient was placed under the operating microscope and a lid speculum was inserted, the epithelium was marked in the inferiorly paracentral area to be treated (diameter 8-9 mm) and the epithelium was removed with a blunt spatula. Before starting UV-A irradiation, the cornea was instilled with Riboflavin 0.1%, Dextran 500 20% solution for 15 minutes (single use isotonic eye drops; Medio Cross Medizin Produkte GmbH, Germany) with a frequency of every five minutes for the drops. In cases of corneas with a CCT of under 400 µm, we used hypotonic riboflavin solution (single use hypotonic eye drops; Medio Cross Medizin Produkte GmbH, Germany) for one hour before irradiation to swell the cornea. Then the cornea was exposed to a UVA 370 nm light (In-Pro CCL-Lix UV, Norderstadt, Germany) for 30 minutes at an irradiance of 3.0 mW/cm² (=5.4 J/cm$^2$). During UVA exposure, isotonic riboflavin drops were continued every two minutes or more often if the corneal surface appeared visibly dry.

After the first 15-minute-treatment the cornea was rinsed with saline to prevent riboflavin altering measurement values and topical anaesthetics was used as most patients complain about eye sensitivity at this stage. The demonstrating 15-minute-Pentacam HR images were captured at this stage. In one case, intraoperative images were captured also with

a Visante optical coherence tomograph (Visante OCT, Carl Zeiss Meditec, Jena, Germany). Then the subject was laid back again and riboflavin UV-A treatment was continued for a further 15 minutes with instillation every two minutes. After treatment, the eye surface was washed with antibiotic drops, a process which was followed by the placement of a bandage soft contact lens (Air Optix Night and Day) Postoperatively, antibiotic (3 mg/ml tobramycin) drops 5/day were administered for five days, corticosteroid (1 mg/ml flurometholon) drops 5/day for a minimum of one month starting at postoperative day 5. The bandage contact lens was removed on postoperative day 3, at which point slit-lamp examination was performed to confirm the presence of complete corneal re-epithelization. Routine examinations were performed 3 days, one week, one month, three months, six months and 12 months postoperatively.

All treatments were carried out by the same experienced operator. All patients were informed about the details of the examinations and the operation and signed an informed consent. All examinations were conducted according to the protocol of the Declaration of Helsinki.

Statistical analysis was performed with MedCalc 10.0. Descriptive statistical results were described as mean, standard deviation (SD) and 95% confidence interval (95% CI) for the mean. A Wilcoxon test was carried out for comparisons between groups or variables, and a Spearman rank test for correlation. A p value below 0.05 was considered statistically significant.

## Results

Forty one eyes of 41 patients were treated with collagen cross-linking technique. The mean age was 27.97±6.97 years (95 % CI: 25.77 - 30.17, range: 18-44.06 year).

Compared to the preoperative data, corneal thickness was significantly thinner at 15 minutes and 30 minutes during treatment ($p<0.001$) using Pentacam HR. The average decrease was 108.95±48.6 µm at 15 minutes and 112.35±47.3 µm at 30 minutes intraoperatively using Pentacam HR. The rate of the decrease showed no correlation with the values of the initial corneal thickness values ($r=0.16$; $p=0.7$). Three days after the operation no statistically significant deviation was found between the measured points and the initial values using Pentacam ($p=0.17$) and ultrasound device ($p=0.32$). No further changes were detected during the follow-up period as well. Our corneal pachymetry data are summarized in Table 1 and Table 2 and illustrated in Figure 1. Intraoperative images were captured by Pentacam HR and Visante OCT (Figure 2.). A hyper-reflective corneal tissue zone can be clearly seen in Pentacam HR pictures and not distinctly visible in Visante OCT.

## Discussion

The purpose of collagen cross-linking (CXL) treatment is to establish new chemical bonds in corneal stroma induced by riboflavin and 370 nm UV-A light. On one hand, riboflavin acts as a photosensitizer during treatment; on the other hand, it protects the areas below treatment zone from the effects of the UV-A radiation (5,6). The aim of the treatment is to increase the rigidity and the biomechanical strength of the cornea. This therapy has been widely used for the treatment of progressive keratoconus (1-4), although its other diagnostic

abilities are also well-known (13). Both objective (14,15) and subjective (16) improvements were reported in eyes with keratoconus after CXL treatment, even in subjects under 18 (17).

As collagen bonds are established at a depth of 250-350 µm in the anterior stroma, called the treatment zone (5,18-21), and for the protection of the endothelium, a minimum of 400 µm stromal thickness is suggested (5,22). Although according to other publications, a minimum of 330 µm of initial thickness is also sufficient for CXL after corneal swelling (23). Transepithelial CXL or customized pachymetric guided epithelial debridement may be beneficial in cases, where corneal thickness is thinner than 400 µm (24,25). Our study was comprised of two patients who had refused any surgical procedure, where the initial corneal thickness value was under 300 µm. According to our experience it is still safe to use isotonic riboflavin after corneal swelling is achieved with hypotonic riboflavin solution. However, in our practice a preoperative treatment limit of at least 400 µm is used.

Our study aimed to reveal the process of how the well-known changes in corneal thickness after collagen cross-linking therapy (7,10,26,27) occur at a less investigated intraoperative stage and in the follow-up period.

Administering isotonic riboflavin, Greenstein et al. (28) measured pupil-, apical-, and the thinnest corneal thickness as being 23-24.6 µm thinner one month after treatment using Scheimpflug analysis. At one year, apex and pupil-center thicknesses returned to the baseline; however, the thinnest pachymetry remained slightly lower than the baseline and similar values were found for pupil-, apex- and thinnest pachymetry thicknesses (28). The gradually increasing values did not reach preoperative readings until one year after the operation. Data were compared with ultrasound pachymetry, but were analysed only preoperatively (28). A number of other studies reported changes in corneal thickness after CXL treatment and, in every case, a decrease was observed (7-10,26,27). Despite the findings mentioned above, corneal thickening was reported three days after CXL in rabbit cornea, which ceased only one

week later (29). So, it seems that corneal tissues may react differently to the treatment due to structural differences.

It has also been suggested that changes in corneal thickness observed using Scheimpflug analysis can be an artefact (30,31) resulting from postoperative corneal haze (8,9,32). In our study ultrasonic measurements were also carried out subsequent to treatments, which showed similar results preoperatively and from one week after CXL. However, intraoperatively and at the three-day-visit, ultrasound pachymetry measured smaller CCT than Pentacam. We think that the possibly altered refractive index of cross-linked corneas can be the background of this difference.

However, they clearly demonstrate that results obtained with Scheimpflug analysis and ultrasound measurements correlate well with each other. The thinning of the cornea and stromal haze can be explained by structural and physiologic wound-healing changes developing after treatment (33), though the mechanism of corneal re-thickening still remains unexplained.

A limitation of our study is that subsequent to de-epithelization no Pentacam HR images were captured. The normal epithelial thickness was 53.4±4.6 µm (34), a value which is supported by the value of Kymionis' data of 43 µm (35). When this mean epithelial thickness is subtracted from the thickness that we measured preoperatively, the decreasing of the corneal thickness remains highly significant ($p<0.01$).

During pachymetric measurements corneal thickness was analysed at the thinnest point and in the pupil centre. Henriquez readings show a mean corneal thinning from 56 µm to 13 µm at the thinnest point of the cornea one and 12 months after treatment (36). Vinciguerra et al. (14) found a significantly thinner cornea in the pupil centre even one year after treatment; however it showed no significant change to the thinnest pachymetry location in eyes with keratoconus one year after the CXL operation. Both of them performed central

CXL operation. In our study, the corneal thickness returned to the preoperative level on postop day three, which remained stable in each of the 3 measured points even at the end of the 1-year follow-up.

Publications suggest corneal thinning in the early postoperative stage, so UV-A light can reach corneal endothelium intraoperatively. Therefore, hypoosmolar riboflavin solution is suggested in order to achieve corneal iatrogenic swelling (37), although according to previous research it has only a short term effect and does not even last until the end of the treatment (38).

Publications found in the literature clearly describe the CCT decrease observed after CXL treatment, though only a few and contradicting reports were carried out on the characteristics of CCT decrease in the intraoperative phase (35,38-39). Using isotonic solution Kymionis et al. detected a mean of 75 µm CCT decrease between epithelial removal and riboflavin instillation with ultrasound pachymetry measurements; however, they found no further changes in corneal thickness during irradiation (35). These data correlate well with our data regarding the decrease in thickness. Kaya (38) measured corneal thickness at five different times: the thinnest pachymetry data decreased to a mean of 376 µm after the epithelial removal and by a mean of 55 µm after the 30-minute application of the isotonic riboflavin solution. The 10-minute application of hypotonic riboflavin resulted in the cornea thickening by a mean of 59 µm. 10 and 30 minutes after the instillation of isotonic riboflavin solution the thinnest pachymetric readings showed a decrease with a value of 50 and 65 µm. Holopainen (39) measured corneal thickness both preoperatively and postoperatively using ultrasound pachymeter and found a thinning of a mean 87 µm during the 1-hour operation. Depending on the initial corneal thickness he administered isotonic or hypotonic solution. Compared to the preoperative CCT, he reported a significantly lower value one month after

the operation and found the same value six months after treatment. In our study, corneal thickness already reached the initial value three days after treatment.

In summary, a significant decrease in corneal thickness can be observed during collagen cross-linking treatment applied for treating keratoconus. However, this change is temporary and the value measured initially is already reached three days after treatment. Further investigations are suggested in order to determine whether this change may have any consequences or whether it is of any significance.

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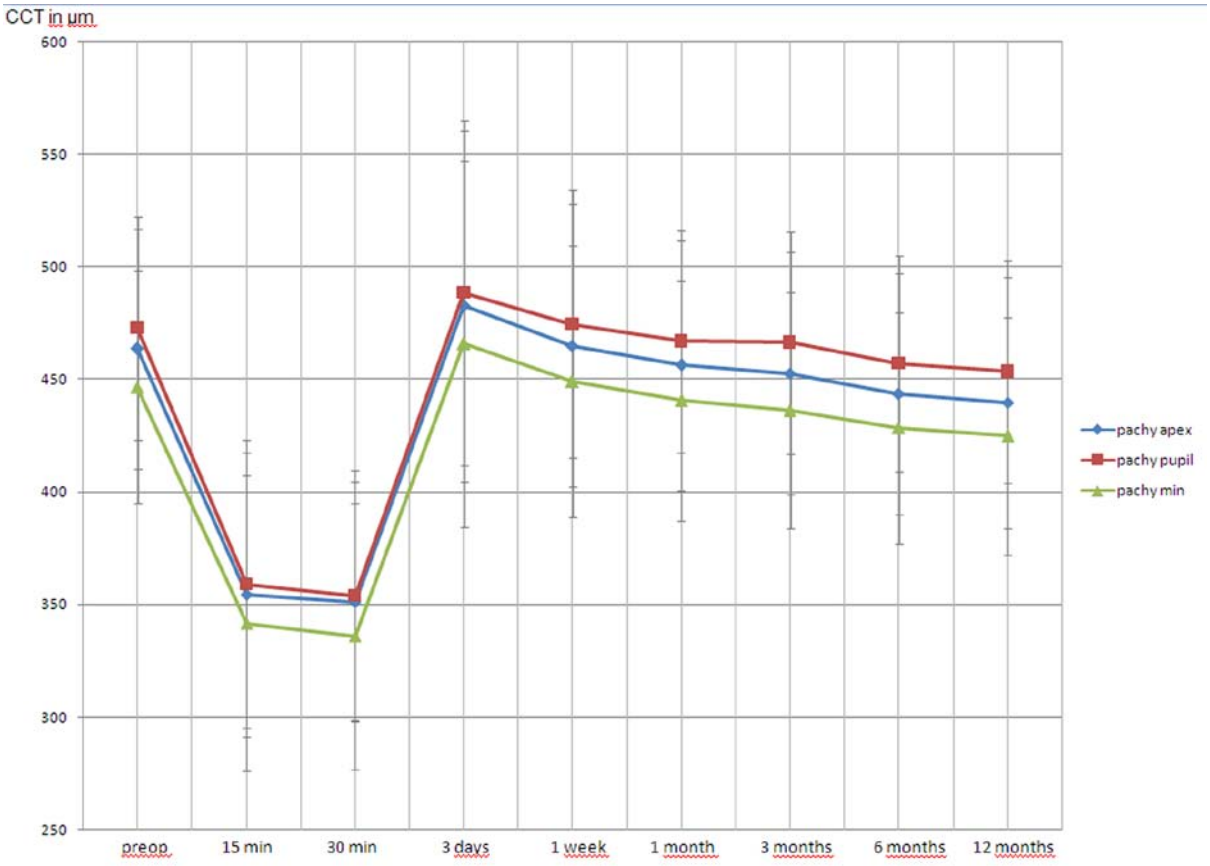


Figure 1: Mean corneal thickness changes at 15 minutes and 30 minutes during collagen cross-linking and 3 days, 1 week, 1,3,6 and 12 months after treatment. In every case, thickness measurements at the corneal apex (apex), pupil centre (pupil) and the thinnest point of the cornea (min) were recorded.

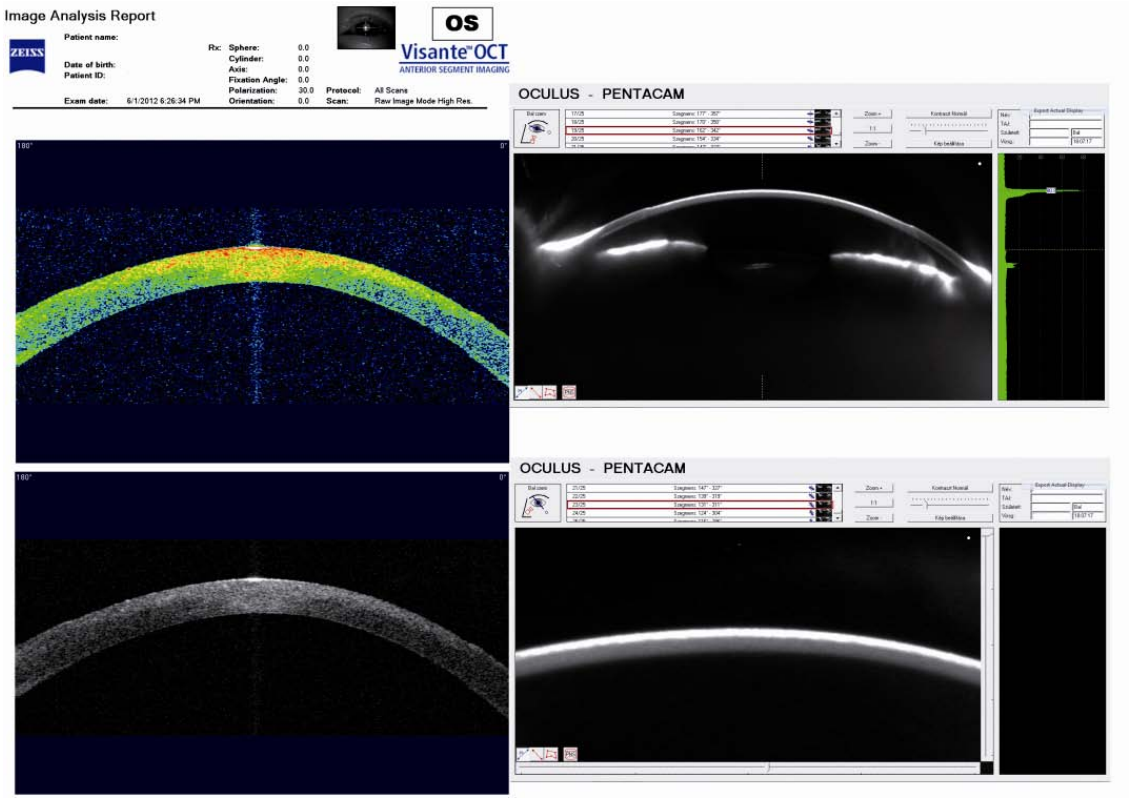


Figure 2: Pentacam HR and Visante OCT images captured at 15 minutes during collagen cross-linking treatment. Hyperreflective corneal tissue zone can be seen in Pentacam HR pictures (right) and not distinctly visible in Visante OCT pictures (left).

| | | Mean | SD | 95% CI | Minimum | Maximum |
|---|---|---|---|---|---|---|
| **preoperative** | apex | 463.61 | 53.454 | 446.513 - 480.703 | 299.33 | 564.67 |
| | min | 446.74 | 51.692 | 430.209 - 463.273 | 298 | 559.67 |
| | pupil | 472.83 | 49.577 | 456.978 - 488.689 | 321.33 | 569.33 |
| **15 minutes** | apex | 354.66 | 63.088 | 334.482 - 374.835 | 199.67 | 468.33 |
| | min | 341.99 | 65.61 | 321.009 - 362.975 | 165.67 | 459 |
| | pupil | 359.13 | 64.101 | 338.633 - 379.634 | 192 | 481.67 |
| **30 minutes** | apex | 351.25 | 53.277 | 334.212 - 368.289 | 233.67 | 458 |
| | min | 336.43 | 59.024 | 317.556 - 355.310 | 208.67 | 453 |
| | pupil | 354.16 | 55.581 | 336.383 - 371.934 | 240 | 464 |
| **3 days** | apex | 484.53 | 78.136 | 459.201 - 509.859 | 275.67 | 666.33 |
| | min | 466.56 | 81.392 | 440.171 - 492.940 | 222.67 | 646.33 |
| | pupil | 490.33 | 76.608 | 465.500 - 515.167 | 295.33 | 667.67 |
| **1 week** | apex | 467.45 | 62.92 | 447.057 - 487.849 | 298.33 | 592 |
| | min | 450.72 | 60.394 | 431.141 - 470.296 | 290.67 | 561 |
| | pupil | 476.83 | 59.366 | 457.585 - 496.073 | 322 | 593.67 |
| **1 month** | apex | 456.68 | 55.596 | 438.895 - 474.456 | 285.33 | 560 |
| | min | 441 | 53.345 | 423.939 - 458.060 | 276.67 | 540 |
| | pupil | 467.25 | 49.241 | 451.502 - 482.998 | 334 | 570 |
| **3 months** | apex | 453.78 | 53.843 | 434.369 - 473.194 | 291.33 | 567 |
| | min | 437.65 | 52.382 | 418.759 - 456.531 | 283.67 | 558.33 |
| | pupil | 467.24 | 49.303 | 449.464 - 485.015 | 327.67 | 573.33 |
| **6 months** | apex | 444.29 | 53.633 | 422.154 - 466.432 | 296.33 | 526.33 |
| | min | 429.39 | 51.364 | 408.186 - 450.589 | 293 | 506 |
| | pupil | 457.76 | 47.916 | 437.983 - 477.540 | 329.67 | 532.67 |
| **12 months** | apex | 441.02 | 55.775 | 412.343 - 469.696 | 294 | 523 |
| | min | 426.35 | 52.725 | 399.244 - 453.462 | 289.67 | 502.67 |
| | pupil | 454.59 | 49.264 | 429.258 - 479.917 | 329.33 | 531 |

Table 1: Corneal thickness measurements in µm at the corneal apex (apex), the thinnest corneal point (min) and pupil centre (pupil) prior to collagen cross-linking, intraoperatively and in the postoperative follow-up period, using Pentacam HR. SD: standard deviation, 95% CI: 95% confidence interval of the mean.

| | Mean | SD | 95% CI | Minimum | Maximum |
|---|---|---|---|---|---|
| **preoperative** | 475.80 | 58.763 | 457.007 - 494.593 | 317 | 590 |
| **15 minutes** | 339.88 | 57.529 | 321.476 - 358.274 | 205 | 460 |
| **30 minutes** | 330.03 | 44.748 | 315.714 - 344.336 | 207 | 460 |
| **3 days** | 474.71 | 69.928 | 450.693 - 498.735 | 311 | 580 |
| **1 week** | 475.80 | 56.937 | 457.591 - 494.009 | 310 | 565 |
| **1 month** | 470.49 | 56.764 | 451.560 - 489.413 | 310 | 567 |
| **3 months** | 468.74 | 54.216 | 447.294 - 490.188 | 320 | 557 |
| **6 months** | 463.36 | 48.423 | 443.372 - 483.348 | 325 | 555 |
| **12 months** | 460.12 | 54.690 | 431.999 - 488.237 | 325 | 554 |

Table 2: Corneal thickness measurements in µm at the centre of the cornea prior to collagen cross-linking, intraoperatively and in the postoperative follow-up period, using an ultrasound pachymeter. SD: standard deviation, 95% CI: 95% confidence interval of the mean.

**Summary statement**

Corneal thickness decreased significantly during collagen cross-linking treatment with isotonic riboflavin solution measured intraoperatively.

At postoperative day 3, the corneal thickness reached preoperative data for at least 12 months after collagen cross-linking.