Comparative evaluation of corneal stromal demarcation line following accelerated corneal collagen crosslinking protocols using different riboflavin formulations and soaking durations

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INTRODUCTION AND AIM

- Corneal crosslinking (CXL) is the only treatment in halting the progression of keratoconus.
- The optimal riboflavin formulation and soaking duration for accelerated CXL (ACXL) protocols remain unknown.
- Riboflavin solution with hydroxypropyl methylcellulose (HMPC) as a carrier, is one of the alternative formulations which has been documented to accelerate stromal penetration of riboflavin, and increase stromal ultraviolet-A (UVA) absorption and stromal hydration compared to dextran.[1]
- Our purpose in this study was to compare the structural features of the crosslinked cornea and depth of stromal demarcation line (DL) following ACXL using riboflavin solutions with different carrier agents (HPMC or dextran) and soaking durations. We also sought to investigate these features with ACXL efficacy at postoperative year-1.

METHODS

- Prospective, comparative study
- Consecutive progressive keratoconus patients who were scheduled for CXL
- Exclusion criteriae : severe axial corneal scarring, previous refractive or other corneal surgery, a history of herpetic keratitis, any corneal/ocular disease or any autoimmune condition.

Group 1	HPMC-based RF, 10 min.	UVA (9 mW/cm²) and HPMC-based RF, 10 min.
Group 2	HPMC-based RF, 20 min.	UVA (9 mW/cm²) and HPMC-based RF, 10 min.
Group 3	Dextran-based RF, 30 min.	UVA (9 mW/cm²) and HPMC-based RF, 10 min.
Group 4	Dextran-based RF, 30 min.	UVA (3 mW/cm²) and Dextran-based RF, 30 min.

RF: riboflavin; UVA: ultraviolet-A; HPMC: hydroxypropyl methylcellulose; Riboflavin 0.1% in 1.1% HPMC solution (Vibex Rapid, Avedro Inc, Waltham, MS, USA); Riboflavin 0.1% in 20% dextran T500 solution (MedioCross, Kiel, Germany); UVA light (Avedro, Waltham, MS, USA)

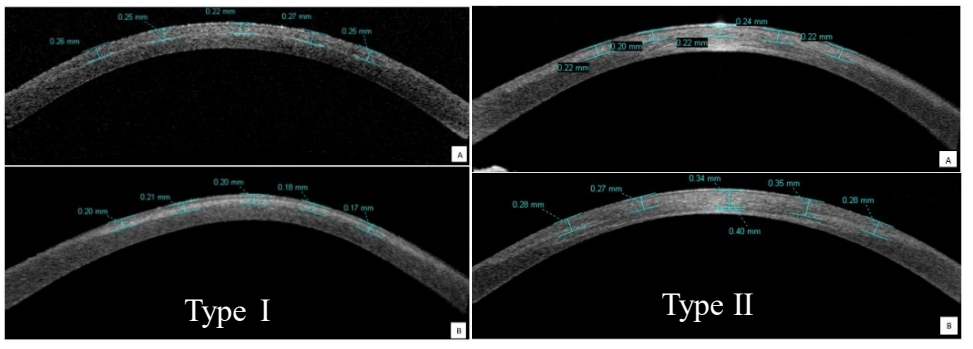
Preoperative, Postoperative Month-1 and Month-12

- Uncorrected and corrected distance visual acuity (UDVA and CDVA)
- Manifest refraction (MR),
- Corneal tomography, pachymetry and aberrometry (Pentacam, Oculus GmbH, Wetzlar, Germany),
- In vivo confocal microscopy (IVCM) (HRT II, Rostock Cornea Module,Heidelberg, Germany)
- Anterior segment optical coherence tomography (AS-OCT) (Visante, Carl Zeiss Meditec, Dublin, CA, USA).

Demarcation Line Depth and Morphology

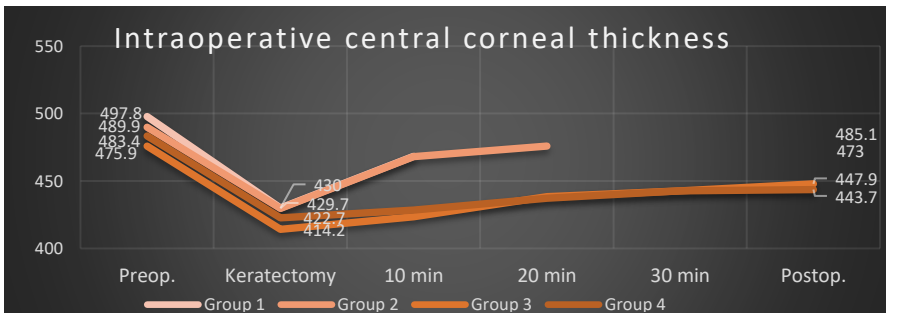
Table 1. Morphological classification of crosslinked cornea and demarcation line as measured using the anterior segment optic coherence tomography

Type	Definition
I	Homogenous, hyper-reflective crosslinked cornea with, linear, uninterrupted hyper-reflective demarcation line that is easily detectable
II	Heterogenous, patchy crosslinked cornea with, barely detectable demarcation line that is deeper at the central 3 mm corneal zone.



RESULTS

- 104 eyes of 104 patients (26 patients in each group), mean age: 24.1±5.1 (15-33) years



Demarcation Line and Structural Features of the Crosslinked Area

Table 2: The mean central demarcation line depth and its percentage relative to total corneal thickness in the study groups

	Group 1	Group 2	Group 3	Group 4	p
DL depth (µm)	358.8±95.6	341.5±84.1	261.2±62.3*	310.3±82.3	<0.001
DL %	68.03±16.90	65.67±16.36	49.41%±11.15*	60.45±11.52	<0.001

*: significantly lower in group 3, compared to other groups

- **Type I** morphology was statistically significantly more common in eyes that had undergone CXL using **dextran-based riboflavin** (83.9% vs 35.9 %), whereas **Type II** morphology was more common in **HPMC-based riboflavin** group (64.2% vs 16.1%).

At postoperative month-12, we documented;

- No statistically significant between-group differences in the improvements in UDVA, CDVA, or spherical/cylindrical MR measurements (p>0.05).
- **Stable** mean Kmax in **group 1** (p=0.324), statistically significant **flattening** of Kmax in **groups 2, 3 and 4** (p=0.001, 0.031, 0.001, respectively). Positive correlation between % of crosslinked cornea and Kmax improvement (p<0.001, r=0.382).
- Statistically significant **improvement** of 5/7 tomographic indices in **group 2 and 4** (p<0.05).
- No significant between-group differences in regards to improvement of Kmax, tomographic indices, vertical coma, and spherical aberration at 12 month follow-up (p>0.05).
- No endothelial cell loss, prolonged corneal haze or any other clinically significant adverse at 12 month follow-up.

DISCUSSION

- **In our study,** demarcation line depth (DLD) reached 60–68% in groups 1, 2, and 4, while it was significantly shallower in the dextran-based group (group 3). HPMC-based groups showed more heterogenous and patchy crosslinked tissue and yielded comparable visual, refractive, and tomographic outcomes to conventional CXL, with no significant endothelial cell loss or adverse events at 12 months.
- **Previous studies** using 10 minutes of HPMC-based riboflavin soaking have reported DLD of 203-355 µm postoperatively,[2-5] whereas 20 minutes of HPMC-based riboflavin soaking with similar protocols revealed 160-273 µm of depth.[6,7] Since UVA intensities and durations in these studies were also variable, comparative evaluation between outcomes is hard to achieve.
- Due to its low surface tension and contact angle,[1] HPMC enables rapid and widespread corneal distribution, which may explain the more heterogeneous, patchy appearance observed after ACXL with HPMC-based riboflavin.
- The DL should be sufficiently deep for efficacy without compromising endothelial safety. It was hypothesized that transient accommodation of excess HPMC in the posterior stroma allows safe UVA exposure, with postoperative de-swelling bringing the DL closer to the endothelium;[8] accordingly, in our study no endothelial cell loss was observed at 12 months, even in eyes with a deep DL.
- **In conclusion,** ACXL (9 mW/cm², 10 min) with HPMC-based riboflavin soaking (10–20 min) provides a crosslinked corneal volume comparable to conventional CXL, whereas dextran-based soaking results in shallower DLD. Despite early stromal differences, 1-year efficacy was similar across all groups. Further large-scale, long-term studies are needed to clarify the relationship between AS-OCT findings and CXL outcomes.

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