Transepithelial cross-linking

PROCEDURE FOR TRANSEPITHELIAL CROSS-LINKING (TE-CXL)

5' 5' 5' 5' 5' 5'

Instill one drop of “pilocarpine 2%” 30 minutes before UV-A treatment.

Place the patient in clinostatic position.

Clean the area around the eye with a suitable antiseptic solution.

Instill RICROLIN® TE starting 30 minutes before UV-A irradiation: one drop every 2 minutes (at least 16 drops over 30’), using a syringe without the needle.

Instill “topic anaesthetic” (single dose) starting 20 minutes before the treatment: 1 drop every 4 minutes, repeated 4 times.

Position the blepharostat.

Accurately focus the light spot on the cornea.

Proceed with irradiation for a total of 30 minutes (six five-minute steps), adding one drop of RICROLIN® TE at the start of each irradiation step using a syringe fitted with a canula needle. During the irradiation phase, if the cornea tends to dry up it can be kept moist with BSS.

At the end of the 30 minutes of irradiation, wash the cornea thoroughly with BSS.

A therapeutic contact lens can be used for 3-4 days, according to the discretion of the ophthalmologist.

UV-A exposure: 6 x 5 min

“Topic anesthetic” (single dose)

Starting 30 minutes before the treatment:
1 drop every 4 minutes, repeated 4 times

November 29th, 2011 - UPDATE
PROCEDURE
FOR TRANSEPITHELIAL CROSS-LINKING (TE-CXL)

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- At the end of the 30 minutes of irradiation, wash the cornea thoroughly with BSS.
- A therapeutic contact lens can be used for 3-4 days, according to the discretion of the ophthalmologist.
Transepithelial cross-linking

Collection of scientific studies
Massimo Filippello MD, Edoardo Stagni MD
Di Stefano - Velona Clinic, Catania, Italy

PURPOSE
To evaluate the efficacy of trans-epithelial cross-linking (TE-CXL) in patients with bilateral progressive keratoconus.

PATIENTS AND METHODS
Twenty patients with a history of bilateral progressive keratoconus were recruited. The worse eye was treated with TE-CXL, while the fellow eye was left untreated as a control. After corneal soaking with RICROLIN® TE (SOOFTitalia S.p.A.), TE-CXL was performed on the intact corneal epithelium (6 steps of 5 minutes each) by UV-A irradiation (VEGA®, CSO, Italy) and further application of RICROLIN® TE (1 drop at the beginning of each step).

RESULTS
In treated eyes there were statistically significant improvements in uncorrected and best corrected visual acuity and topographically derived keratometry, cone apex power and high order aberrations (p<0.05). In untreated control eyes there was a general trend of worsening of these parameters. No complications were reported.

CONCLUSIONS
TE-CXL treatment appeared to halt keratoconus progression, with a statistically significant improvement of our measured visual and topographic outcome parameters. The treatment was safe and well tolerated. Its non-invasive nature makes it a potentially useful treatment in those cases where epithelial debridement is ideally avoided, such as pediatric cases, uncooperative patients and in thin corneas with thickness less than 400 microns.
## RESULTS

<table>
<thead>
<tr>
<th></th>
<th>Pre CXL</th>
<th>1 month</th>
<th>3 months</th>
<th>6 months</th>
<th>12 months</th>
<th>18 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sim kS</td>
<td>51.02±1.10</td>
<td>51.12±1.02</td>
<td>49.05±0.92</td>
<td>48.65±0.89</td>
<td>51.42±0.96</td>
<td>47.82±0.78</td>
</tr>
<tr>
<td>Sim kF</td>
<td>45.13±0.97</td>
<td>46.05±0.93</td>
<td>44.46±0.65</td>
<td>44.13±0.89</td>
<td>46.52±0.91</td>
<td>44.57±1.11</td>
</tr>
</tbody>
</table>

After 18 months of follow up there is a significant (p<0.05) decrease of keratometric values: Sim kS from 51.02 to 48.05; Sim kF from 45.13 to 44.43; Sim Cyl from 5.89 to 3.62.
Transepithelial Cross-linking (TE-CXL): Results after 12 months

Antonio Laborante MD

*Ophthalmology Department - Casa Sollievo della Sofferenza Hospital, S. Giovanni Rotondo, Foggia, Italy*

**OBJECTIVE**

To evaluate the effectiveness of the epi-on (CXL-TE) technique in two different groups of patients affected by progressive keratoconus (Group A) and by forme fruste keratoconus (Group B). Uncorrected visual acuity (UCVA), best spectacle-corrected visual acuity (BSCVA), endothelial cell counts, along with keratometric and aberrometric parameters were all evaluated.

**PATIENTS AND METHODS**

**Group A**: 15 eyes from 15 different patients with stage II and III keratoconus (according to Krumeich’s classification) and Group B: 10 eyes from 10 different patients with stage I keratoconus (Krumeich) and forme fruste keratoconus were treated with TE CXL using RICROLIN® TE (riboflavin 0.1% + enhancer). The VEGA® UVA emitter was employed. The procedure involved a 30-minute inhibition phase (1 drop of RICROLIN® TE every 2 minutes) and a 30-minute irradiation phase (6 steps, 5 minutes each). UCVA and BSCVA, endothelial cell counts along with keratometric and aberrometric parameters were evaluated before the treatment as well as 6 months and 12 months after treatment.

**RESULTS**

**Group A**: a mean improvement of 2 lines in UCVA and 3 lines in BSCVA after six months; and of 3 lines in UCVA and 3 lines in BSCVA after 12 months. Mean K improved by 0.5 ± 0.15 dioptres after 6 months and 0.75 ± 0.20 dioptres after 12 months. COMA exhibited a statistically significant decrease, going from 2.42 μm ± 0.87 μm (pre-treatment), to 1.82 μm ± 0.97 μm (after six months) and to 1.75 μm ± 0.87 μm (after 12 months). **Group B**: a mean improvement of 3 lines in UCVA and 3 lines in BSCVA after six months; and of 3 lines in UCVA and 3 lines in BSCVA after 12 months. Mean K improved by 0.5 ± 0.25 dioptres after 6 months and 0.75 ± 0.25 dioptres after 12 months. COMA exhibited a statistically significant decrease, going from 1.48 μm ± 0.91μm (pre-treatment), to 1.25 μm ± 0.85 μm (after 6 months) and to 1.20 μm ± 0.82 μm (after 12 months). Cross-linking effect takes place in the corneal stroma approximately 100 μm below the surface of the epithelium.

**CONCLUSION**

Transepithelial technique is safe and effective. Group B patients displayed clearer treatment effects than Group A patients who had a more severe disease with thinner corneas (Krumeich stage II and III). Transepithelial treatment benefits are: preservation of visual acuity prior to surgery; the possibility of treating children, disabled and non-cooperative patients and that no operating theatre is required. It is a painless technique, without de-epithelialisation related complications and it can be performed also with a corneal pachymetry of less than 400 μm.
Transepithelial Cross-linking (TE-CXL): Results after 12 months

**OBJECTIVE**

To evaluate the effectiveness of the epi-on (CXL-TE) technique in two different groups of patients affected by progressive keratoconus (Group A) and by forme fruste keratoconus (Group B). Uncorrected visual acuity (UCVA), best spectacle-corrected visual acuity (BSCVA), endothelial cell counts, along with keratometric and aberrometric parameters were all evaluated.

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**RESULTS**

**GROUP A**

- A mean improvement of 2 lines in UCVA and 3 lines in BSCVA after six months; and of 3 lines in UCVA and 3 lines in BSCVA after 12 months.
- Mean K improved by 0.5 ± 0.15 dioptres after 6 months and 0.75 ± 0.20 dioptres after 12 months.
- COMA exhibited a statistically significant decrease, going from 2.42 μm ± 0.87 μm (pre-treatment), to 1.82 μm ± 0.97 μm (after six months) and to 1.75 μm ± 0.87 μm (after 12 months).

**GROUP B**

- A mean improvement of 3 lines in UCVA and 3 lines in BSCVA after six months; and of 3 lines in UCVA and 3 lines in BSCVA after 12 months.
- Mean K improved by 0.5 ± 0.25 dioptres after 6 months and 0.75 ± 0.25 dioptres after 12 months.
- COMA exhibited a statistically significant decrease, going from 1.48 μm ± 0.91 μm (pre-treatment), to 1.25 μm ± 0.85 μm (after 6 months) and to 1.20 μm ± 0.82 μm (after 12 months).

Cross-linking effect takes place in the corneal stroma approximately 100 μm below the surface of the epithelium.

Transepithelial technique is safe and effective. Group B patients displayed clearer treatment effects than Group A patients who had a more severe disease with thinner corneas (Krumeich stage II and III). Transepithelial treatment benefits are: preservation of visual acuity prior to surgery; the possibility of treating children, disabled and non-cooperative patients and that no operating theatre is required.

It is a painless technique, without de-epithelialisation related complications and it can be performed also with a corneal pachymetry of less than 400 μm.

**Uncorrected visual acuity (UCVA) and best spectacle-corrected visual acuity (BSCVA) variation**

- **GROUP A**
  - 6 months: UCVA, BSCVA
  - 12 months: UCVA, BSCVA

- **GROUP B**
  - 6 months: UCVA, BSCVA
  - 12 months: UCVA, BSCVA

**Clinical study update**

Transepithelial CXL in paediatrics

Luca Buzzonetti MD, Gianni Petrocelli MD
Ophthalmology Department - Bambin Gesù Hospital, Rome, Italy

OBJECTIVE
Early clinical assessment of the effects of transepithelial corneal cross-linking (TE-CXL) in paediatric patients.

PATIENTS AND METHOD
11 eyes from 11 different paediatric patients (14 years old on average, age range 8-18) with progressive keratoconus were treated. After soaking the cornea with a RICROLIN® TE solution, a UVA irradiation phase was performed for 30 minutes (6 steps, 5 minutes each). Patients were assessed preoperatively and 1, 3, 6 and 9 months after surgery: BSCVA, spherical equivalent, Kmax, Kmin, Kave, COMA as well as higher order aberrations for 3 mm and 5 mm. Statistical analysis was performed employing Student's t-test.

RESULTS
Pre-operative data and post-operative data after 1, 3 and 6 months are the following:

<table>
<thead>
<tr>
<th></th>
<th>Pre CXL-TE</th>
<th>3 months</th>
<th>6 months</th>
<th>9 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSCVA</td>
<td>6.4±2.3 D</td>
<td>7.2±2.1 D</td>
<td>6.4±3.4 D</td>
<td>8.2±2.2 D</td>
</tr>
<tr>
<td>Spherical Equivalent</td>
<td>-4.1±3.1 D</td>
<td>-2.4±2.5 D</td>
<td>-3.0±2.5 D</td>
<td>-3.0±2.5 D</td>
</tr>
<tr>
<td>Kmax</td>
<td>49.8±4.6 D</td>
<td>48.3±3.0 D</td>
<td>48.9±5.0 D</td>
<td>50.2±4.0 D</td>
</tr>
<tr>
<td>Kmin</td>
<td>45.4±3.3 D</td>
<td>44.5±2.4 D</td>
<td>45.0±3.0 D</td>
<td>45.0±3.0 D</td>
</tr>
<tr>
<td>Kave</td>
<td>47.9±3.8 D</td>
<td>46.3±2.6 D</td>
<td>46.8±5.5 D</td>
<td>47.8±3.4 D</td>
</tr>
<tr>
<td>COMA 3 mm</td>
<td>0.48±0.2 μm</td>
<td>0.5±0.3 μm</td>
<td>0.6±0.4 μm</td>
<td>0.5±0.1 μm</td>
</tr>
<tr>
<td>COMA 5 mm</td>
<td>1.73±0.8 μm</td>
<td>1.51±0.8 μm</td>
<td>1.7±1.0 μm</td>
<td>2.1±0.8 μm</td>
</tr>
<tr>
<td>Aberrations AO 3 mm</td>
<td>0.58±0.3 μm</td>
<td>0.65±0.3 μm</td>
<td>0.7±0.8 μm</td>
<td>0.6±0.1 μm</td>
</tr>
<tr>
<td>Aberrations AO 5 mm</td>
<td>2.0±0.9 μm</td>
<td>1.8±0.8 μm</td>
<td>2.1±1.0 μm</td>
<td>2.4±0.9 μm</td>
</tr>
</tbody>
</table>

CONCLUSIONI
TE-CXL shows a moderate positive effect on keratoconus stabilization in paediatric patients. Six months after surgery, no side effects were found in the young patients.
Luca Buzzonetti MD, Gianni Petrocelli MD
Ophthalmology Department - Bambin Gesù Hospital, Rome, Italy

OBJECTIVE
Early clinical assessment of the effects of transepithelial corneal cross-linking (TE-CXL) in paediatric patients.

PATIENTS AND METHOD
11 eyes from 11 different paediatric patients (14 years old on average, age range 8-18) with progressive keratoconus were treated. After soaking the cornea with a RICROLIN® TE solution, a UVA irradiation phase was performed for 30 minutes (6 steps, 5 minutes each). Patients were assessed preoperatively and 1, 3, 6 and 9 months after surgery: BSCVA, spherical equivalent, Kmax, Kmin, Kave, COMA as well as higher order aberrations for 3 mm and 5 mm.

Statistical analysis was performed employing Student's t-test.

RESULTS

BSCVA, Spherical Equivalent and Kave evaluation during the follow up period:

- **BSCVA**: Pre CXL-TE 3 months 6 months 9 months
  - Pre CXL-TE: 6.4
  - 3 months: 7.2
  - 6 months: 6.4
  - 9 months: 8.2
  - p > 0.05

- **Spherical Equivalent**: Pre CXL-TE 3 months 6 months 9 months
  - Pre CXL-TE: -4.1
  - 3 months: -2.4
  - 6 months: -3.0
  - 9 months: -3.0
  - p > 0.05

- **Kave**: Pre CXL-TE 3 months 6 months 9 months
  - Pre CXL-TE: 47.9
  - 3 months: 46.3
  - 6 months: 46.8
  - 9 months: 47.8
  - p > 0.05
Refractive, biomechanical and topoaberrometric changes after transepithelial cross-linking (TE CXL): 2 year follow-up

Luca Gualdi MD
Gualdi Ophthalmology Centre, Rome, Italy

OBJECTIVE
Evaluation of refractive, biomechanical and topoaberrometric changes, induced by transepithelial corneal cross-linking (TE CXL) after a 2 year follow-up period of 82 eyes from patients with keratoconus.

PATIENTS AND METHOD
TE CXL surgery treatment was performed on 82 eyes (52 patients). The following pre-operative and post-operative investigations were carried out: uncorrected and best spectacle-corrected visual acuity (UCVA and BCVA), corneal topography (CSO EYE TOP®), aberrometry (OPD-Scan II®), corneal topography (Pentacam® HD, Sirius®, Visante® OCT), corneal hysteresis and corneal resistance factor (Ocular Response Analyser®), endothelial cell counts (CSO), confocal microscopy (Confoscan 4®). Follow-up >2 years.

RESULTS
After over 2 years of follow-up, no patient lost any Snellen lines. The average improvement in uncorrected visual acuity (UCVA) and best spectacle-corrected visual acuity (BCVA) was respectively 0.56 and 0.30 Snellen lines (figure 1). Mean apical keratometry (AK) decreased significantly (figure 2); RMS at 4.8 mm improved on average by 0.62 μm, mainly due to COMA decrease (from 1.12 μm to 0.92 μm, figure 3) and to spherical aberration (from -1.80 μm to -1.58 μm). There was no statistically significant difference detected in the thinnest point (figure 4), in corneal hysteresis (CH = 8.8 mean pre-op, post-op = 8.9) and in corneal resistance factor (pre-op mean CRF = 9.1 and post-op = 9.1). Through corneal OCT, the observed “demarcation line” was on average around 90 μm (between 50 μm and 200 μm range). Side effects: rarely blurred vision and/or feeling of grit in the eye for a few hours There was no pain or other complications in any patients.

CONCLUSION
TE CXL benefits undoubtedly include: the possibility of treating corneas which are thinner than 400 μm; very young and/or non-cooperative patients (e.g. those with Down’s syndrome) or those requiring a very quick post-operative recovery. This technique is also characterized by the absence of pain, a quick rehabilitation, safety, the possibility of repeating, reduced risk of complication and less need for post-operative check-ups with a consequent cost reduction (surgery does not necessarily require an operating theatre, eyewash therapy only lasts a few days and does not require TCL application and removal). UVA radiation penetration in the stroma, without the removal of the epithelium was lower than standard treatment. Despite this, TE CXL was safe and effective in treating keratoconus and other ectatic diseases. There are also new techniques are on the horizon to take full advantage of the treatment’s effects in posterior stroma, such as iontophoresis, which will allow a greater distribution of stromal riboflavin. We are also exploring shorter exposure periods at different wavelengths and irradiation fluences. To date, both epi-of and epi-on techniques should continue to co-exist and every ophthalmologist should refer the patient to the best choice of treatment for each individual case.
Results

Parameters value during the follow-up period:

### Visual Acuity Improvement

<table>
<thead>
<tr>
<th>Snellen</th>
<th>UCVA</th>
<th>BCVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE-TREATMENT</td>
<td>0.56</td>
<td>0.30</td>
</tr>
<tr>
<td>POST-TREATMENT</td>
<td>0.56</td>
<td>0.30</td>
</tr>
</tbody>
</table>

### Keratometric Apex (AK)

<table>
<thead>
<tr>
<th>Dioptres</th>
<th>PRE-TREATMENT</th>
<th>POST-TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>56.12</td>
<td>54.48</td>
<td></td>
</tr>
</tbody>
</table>

### Corneal Aberrometry (COMA)

<table>
<thead>
<tr>
<th>Micron</th>
<th>PRE-TREATMENT</th>
<th>POST-TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.12</td>
<td>0.92</td>
<td></td>
</tr>
</tbody>
</table>

### Thinnest Point

<table>
<thead>
<tr>
<th>Micron</th>
<th>PRE-TREATMENT</th>
<th>POST-TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>488</td>
<td>492</td>
<td></td>
</tr>
</tbody>
</table>

Use of transepithelial corneal cross-linking (TE CXL) in the treatment of post-radial keratotomy ectasia.

Claudio Panico MD, Romolo Protti MD, Erika Savio MD
Ophthalmic B Division, - Ophthalmology Hospital, Turin, Italy

OBJECTIVE
Evaluation of the effectiveness of ribo avin UVA transepithelial corneal cross-linking in treating post-radial keratotomy (RK) corneal ectasia.

PATIENTS AND METHODS
TE CXL surgery was performed on six patients with corneal ectasia and progressive myopic shift which occurred after 5-15 years after RK was performed on both eyes. Each eye received: 1 drop of 1% pilocarpine 30 minutes before surgery, 4% lidocaine 15 minutes before surgery, cornea imbibition with 0.1% ribo avin + enhancer (RICROLIN® TE SOOFT Italia) for 30 minutes and RICROLIN® TE application every 2 minutes during the 30-minute exposure to UVA (6 steps, 5 minutes each) with UVA VEGA® emitter (3 mW/cm² of power emitted and 9 mm diameter irradiated area).

RESULTS
The resulting data shows that, after 18 months, aberrometric values (coma, spherical aberration) and keratometry are reduced while BSCVA improves and pachymetry stabilises. This data is statistically significant, although it is important to increase the patient sample to increase its statistical value. The ectatic process is therefore halted or stabilised without significant progression signs. Since CXL treatment is designed to halt or prevent the evolution of ectasia, end-point seems to have been reached in this case.

CONCLUSIONS
TE-CXL is an effective and safe therapeutic option in treating corneal ectasia after radial keratotomy. A lengthier follow-up is required in order to determine its long term effectiveness. Considering the data gathered, it would appear that performing TE-CXL treatment on all RK patients 3-6 months after surgery can increase the corneal brils structure stability by reducing the risk of wearing out.
Use of transepithelial corneal cross-linking (TE CXL) in the treatment of post-radial keratotomy ectasia.

OBJECTIVE
Evaluation of the effectiveness of riboavin UVA transepithelial corneal cross-linking in treating post-radial keratotomy (RK) corneal ectasia.

PATIENTS AND METHODS
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18 months after TE-CXL treatment, follow-up data shows:

- a coma reduction compared to pre-treatment measurements.
- a spherical aberration reduction compared to pre-treatment measurements.
- an improved best spectacle-corrected visual acuity (BSCVA) compared to pre-treatment measurements.
- stabilization of pachymetry values.
Combined treatment ICRs and transepithelial cross-linking in keratoconic eyes: preliminary results

Alessandro Mularoni MD
Department of Ophthalmology, Maggiore Hospital, Bologna, Italy

PURPOSE
To evaluate the efficacy of transepithelial cross-linking (TE-CXL) in keratoconic eyes after ICR implantation.

PATIENTS AND METHODS
Twelve eyes of 11 patients with bilateral keratoconus underwent INTACS (standard or SK) implantation with subsequent transepithelial cross-linking (TE-CXL with Ricrolin® TE) treatment. Inclusion criteria were: clear cornea, contact lens intolerance, corneal thickness higher than 400 microns. Preoperative and 1-3-6 months postoperative uncorrected visual acuity (UCVA), best-corrected visual acuity (BCVA), manifest refractions, and mean and simulated keratometric (K) values were reviewed retrospectively. The results of ICR group were compared to ICR + TE-CXL group.

RESULTS
There were 8 males and 3 females; average age was 30.14 +/- 7.11 (range 16-39) years old. Average time between implantation of INTACS and TE-CXL was 3.7 months. TE-CXL after ICRs resulted in an additional improvement in UCVA, BCVA and in an additional reduction in sphere, cylinder, and keratometry.

CONCLUSIONS
ICRs implantation with transepithelial CXL is effective in eyes with keratoconus. TE-CXL has an additive effect on eyes implanted with ICRs and could be considered as a stabilizing procedure.
Purpose:
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Twelve eyes of 11 patients with bilateral keratoconus underwent INTACS (standard or SK) implantation with subsequent transepithelial cross-linking (TE-CXL with Ricrolin® TE) treatment. Inclusion criteria were: clear cornea, contact lens intolerance, corneal thickness higher than 400 microns. Preoperative and 1-3-6 months postoperative uncorrected visual acuity (UCVA), best-corrected visual acuity (BCVA), manifest refractions, and mean and simulated keratometric (K) values were reviewed retrospectively. The results of ICR group were compared to ICR + TE-CXL group.

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Further reduction of apical keratometry (-1.41 D) is observed in an eye implanted with ICRs and treated with TE-CXL.
Traditional and transepithelial cross-linking: 
in vivo morphological study.

Manuela Lanzini MD, Roberta Calienno MD, Mario Nubile MD, Martina Colasante MD, Leonardo Mastropasqua MD
Regional Center of Excellence in Ophthalmology, University "G. d'Annunzio" of Chieti-Pescara, Italy

AIM OF THE STUDY
In vivo assessment, through confocal microscopy and OCT of the anterior segment, of the corneal changes induced by corneal cross-linking (CXL) using traditional and transepithelial techniques.

PATIENTS AND METHODS
The study assessed 80 eyes of 72 patients with progressive keratoconus, randomized for treatment of corneal cross-linking with traditional or transepithelial method. Inclusion criteria: Documented progression of keratometric indexes through altitudinal topography in a period of less than one year, corneal thinnest point greater than 400 microns, corneal transparency, age less than 40 years. Patients were assessed at one week, 1, 3, 6 and 12 months after treatment. At each control point, they were subjected to laser confocal microscopy of the cornea and OCT of the anterior segment. The level of inflammation, expressed as an absolute numerical index, and the keratocyte density were evaluated by confocal microscopy.

The presence and depth of a hyper-reflective stromal line previously described in the literature (demarcation line) is assessed by OCT of the anterior segment.

RESULTS
During the follow-up period, in vivo confocal microscopy showed both a significant reduction of keratocyte density (p = 0.01) and a significant increase of stromal inflammation (p = 0.001) in patients treated using the traditional technique, while no significant changes were observed in patients treated using the transepithelial technique (p > 0.05).

The OCT study of the anterior segment identified the presence of a demarcation line that was deeper and more persistent in patients receiving the traditional treatment (p < 0.001). In fact, in patients treated with the transepithelial technique that line first appeared nearly indistinguishable in the first check and absent in subsequent checks.

CONCLUSIONS
The results obtained show that corneal changes in terms of stromal inflammation and reduction of keratocyte density are much more pronounced and evident in patients treated with traditional techniques, suggesting that the tran-septithelial technique turns out to be much less invasive and damaging for the corneal tissue itself.

Both techniques are equally efficient in the stabilization of keratoconus. The CXL appears to be much less invasive than the traditional technique and allows a faster improvement of visual acuity.
Traditional and transepithelial cross-linking: in vivo morphological study.

**RESULTS**

Stromal changes induced by traditional Cross-Linking shown in confocal microscopy at different time intervals after surgery. 

A: 7 days; B: 1 month; C: 3 months; D: 1 year.

Stromal changes induced by transepithelial Cross-Linking shown in confocal microscopy at different time intervals after surgery. 

A: 7 days; B: 1 month; C: 3 months; D: 1 year.

Hyper-reflective stromal line (demarcation line) shown in OCT of the anterior segment 7 days after traditional (A) and trans-epithelial Cross-Linking.
Transepithelial corneal cross-linking (TE CXL) in patients with ultrathin keratoconic corneas

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**OBJECTIVE**
Analysis of the transepithelial corneal cross-linking (TE-CXL) results obtained by the combined action of riboflavin + enhancer (RICROLIN® TE) and UVA irradiation in 8 patients with progressive keratoconus, all with thinnest pachymetry values of less than 400 µm who are not treatable with de-epithelialisation standard technique.

**PATIENTS AND METHODS**
Eight patients with progressive keratoconus and with thinnest pachymetry values from 356 to 386 µm underwent TE-CXL treatment on one eye using a 0.1% riboflavin, 15% dextran T500 + enhancer (0.01% EDTA and trometamol) solution in order to improve riboflavin (RICROLIN® TE) transepithelial penetration. Patients underwent an ophthalmologic examination before treatment and other examinations 1 day, 1 week, 1 month and 6 months after surgery. The visit included endothelial cell density measurements and computerized videokeratography. Six months after TE-CXL treatment, a patient underwent a triple procedure (penetrating keratoplasty, open sky extracapsular cataract extraction and intraocular lens implantation). The explanted cornea was sent for immunohistochemical analysis where connexin-43 and CD34 protein expression was evaluated.

**RESULTS**
A full recovery of the corneal epithelium was achieved in all patients after one day’s use of a soft contact lens bandage. No side effects or damage to the limbal region were observed during the entire follow-up period. All patients showed a significant improvement in uncorrected visual acuity (UCVA) and best spectacle-corrected visual acuity (BSCVA); there was a reduced keratometric astigmatism (from 0.1 to 2.2 D) and a reduced cone’s apex dioptric power (K max values reduction ranged between 1 and 3 D). The density of endothelial cells did not show significant changes. Six months after surgery, the explanted cornea that had undergone immunohistochemical analysis, revealed epithelium, stroma and endothelium structural and functional integrity.

**CONCLUSIONS**
Performing TE-CXL with the use of a specific riboflavin formula enriched with substances promoting riboflavin penetration through an intact epithelium has proven to be extremely safe and effective with ultra thin keratoconic corneas, thus allowing us to treat also patients with more advanced stages of keratoconus.
Transepithelial corneal cross-linking (TE CXL) in patients with ultrathin keratoconic corneas

OBJECTIVE

PATIENTS AND METHODS

RESULTS

CONCLUSIONS

TRANSEPITHELIAL CROSS-LINKING

CORNEAL TOPOGRAPHY BEFORE TE CXL TREATMENT

Right eye computerized corneal topography (relative scale, tangential algorithm) of a 57 year old woman before TE CXL treatment.

Topographic pattern displays the presence of a keratoconus.

Apex power of keratoconus (central cornea) of 67.5 D.

CORNEAL TOPOGRAPHY 6 MONTHS AFTER TE CXL TREATMENT

Right eye computerized corneal topography (relative scale, tangential algorithm) of a 57 year old woman 6 months after TE CXL with riboflavin + enhancer and UVA irradiation treatment.

Topographic pattern shows a significant improvement in the corneal profile and a keratoconus with an apex power reduction of 65.0 D. (-2.5 D vs. pre-treatment).

L.A.A., 57 years old, surgery performed on 13 September, 2001

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Transepithelial corneal cross-linking (TE CXL): histological and immunohistochemical analysis of human corneas

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OBJECTIVE

Evaluation of transepithelial corneal cross-linking effects (performed with RICROLIN® TE and VEGA® UVA emitter) in the treatment of keratoconus, epithelium, keratocytes, collagen fibres and nerve fibres.

PATIENTS AND METHODS

15 human corneas were part of the clinical study: 10 were from patients with keratoconus who underwent penetrating keratoplasty (PKP); 5 of these were treated with TE-CXL 2 hours before PKP, while the remaining 5 were treated with TE-CXL 3 months before. 5 normal corneas taken from the cornea bank were employed as controls. All samples were prepared for epithelium and stroma morphological evaluation through immunohistochemical analysis (β-catenin, connexin 43, CD34, collagen type I) and through TUNEL assay to research and study keratinocyte apoptosis.

RESULTS

Changes in the epithelial cell expression of connexin 43 and of β-catenin were observed right after treatment; samples taken three months after treatment were almost completely deteriorated. Normal corneas displayed no TUNEL positive keratocytes while corneas of patients with keratoconus and those of patients treated with TE-CXL showed apoptotic cells in the anterior stroma. Keratocytes of corneas that underwent TE-CXL showed evenly distributed CD34 positivity throughout the thickness of the cornea; the same pattern was found in normal corneas (controls). 3 months after TE-CXL, collagen I immunohistochemical analysis showed a normal pattern in the anterior stroma. There were no changes in the corneal subepithelial nerve plexus.

CONCLUSIONS

CXL is a safe and minimally invasive technique. Histological data of the human cornea conforms changes that damage the epithelium, keratocytes and stromal collagen structure.
CD 34 immunohistochemical analysis
CD34 protein constitute keratocytes. In a normal cornea they are uniformly distributed throughout the corneal stroma. In patients with keratoconus (KC) they are not uniform since keratoconus reduces CD34 immunoreactivity. Corneas treated with TE-CXL display CD34 uniformly distributed in the corneal stroma.

Immunohistochemical analysis of collagen fibres
Type I collagen control colour (CTL), keratoconus (KC) and transepithelial cross-linking (TE-CXL). Image shows how cross-linked corneas collagen fibres are more regular 3 months after surgery compared to KC ones.