Micromorphological evaluation of Crosslinked cornea by Confocal laser scanning In vivo Microscopy

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According to Wollensak, Seiler and Spöerl, we introduced the CCL in Italy in the 2004 at Siena University.

First Italian CCL Trial

Parasurgical therapy for keratoconus by riboflavin–ultraviolet type A rays induced cross-linking of corneal collagen

Preliminary refractive results in an Italian study

Aldo Caporossi, MD, Stefano Baiocchi, MD, Cosimo Mazzotta, MD, Claudio Traversi, MD, Tomaso Caporossi, MD

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Conservative treatment of keratoconus by riboflavin-UVA-induced cross-linking of corneal collagen: Qualitative investigation of corneal epithelium and subepithelial nerve plexus regeneration by in vivo HRT II system confocal microscopy in humans

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In all crosslinked corneas after therapeutic soft contact lens removal 5 dd post op the micromorphology of epithelium is regular. Quality of epithelium in the post operative period improve progressively increasing patient visual outcome.
Sub epithelial nerves regeneration start 1 month after CCL

Nerve fibres of sub-epithelial plexus: pre operative aspect

15 day postop: nerve fibres loss in the 7 mm central irradiated oedematous area

Central area

Peripheral ring area beyond 9 mm

1m post op: regular nerve fibres in the untreated peripheral ring area beyond 9 mm

1m post op: starting of nerve fibres regeneration in 7 mm central irradiated area
Sub epithelial nerves regeneration become complete 6 months after CCL restoring rapidly patient corneal sensitivity.
Stromal Nerves Regeneration

start one month after CCL from the deeper fibres and centripetally

Pre op Anterior stromal nerves

1 month post: stromal nerves disappearance

stromal nerves regeneration after 3 months

C. Mazzotta MD, PhD
First International In Vivo Confocal Report after CCL

Mazzotta et al

Clinical Science

Treatment of Progressive Keratoconus by Riboflavin-UVA–Induced Cross-Linking of Corneal Collagen

Ultrastructural Analysis by Heidelberg Retinal Tomograph II
In Vivo Confocal Microscopy in Humans

Cosimo Mazzotta, PhD,* Angelo Balestrazzi, PhD,* Claudio Traversi, MD,* Stefano Baiocchi, PhD,* Tomaso Caporossi, MD,† Cristina Tommasi, MD,* and Aldo Caporossi, MD*

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Depth 50 -100µm: Anterior corneal stroma 1,3 and 6 months after collagen cross-linking by in vivo real time HRT II confocal microscopy Rarefaction of keratocytes with spongy or honeycomb-like edema (A). Initial repopulation with activated keratocytes 3 months after the operation (B). No changes in cells density in the peripheral untreated area (C). Dense population of activated keratocytes, regenerated nerve fibres, increased tissue density at 6 months (D).
Depth 100 -150µm: Corneal stroma after collagen cross-linking by HRT II in vivo real time confocal microscopy. Rarefaction of keratocytes and edema after the treatment with appeared trabecular network (A). Inside trabecular network are detectable elongated (masked necrotic keratocytes) nuclei and smaller nuclei (keratocyte apoptotic bodies) (A). Gradual keratocytes stromal repopulation at this depth after 3 months (B), almost completed at 6 month (C), with good corneal biomicroscopic clinical appearance (D).

C. Mazzotta MD, PhD
Depth 170-180µm: Intermediate stroma after combined riboflavin-UVA-induced corneal collagen cross-linking by HRT II in vivo real time confocal microscopy, (Corneal edema at 1 month in the intermediate stroma with ghost nuclei (apoptosis bodies) in the fibrillar network, elongated nuclei and absence of keratocytes (A). Initial keratocytes repopulation after 3 months (B). Dense extracellular matrix, cell repopulation with activated keratocytes and dark micro-striae at 3 (C) and 6 months (D)
**Depth 180-300µm:** Intermediate corneal stroma after riboflavin-UVA-induced corneal collagen cross-linking. In vivo HRT II confocal microscopy, Keratocytes disappearance and stromal edema were evident, with small nuclei (keratocyte apoptosis bodies, arrows) in the fibrillar network (A). Initial signs of cell repopulation were observed at 3 months (B). Keratocytes repopulation was clearly evident and complete at 6 months (C) with good corneal clinical aspect (D).
The depth of treatment *in vivo* is more frequent at 310 micron, from 270 to 350 microns (VTA).

The vertical transition area (VTA) consisted in a merging from an oedematous zone poor in cells and with low reflectivity (A) to a deeper stromal zone regularly populated with keratocytes and slight oedema diffusion (B-D).

*C. Mazzotta MD, PhD*
In vivo micromorphological safety evaluation of CCL by focusing real depth of treatment 1 month post op: Vertical Transition Area
Evaluation of lateral transition area (LTA) by In vivo HRT II confocal microscopy after corneal collagen cross-linking. Laterally, beyond the central irradiated area, keratocytes had a normal appearance (A-B, green arrows,) with minimal spread of edema (A-B, yellow arrows). Typical keratoconus dark micro-striae were evident in both section (A-B, red arrows). Lateral transition (LTA) consist in a crossing lateral area regularly populated with keratocytes and slight edema diffusion with a central edematous zone poor in cells, with low reflectivity (B, blue arrows).
Depth 350 – 450 µm: The deep stroma beyond 350 µm (A) was not reached by cross-linking except for slight spread of stromal edema which did not extend more than 10-20 µm beyond the vertical transition zone. Activated keratocytes (yellow arrows) are evident with slight edema (red arrows) at 1 (B), 3 (C) and 6 months (D).
In vivo real time HRT II confocal microscopy after collagen cross-linking. At 1 month corneal thickness is increased by edema (A). Endothelium had regular cell density and morphology at 1 (A) 3 (B), 6 (C) and 12 months (D).
Treatment of progressive keratoconus by riboflavin-UVA-induced cross-linking of corneal collagen: healing detection by in vivo HRT II system confocal microscopy in humans

C. Mazzotta MD, An. Balestrazzi MD, C. Traversi MD, S. Baiocchi MD, O. Caporossi MD and Aldo Caporossi MD; Clinic Exp Ophthalmol 2007, IN PRESS
Keratoconus dark microstriae

keratoconus typical banding pattern

C. Mazzotta MD, PhD
Sub-clinical In vivo healing process:
haze is detectable by in vivo HRT II confocal microscopy in a case of advanced keratoconus with large dark micro-striae and deep stromal Vogt striae (A). Corneal edema at 1 month (B). Initial keratocytes repopulation after 3 months (C) Activated keratocytes nuclei (D, green arrows) with increased stromal reflectance (D, red arrows) and microscopically detectable haze after 6 months.
In vivo increased stromal reflectivity after CCL

In vivo increased density of extracellular matrix after CCL represent a micromorphological indirect sign of a Cross-Linked Cornea

C. Mazzotta: I and II International CCL Meetings, Zurich - December 2005 - 2006
### Confocal Analysis Summary

**Total analyzed: 40 cases**

**Follow up > 6 m: 34 cases**

<table>
<thead>
<tr>
<th>N cases</th>
<th>Confocal</th>
<th>Microsc</th>
<th>F up &gt; 6m</th>
<th>Confoc pre-op</th>
<th>Dark microstrie</th>
<th>Preop Reticular Pattern</th>
<th>Dark microstrie</th>
<th>Slit lamp Preop</th>
<th>Preop Vogt Striae</th>
<th>Epithelial Regeneration</th>
<th>After 5 days of Soft contact lens</th>
<th>Subepithelial &amp; anterior stromal nerves</th>
<th>Repopulation</th>
<th>Post op lacunar Edema resolution</th>
<th>Post op Keratocytes</th>
<th>Hyper reflect extra cell matrix</th>
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<tr>
<td>34</td>
<td>34</td>
<td>100 %</td>
<td>4</td>
<td>6</td>
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<td>Haze</td>
<td>Early on</td>
<td>5 (15%)</td>
<td>4 (11%)</td>
<td>1- 3 m</td>
<td>Late on 1- 3 m</td>
<td>1 (4%)</td>
<td>12 m</td>
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No changes in endothelial cells density and micromorphology after 18 months

Treatment Depth: m 320 µ