Iontophoresis is the transfer of charged molecules throughout the tissues to be treated, by means of a low intensity electric field. Many types of drugs can be used as molecules. The rate at which the charged molecules pass throughout, can be increased by changing the intensity of the applied current or according to the characteristics of the employed solution/preparation.

By exploiting the physical principle of ions migrating from one pole to the other, specific polarized drugs are prepared containing either positively or negatively charged ions, or both (bipolar). These polarized drugs are applied to the electrodes according to their polarity. If, for example, the drug bears positive charges, it is applied at the positive electrode. If it bears negative charges, the drug is applied at the negative electrode. It is indifferent when bipolar charged drugs are used. In either case, the other pole is placed on an area adjacent to the district to be treated. Therefore, by applying the electrode with the drug on the area to be treated and the other one no more than at a distance of 10/20 cm, the electric current will deliver the drug throughout the tissues since the drug’s ions will migrate toward the opposite pole until the drug is fully absorbed.

Iontophoresis is currently the preferred technique to introduce a drug into the body through the skin. This treatment allows to directly treat those areas that are affected by a painful illnesses (algia) caused by rheumatic diseases (arthritis, sciatica, back pain, neck pain, etc.) as well as other pathologies such as cellulites, adiposis, etc., achieving quick results. The intake of oral drugs is thereby avoided which could produce well known systemic side effects.
For ocular iontophoresis, the treatment is performed by applying on the patient two electrodes that are connected to a power generator.
A special rubber ring contains the main electrode (- terminal) which is applied to the cornea to be treated by suction; the other electrode (+ terminal) is a “small plaster” (patch) that is placed on the patient’s forehead.
The electric current flow (low intensity) between the two electrodes allows a specific formulation of riboflavin (RICROLIN®+), especially developed for iontophoretic drug delivery, to quickly penetrate into the corneal stroma, through the intact epithelium (therefore without de-epithelialisation), ensuring optimal imbibition.

The flow of electric charges is due to a power generator.
Iontophoresis is performed using a 1 mA/min intensity (5-minute treatment).
It is a continuous current output powered by batteries.
Treatment duration is automatically monitored by a suitable software of the power supply.
Iontophoresis is automatically stopped when the 5-minute treatment limit is reached.
Iontophoresis for ophthalmic applications, has been studied for several years and extensively published. It is possible to refer to the research carried out by Frucht-Pery et al. regarding transcorneal and transconjunctival dexamethasone delivery or to the studies performed by the USA company Eye-Gate.
Experimental studies with riboflavin delivery by means of iontophoresis (P. Roy, 2011) have demonstrated that the molecule penetrates into the corneas of animals (in vivo) and into human corneas (ex vivo). Penetration was evaluated both directly (i.e. measured by determining the concentration of riboflavin in the corneal stroma and aqueous humor) and indirectly (i.e. through the evaluation of the increase of stromal biomechanical rigidity after CXL-TE). During biomechanical studies, all treated corneas were exposed to a 3 mW/cm² dose of ultraviolet rays (UV-A) after imbibition.

Data clearly show that 5 minutes of iontophoresis treatment produce a greater stromal imbibition than 15 minutes of passive imbibition.
The stress-strain test demonstrates that corneas imbibed with RICROLIN®+, using iontophoresis, thanks to an improved stromal imbibition, have higher biomechanical strength compared to those treated with RICROLIN® TE with passive imbibition for 30 minutes.

CONCLUSIONS

A higher amount of riboflavin is delivered into the corneal stroma through iontophoresis compared to the amount delivered using passive imbibition.

A 5-minute iontophoresis treatment actually delivers into the corneal tissue a higher amount of riboflavin compared to 15’ of passive imbibition.

The amount of riboflavin available in the stroma is proportional to the iontophoresis treatment time.

Since iontophoresis is performed with an intact epithelium, corneal cross-linking treatment provides all of the advantages of the transepithelial technique (no haze risk, no visual acuity decrease after surgery, no infection risk).
Comparative Stress Strain Measurements Of Human Corneas After Transepithelial UV-A Induced Cross-linking: Impregnation With Iontophoresis, Different Riboflavin Solutions And Irradiance Power.


* Ophthalmology, Istituto Clinico Humanitas, Milan, Italy; ** Ophthalmology, University of Dresden, Dresden, Germany.

OBJECTIVE
The aim of this study was to evaluate the iontophoresis delivery method to administer Riboflavin into the corneal stroma without disepithelialization and compare it to other transepithelial techniques. The effect on corneal structures (collagen fibers) was evaluated by biomechanical methods on human corneas (ex-vivo) after the treatment of UV-A riboflavin induced cross-linking.

METHODS
Twelve post-mortem human corneas were treated with TE CXL dividing the corneas in four groups, according to the method of impregnation and the UV-A power used.

<table>
<thead>
<tr>
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<th>RTE</th>
<th>RB3</th>
<th>RB10</th>
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<td>3</td>
<td>3</td>
<td>2*</td>
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<td>Ricolin Te</td>
<td>Ricolin Te</td>
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</tbody>
</table>

Impregnation methods and UV-A irradiation power.

- RTE=Group A: irradiance power of 3 mW/cm² for 30 minutes and impregnation with “standard” transepithelial riboflavin solution (RICROLIN® TE).
- RB3=Group B: irradiance power of 3 mW/cm² for 30 minutes and impregnation with hypotonic + enhancer riboflavin solution.
- RB10=Group C: irradiance power of 10 mW/cm² for 9 minutes and impregnation with hypotonic + enhancer riboflavin solution.
- RBI10=Group D: irradiance power of 10 mW/cm² for 9 minutes, impregnation with iontophoresis and hypotonic + enhancer riboflavin solution.
The IONTOPHORESIS device for corneal application (8mm in diameter) is placed on the cornea using an annular suction ring (low suction created by a syringe connected to the suction annulus).

The device is filled with approximately 0.5ml solution from the open proximal side, until the electrode (stainless steel mesh) is covered. The device is connected to a constant current generator (I-ON CXL®) set at 1mA/min for 5 minutes (the total dose of 5mA x min is monitored by the generator). Since the corneas are placed on a MORIA artificial chamber, the return electrode is a stainless steel wire inserted into one of the pressure tubes, underneath the corneas, in the artificial anterior chamber. The 2 tubes are connected to a perfusion line allowing circulation of BSS during the experiment. Attention is paid to assure the strict absence of air bubbles into the circuit.

RESULTS
The stress-strain curves showed the typical exponential increase of a bioviscoelastic solid. Stress strain measurement showed an increase in corneal rigidity after cross-linking compared to standard CXL-TE, indicated by a rise in strain and in Young's modulus calculated at 10% strain. The stress values, using 10% strain, are summarized in the picture. Considering group A (TE CXL) as standard of comparison, group B showed an increase in Young's modulus was by a factor of 1.45, group C by a factor of 1.26, group D by a factor of 1.81. None of these differences was statistically significant, possibly because of the small sample size of each group.

CONCLUSION AND DISCUSSION
We evaluated the difference between different transepithelial cross-linking (TE CXL) techniques in terms of stress strain. The maximal effect was measured in the Iontophoresis group with 10 mW/cm² of irradiation power. The reported increase in stability was similar to the data published by Wollensak et al. However, there was no statistically significant difference between corneas treated with such technique versus those undergoing standard TE CXL, possibly because of the small sample size of each group. Stress strain measurement in the other groups showed a better results using riboflavin solution without dextran and 3mW/cm² of irradiance power. Pachymetry results showed a similar mean value between the groups.

In conclusion, Iontophoresis with an irradiation power of 10 mW/cm², was more effective in increasing stress strain compared to other impregnation techniques and irradiation powers. Iontophoresis has the potential to become a valid alternative to increase corneal biomechanical properties while reducing treatment time, postoperative pain and risk of infection. However, more studies are needed to evaluate its safety and efficacy as compared to the standard protocol which entails the removal of the epithelium.
Effects of UVA Crosslinking in human corneas after imbibition with iontophoresis and hypotonic riboflavin solution: an ex vivo study.

Rita Mencucci *MD, Iacopo Paladini* MD, Eleonora Favuzza*, Mirca Marini** MD, Giulia Raugei*, Ugo Menchini*, Barbara Vannelli**

*Eye Clinic – University of Florence, Florence, Italy
**Department of Anatomy – University of Florence, Italy

OBJECTIVE
To evaluate whether different UV-A irradiation intensities (3 and 10 mW/cm²) on human corneas (ex vivo) soaked through IONTOPHORESIS procedure performed with a new hypotonic solution of riboflavin + enhancer (RICROLIN® +) determine different morphological and biochemical responses on corneal tissues (epithelium, keratocytes, collagen and nerve fibers and endothelium).

METHODS
Fifteen human corneas coming from eye bank were divided into 3 different groups according to methods of soaking and UV-A intensity used:

Group 1 - five corneas soaked through 5 minutes iontophoresis treatment using RICROLIN®+ and followed by 30 minutes of UV-A irradiation at 3 mW/cm²;

Group 2 five corneas soaked through 5 minutes iontophoresis treatment using RICROLIN®+ and followed by 9 minutes of UV-A irradiation at 10 mW/cm²

Group 3 - five corneas soaked through 5 minutes iontophoresis treatment using RICROLIN®+ without UV-A irradiation;
Three more untreated corneas have been utilized as control.

All samples were prepared for epithelium and corneal stroma morphological evaluation using immunohistochemical analysis (CD34, type I collagen) and for keratocytes apoptosis through TUNEL assay. Hematoxylin eosin stain was used to investigate any signs of corneal fibrosis.

The endothelium evaluation has been conducted through hematoxylin eosin stain too, while for nerve fiber a zinc iodide-osmium tetroxide impregnation was used.

Every samples were analyzed 48 hours after the treatment.

RESULTS
Group 3 doesn’t show any difference from the control group.

The two cross-linked groups of corneas (Group 1 and Group 2) showed variable changes in the stroma, mainly due to keratocytes apoptosis related to the treatment intensity.
No corneas showed signs of fibrosis. No endothelial damage has been evidenced in treated groups, nor nerve fiber alterations.

CONCLUSIONS
On the basis of this ex-vivo preliminary study, iontophoresis can be considered as an effective technique to improve riboflavin penetration into corneal stroma. 10 mW/cm² power intensity can be considered safe for irradiated tissues.
**Patient Preparation**

1. The patient shall be positioned horizontally and is instructed to stay still during the whole procedure.
2. Anesthetize the eye with a topical anaesthetic according to anaesthetic recommendations. Ensure that the last application is made 5 minutes before starting procedure, in order to avoid inadvertent iontophoresis administration of the anaesthetic.
3. Inspect the forehead area for any damaged or compromised skin. Determine if there is sufficient space on the forehead to apply the return electrode avoiding placement of the electrode on damaged or compromised skin. Do not proceed if there is insufficient space on the forehead to place the return electrode.
4. Thoroughly clean the forehead with a 70% alcohol wipe.
5. Apply the return electrode to the centre of the forehead. Refer to the “Return Electrode Instructions for Use” for further details.
Applicator preparation

6. Open the corneal iontophoresis applicator package.
7. Connect the vacuum syringe on the female luer lock (4) connector and verify that the clamp (3) is open.
8. Position the blepharostat.
9. Position the applicator on the cornea to be treated. Check the proper location by looking through the centre of the applicator; cornea and applicator must be concentric with one another.
10. Perform a tiny aspiration of 1 ml with the syringe and close the clamp (3).
11. Verify that the applicator is fixed well on the patient eye; if not, repeat points 9-11. Disconnect and remove the vacuum syringe.
12. Fill a syringe with RICROLIN®+, connect it on the yellow female luer lock (8) connector and fill the applicator with RICROLIN®+ until the level is above the grid. The electrode should be completely immersed during the whole procedure, to obtain a proper electrical contact.

DO NOT touch the applicator during procedure.

13. Verify that the generator is off.
14. Connect the current generator I-ON CXL® with the applicator at (9) and connect the current generator with the return electrode, too (Refer to the “Current generator and the Return electrode Instruction for Use” for further details).

Treatment Procedure

15. Turn on the current generator, choose 1 mA and press START (Refer to the “Current generator Instruction for Use”).
16. The procedure should last 5 minutes. Bubbles could appear around the grid during the procedure: this is the proof that current is flowing. However this event is not index of temperature rise, which remains constant in both the solution and the tissue.

DO NOT remove the electrode before the end of the treatment.

17. If the patient can’t stand 1 mA current (it rarely happens), switch the current to 0,5 mA. The generator automatically records the variation and recalculates the time left until to achieve the total current emission of 5 mA.
18. At the end of the treatment, remove the RICROLIN®+ from the applicator through the tube (7). Open the clamp (3) to let air enter in the vacuum ring (1) and remove the applicator from the eye. Disconnect the current generator from applicator and return electrode.
19. Rinse the patient eye with a saline solution.
20. Proceed to the UV-A irradiation with a UV-A emitter. Refer to the “UV-A light source Instructions for Use” for further details.
21. Discard the applicator, the return electrode and the vacuum syringe according to standard operating procedures.

Note: If the iontophoresis treatment should be stopped, put the generator in “pause” mode, pressing the STOP button before removing the applicator from the eye.
A novel riboflavin delivery system through the cornea using iontophoresis and a novel riboflavin formulation (RICROLIN®+) “optimized” for iontophoresis, allow to achieve a cutting edge technology in cross-linking, since the corneal imbibition times are greatly reduced. Total treatment time is reduced from one hour to just 14 minutes, without diminishing the safety and effectiveness of the treatment.