PhotoRefractive IntraStromal Cross-Linking (PiXL)

Anastasios John Kanellopoulos, MD[1,2]

George Asimellis, PhD[2]

1. Clinical Professor of Ophthalmology, NYU Medical School, Department of Ophthalmology, New York, NY
2. Laservision.gr Eye Institute, 17 Tsocha Street, Athens, Greece, Postal Code: 11521

Tel + 30 210 7472777

Fax+ 30 210 7472789

Email: ajk@brilliantvision.com

Stabilization of corneal ectasia has been the original intent of the initially described, corneal collagen crosslinking (CXL) by the Dresden protocol.[1,2]. What has become evident through the last two decades of broad clinical CXL applications, in addition to halting the ectatic process,[3,4,5] CXL appears to decrease the corneal curvature irregularity, manifested as mainly as central anterior corneal flattening.[6,7]. The overall increase in corneal biomechanics (the stiffening effect over the entire cornea) is invariably accompanied by corneal curvature reduction.[8,9,10,11,12] We have attributed this flattening effect to the more intense (higher bioavailability) of the invariably centrally-applied pattern of both the riboflavin solution, as well as the UV-A illumination. It has been therefore the mainly centrally-applied CXL that induces central corneal flattening as a collateral effect to the biomechanical stabilization of the cornea.

Our team has contributed many of the evolutionary steps of the initially introduced CXL technique:

1-Higher fluence

2-non-dextran containing riboflavin solution

3-combination of CXL with topography-guided excimer normalization of ectatic corneas (The Athens Protocol)

4-Prophylactic CXL in routine myopic and hyperopic LASIK

5-In-situ CXL through a femtosecond laser created corneal pocket

6-Photorefractive CXL

Specifically: We have introduced the concept of accelerated, high-fluence collagen crosslinking (CXL) in post-LASIK ectasia,[13] as well as the utilization of prophylactic CXL in routine LASIK,[14] and in-situ, femtosecond laser-assisted treatment of corneal ectasia[15], in attempting corneal deturgescence[16] in bullous keratopathy[17] and as a prophylactic intervention adjuvant to Boston keratoprosthesis surgery.[18] The procedure known as the Athens Protocol (AP),[19] involves sequentially excimer-laser epithelial debridement (50 μm), partial topography-guided excimer-laser stromal ablation, and high-fluence UVA irradiation (10 mW/cm^2), accelerated (10′) CXL. Corneal topography data are derived from either the Alcon/WaveLight (WaveLight AG, Erlagen, Germany) Allegro Topolyzer Vario, a wide-cone Placido corneal topographer, or the Alcon/WaveLight Oculyzer, a Pentacam Scheimpflug imaging rotating camera (Oculus Optikgeräte GmbH, Wetzlar, Germany) [20] Early results,[21] as well as anterior-segment optical coherence tomography quantitative findings [22] are indicative of the long-term stability of the procedure.[23]

As our understanding and increased capabilities in the assessment of CXL effects have progressed, we have theorized that customizable, differential CXL application in specific areas of the cornea may be able to induce refractive changes. Spatially selective corneal stromal stiffening can alter corneal curvature, resulting in the achievement of a planned

refractive change. This theory has also been recently supported by computer simulation patterns innovated by B.J. Dupps et. al. at the Cleveland Clinic Foundation.[24]

The key questions are what is the importance of doing so, and what are the indications for such a novel technique? The answer lies in the proposition that –if proven successful- this CXL application may radically enlarge the use of CXL not just in the field of stabilizing ectatic disorders (and to a lesser degree, managing certain corneal infections),[25] but to a much broader arena, that of refractive surgery. This new application may be considered as an alternative to the excimer laser-ablation for small refractive errors. As far back as in the early 1990s, the use of laser as a means of correcting, in a predictable manner, the refractive error by 'reshaping' the cornea was probably as far-reaching and unproven as it is perhaps the use of CXL today for the same purpose. The difference lies not in the reshaping of the cornea by means of localized tissue ablation, but in the reshaping of the cornea by means of localized, differential tissue strengthening.

Thus the indications for this application are:

1- Healthy corneas of patients with lower refractive errors. The 'quantification' of how low may be defined in the future; based on today's experience, it appears that 3 diopters maybe the higher limit for myopia, hyperopia and/or astigmatic corrections with CXL alone.
2- Keratoconic or ectatic corneas of lower ectasia index that CXL is desired to be combined with a customized refractive intervention of myopia and/or cylinder without tissue removal.
3- Both of the above could be approached in an epi-on and epi-off technique, the decision between the two will be affected by the patient age (pediatric cases may be chosen to undergo epi-on techniques), minimal cornea thickness, corneas thinner

than 400 microns of total corneal thickness may be chosen to undergo epi-on CXL in order to minimize potential endothelial damage.

Several key aspects of this novel procedure have been considered before efficacy could be claimed: feasibility, safety and efficacy, predictability and long-term stability are among the chief ones.

Feasibility: as the procedure is adequately comprehended, the application may be custom-applied to not only achieving myopic refractive change, but also astigmatic, and more far-promising and novel, hyperopic refractive change, i.e. selective central corneal steepening. For this purpose, specific, customizable CXL patterns may be employed. For example, to achieve a myopic change, in which a central corneal flattening is desired, we apply a central CXL pattern (Figure 1A).[26] To achieve an astigmatic change, in which a flattening along a specific meridian is desired, we apply a bow-tie CXL pattern (Figure 1B);[27] and to achieve a hyperopic change, an annular shape CXL pattern is applied (Figure 1C).[28] These are all possible today with the differential UV delivery system offered by the KXL II device (Avedro, Waltham, MA).

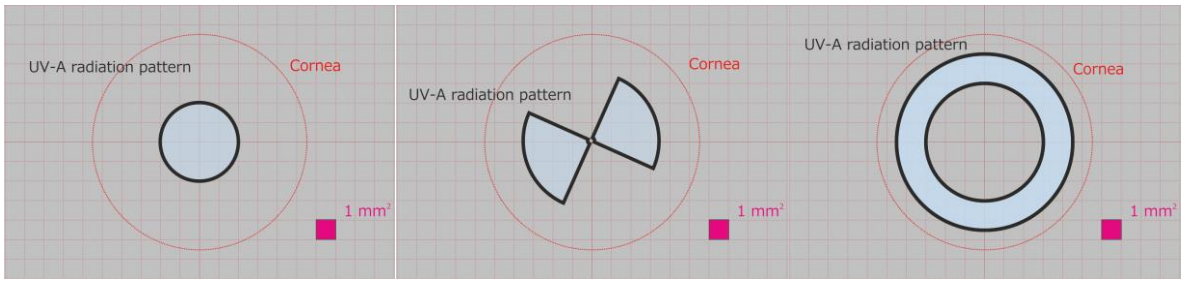

Figure 1: Customizable patterns for predictable refractive changes. A, myopic; B, astigmatic; C, hyperopic.

The second aspect of the feasibility question revolves around the intact epithelium-on (transepithelial) or epithelium-off application debate. As the application is inevitably targeted to healthy corneas, for which the existing, minimally invasive and fully matured applications exist (such as LASIK), the transepithelial application must be considered as the

one offering the least invasive option in comparison to removing the epithelium. According to the Dresden protocol, the epithelium must be removed prior to CXL treatment to permit riboflavin penetration into the corneal stroma and ensure adequate corneal saturation with riboflavin.

The intact epithelial option may be advantageous as relatively simple for the patients and surgeon, requires minimal post-operative adjustment of daily activities by the patient, and produces essentially no pain or discomfort. The rapid recovery and relative safety of transepithelial CXL may additionally offer the possibility to titrate the effect through two or more treatments over time as needed. Owing to the characteristics of this novel application, is termed photorefractive intrastromal crosslinking (PiXL).

The epithelium-on CXL results in a significantly weaker biomechanical effect in comparison to the epithelium-off CXL. The two main reasons for this reduced CXL efficacy are, first the reduced UV-A transmittance to underlying stroma,[29] as the epithelium and Bowman's membrane may be responsible for at least 1/3 of the UV-A being absorbed. The second is the insufficient and inhomogeneous transepithelial riboflavin diffusion into the corneal stroma.[30] The intact epithelium prevents riboflavin penetration[31] (the large molecular weight of riboflavin being responsible for this). Even the use of higher-concentration (0.5%) riboflavin solution through the intact epithelium may not facilitate improved results in comparison to the standard (0.1%) concentration.[32]

To overcome these challenges, two novel approaches have been adopted. First, a higher fluence UV-A is implemented via the KXL II device, reaching up to 45mW/cm^2. Second, a specifically designed approach to achieve riboflavin penetration is implemented. Specifically, the riboflavin penetration through the intact epithelium (transepithelial) into the stroma involves a two-step process. First step is application of Paracel solution (Avedro Inc), a slightly hypotonic specially-formulated 0.25% riboflavin solution with 0.02% benzalconium

chloride. Application time is 4 minutes with two drops approximately every minute. The aim of this mild 'abrasive' solution application was to 'open up' the epithelial cell junctions. The manufacturer cautions against administering ParaCel for over four minutes to minimize the risk of epithelial sloughing. The second step is application of VibeX Xtra (Avedro Inc,), 0.25% riboflavin isotonic saline solution. Application time is six minutes, with a drop every thirty seconds. With this 2-step soaking process, riboflavin passage to the anterior chamber was approximately complete in ten minutes.

Safety and efficacy may be studied in-vivo and ex-vivo. The safety aspect is related to the question, can the human cornea tolerate higher fluence of UV-A light? The possible effect on corneal endothelium is related the increased energy and fluence applied. Endothelial Cell Count (ECC) examination by confocal specular microscopy both pre-operatively as well as one-month postoperatively is integral part of our clinical protocol. Our data have not yet indicated any statistically significant difference in ECC. The efficacy aspect is related to the question, can this intrastromal cross-linking induce sufficient change in the biomechanical properties? To answer this question, we have applied novel ex-vivo investigation to biomechanically address this issue, as in-vivo biomechanical measurements in our experience have shown low specificity and sensitivity. We evaluated the changes in corneal strength following intrastromal CXL by employing objective biaxial stress-strain measurements. This technique may be superior to corneal strip extensiometry utilized in past experiments[33], taking into account the non-uniform topographic distribution of the corneal strength profile.[34] The findings in this study[35] may provide substantial ex-vivo evidence that significant corneal strengthening takes place even when UV-A light is projected through the intact corneal epithelium, Bowman's membrane and superficial stroma, to reach underlying riboflavin-soaked stroma in order to conduct CXL.

Predictability may be assessed by further studies, as currently we are conducting initial, proofing clinical evaluation. Currently we have applied the PiXL application in more than 40 cases, over the course of more than 18 months. We have presented pioneering studies that achieved myopic refractive change;[26] the one-year refractive changes are illustrated in Figure 2. Astigmatic changes have also been reported, [27] as well hyperopic changes.[28]

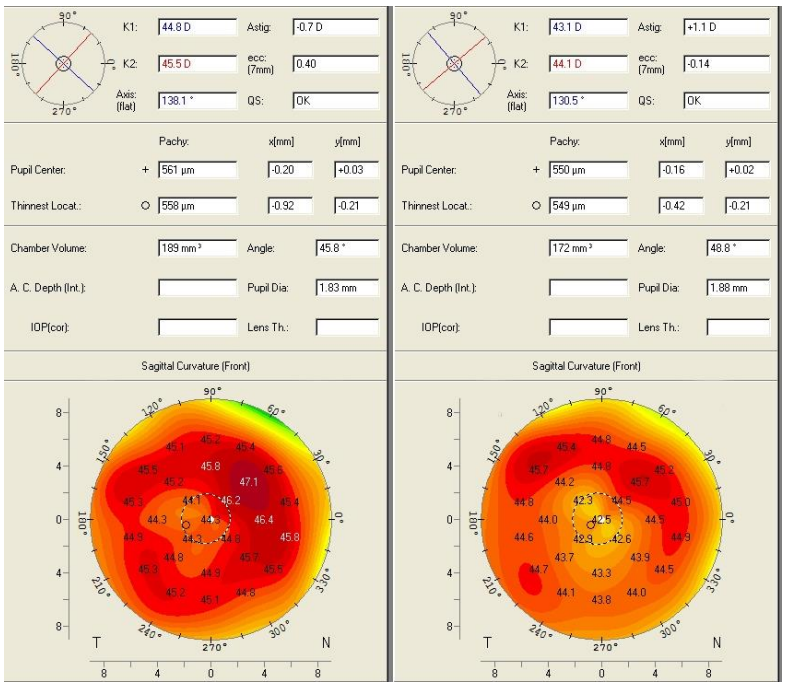

Figure 2: Scheimpflug imaging data of myopic PiXL: pre-operative (left) and at postoperative (right) depicting the significant and regular pattern central corneal flattening effect. The applied pattern corresponds to the one shown in figure 1A.

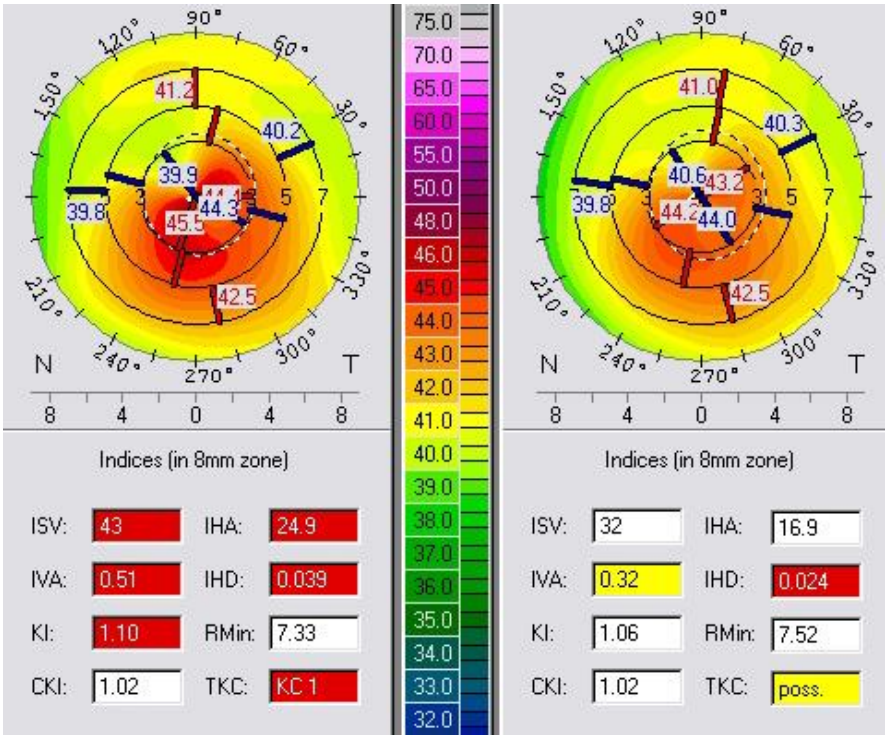

Figure 3: Scheimpflug imaging data showing comparison of pre-operative versus post-operative data depicting significant refractive changes along the axis of the customized cross-linking pattern as well as anterior-surface normalization. The applied pattern corresponds to the one shown in figure 1B.

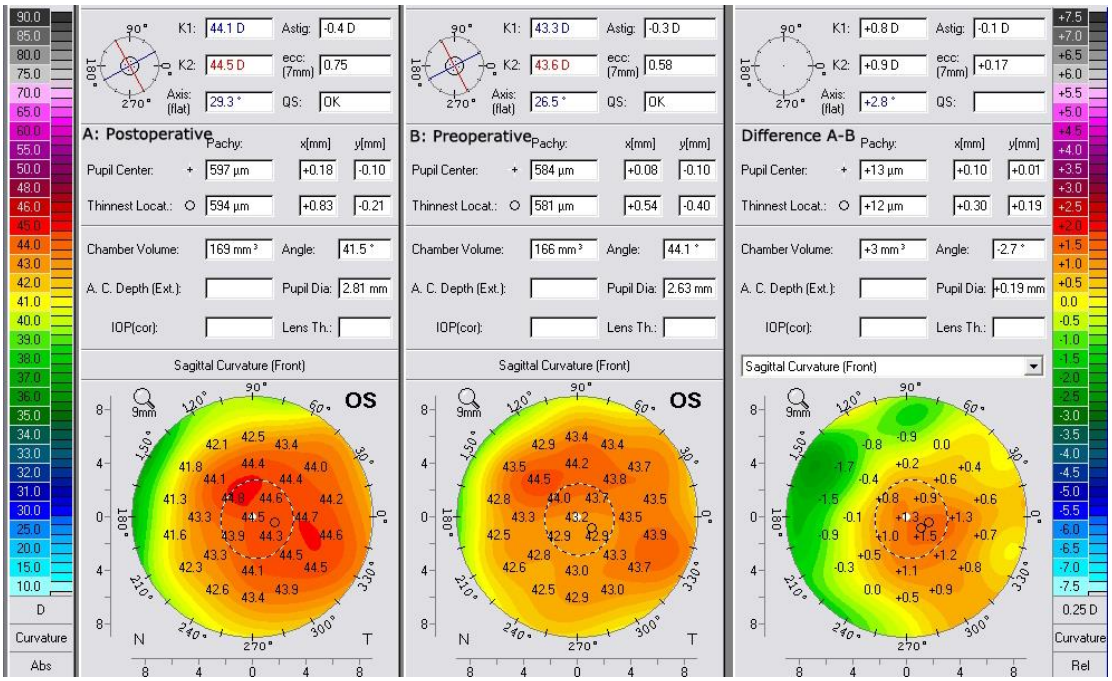

Figure 5: Scheimpflug imaging data showing comparison of pre-operative versus post-operative data depicting hyperopic refractive changes. The applied pattern corresponds to the one shown in figure 1C.

Establishing and refining a nomogram, the optimal optical zone, are of course data that may be attempted and evaluated in future studies. We note, however, that the ease and essentially null morbidity that this procedure offers may additionally offer the possibility to titrate the effect through two or more treatments through time.

We discussed so far the initial clinical results of a novel CXL application with a novel device employing very high fluence variable-customizable pattern collagen crosslinking, in order to achieve myopic, astigmatic, and hyperopic refractive changes in healthy patients.

The second option for the application of the PiXL technique is the customized treatment of mild keratectasia. The nearly unlimited customization options for the topographic determination of the UV-A pattern, along with the possibility of combining different steps of different geometries allows for a specific guidance of the UV-A irradiation to achieve specific goals.

In the example shown in Figure 6, a keratoconic patient was treated with a customizable version of PiXL. Custom CXL pattern was applied in a three-step overlaid patterns, a circular, and two single arcs. Exposure time and fluence (and consecutively, delivered energy) are all customizable. The CXL 'action' was targeted on the thinnest cornea. Comparison of pre-operative to post-operative data indicate a significant 'cone' reduction, and a post-operative cornea appearing a lot more 'regular'. This patient can now be managed with a spectacle refraction, as the postoperative astigmatism has been managed to a significantly more regular, in comparison to the preoperative.

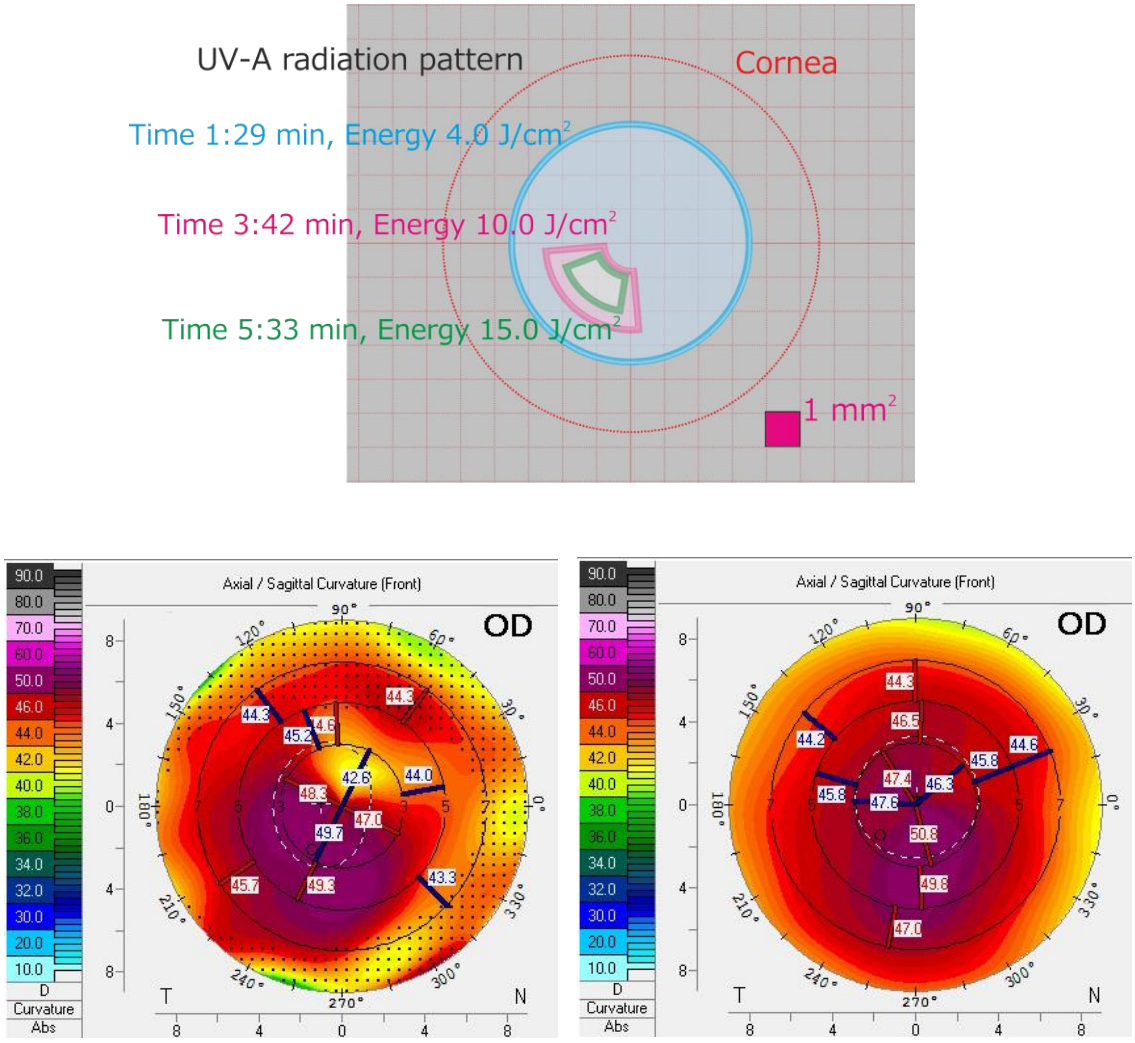

Figure 6: Top: Custom CXL pattern applied in a three-step overlaid patterns, a circular, and two single arcs. Exposure time and fluence (and consecutively, delivered energy) are all customizable. The CXL 'action' was targeted on the thinnest cornea. Bottom: Scheimpflug imaging data showing comparison of pre-operative (left) versus post-operative (right) curvature data depicting significant 'cone' reduction, and a post-operative cornea appearing a lot more 'regular'.

Conclusions:

CXL has come a long way since it was introduced in Ophthalmology clinical practice as a stabilizer of corneal ectasia. Its clinical use has expanded in corneal deturgesence for bullous keratopathy, it has been combined with excimer laser ectasia normalization and ICRS, it has been combined with LASIK as a biomechanical modulator, and lately it has been applied in a customized, variable fluence way in order to productively employ its potential refractive changed to the cornea presumably deriving from CXL

differentials within the same cornea. The anterior segment ophthalmic surgeon has acquired an enhanced armamentarium in effectively treating cornea disease and potentially expanding the refractive surgery options available today.

References

[1] Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-A-induced collagen crosslinking for the treatment of keratoconus. *Am J Ophthalmol*. 2003;135:620-627.

[2] Dupps WJ Jr: Special section on collagen crosslinking: new hope for more advanced ectatic disease? *J Cataract Refract Surg* 2013;39:1131–1132.

[3] Ghanem RC, Santhiago MR, Berti T, Netto MV, Ghanem VC. Topographic, corneal wavefront, and refractive outcomes 2 years after collagen crosslinking for progressive keratoconus. *Cornea*. 2014;33(1):43-8

[4] Arora R, Jain P, Goyal JL, Gupta D. Comparative Analysis of Refractive and Topographic Changes in Early and Advanced Keratoconic Eyes Undergoing Corneal Collagen Crosslinking. *Cornea*. 2013 Aug 22. [Epub ahead of print] PMID: 23974893

[5] Hassan Z, Szalai E, Módis L Jr, Berta A, Németh G. Assessment of corneal topography indices after collagen crosslinking for keratoconus. *Eur J Ophthalmol*. 2013;23(5):635-40.

[6] Touboul D, Trichet E, Binder PS, Praud D, Seguy C, Colin J. Comparison of front-surface corneal topography and Bowman membrane specular topography in keratoconus. *J Cataract Refract Surg*. 2012;38(6):1043-9

[7] Piñero DP, Alio JL, Klonowski P, Toffaha B. Vectorial astigmatic changes after corneal collagen crosslinking in keratoconic corneas previously treated with intracorneal ring segments: a preliminary study. *Eur J Ophthalmol.* 2012;22 Suppl 7:S69-80.

[8] Coskunseven E, Jankov MR II, Hafezi F. Contralateral eye study of corneal collagen cross-linking with riboflavin and UVA irradiation in patients with keratoconus. *J Refract Surg*. 2009;25:371-376.

[9] Vinciguerra P, Albè E, Trazza S, et al. Refractive, topographic, tomographic, and aberrometric analysis of keratoconic eyes undergoing corneal cross-linking. *Ophthalmology*. 2009;116:369-378.

[10] Raiskup-Wolf F, Hoyer A, Spoerl E, Pillunat LE. Collagen crosslinking with riboflavin and ultraviolet-A light in keratoconus: long-term results. *J Cataract Refract Surg*. 2008;34:796-801.

[11] Caporossi A, Mazzotta C, Baiocchi S, Caporossi T. Long-term results of riboflavin ultraviolet a corneal collagen cross-linking for keratoconus in Italy: the Siena eye cross study. *Am J Ophthalmol.* 2010;149:585-593.

[12] O'Brart DP, Kwong TQ, Patel P, McDonald RJ, O'Brart NA. Long-term follow-up of riboflavin/ultraviolet A (370 nm) corneal collagen cross-linking to halt the progression of keratoconus. *Br J Ophthalmol*. 2013;97:433-437.

[13] Kanellopoulos AJ. Post-LASIK ectasia. *Ophthalmology*. 2007;114:1230.

[14] Kanellopoulos AJ, Pamel GJ. Review of current indications for combined very high fluence collagen cross-linking and laser in situ keratomileusis surgery. *Indian J Ophthalmol*. 2013;61:430-2

[15] Kanellopoulos AJ. Collagen cross-linking in early keratoconus with riboflavin in a femtosecond laser-created pocket: initial clinical results. *J Refract Surg.* 2009;25:1034-7.

[16] Krueger RR, Ramos-Esteban JC, Kanellopoulos AJ. Staged intrastromal delivery of riboflavin with UVA cross-linking in advanced bullous keratopathy: laboratory investigation and first clinical case. *J Refract Surg*. 2008;24:S730-6.

[17] Kanellopoulos AJ, Asimellis G. Anterior-Segment Optical Coherence Tomography Investigation of Corneal Deturgescence and Epithelial Remodeling After DSAEK. *Cornea*. 2014;33(4):340-8.

[18] Kanellopoulos AJ, Asimellis G. Long-term safety and efficacy of high-fluence collagen crosslinking of the vehicle cornea in Boston keratoprosthesis type 1. *Cornea*. 2014;33(9):914-8.

[19] Kanellopoulos AJ. Long term results of a prospective randomized bilateral eye comparison trial of higher fluence, shorter duration ultraviolet A radiation, and riboflavin collagen cross linking for progressive keratoconus. *Clin Ophthalmol.* 2012;6:97–101.

[20] Kanellopoulos AJ, Asimellis G. Correlation Between Central Corneal Thickness, Anterior Chamber Depth, and Corneal Keratometry as Measured by Oculyzer II and WaveLight OB820 in Preoperative Cataract Surgery Patients. *J Refract Surg.* 2012;28:895-900.

[21] Krueger RR, Kanellopoulos AJ. Stability of simultaneous topography-guided photorefractive keratectomy and riboflavin/UVA cross-linking for progressive keratoconus: case reports. *J Refract Surg.* 2010;26:S827-32

[22] Kanellopoulos AJ, Asimellis G. Introduction of quantitative and qualitative cornea optical coherence tomography findings, induced by collagen cross-linking for keratoconus; a novel effect measurement benchmark. *Clin Ophthalmol.* 2013;7:329-35.

[23] Kanellopoulos AJ, Asimellis G. Keratoconus management: long-term stability of topography-guided normalization combined with high-fluence CXL stabilization (the Athens Protocol). *J Refract Surg.* 2014;30(2):88-93.

[24] Roy AS, Dupps WJ Jr. Patient-specific computational modeling of keratoconus progression and differential responses to collagen cross-linking. *Invest Ophthalmol Vis Sci* 2011;52:9174–9187.

[25] Tayapad JB, Viguilla AQ, Reyes JM. Collagen cross-linking and corneal infections. *Curr Opin Ophthalmol.* 2013;24(4):288-90.

[26] Kanellopoulos AJ. Novel myopic refractive correction with transepithelial very high-fluence collagen cross-linking applied in a customized pattern: early clinical results of a feasibility study. *Clin Ophthalmol.* 2014;8:697-702

[27] Kanellopoulos AJ, Dupps WJ, Seven I, Asimellis G. Toric topographically customized transepithelial, pulsed, very high-fluence, higher energy and higher riboflavin concentration collagen cross-linking in keratoconus. *Case Rep Ophthalmol*. 2014;5(2):172-80.

[28] Kanellopoulos AJ, Asimellis G. Hyperopic correction: clinical validation with epithelium-on and epithelium-off protocols, using variable fluence and topographically customized collagen corneal cross-linking. . *Clin Ophthalmol*. 2014;8: in press

[29] Bottós KM, Schor P, Dreyfuss JL, et al. Effect of corneal epithelium on ultraviolet-A and riboflavin absorption. *Arq Bras Oftalmol*. 2011;74:348-51.

[30] Leccisotti A, Islam T. Transepithelial corneal collagen cross-linking in keratoconus. *J Refract Surg*. 2010;26:942-8.

[31] Baiocchi S, Mazzotta C, Cerretani D, et al. Corneal crosslinking: riboflavin concentration in corneal stroma exposed with and without epithelium. *J Cataract Refract Surg.* 2009;35:893-9.

[32] Stojanovic A, Zhou W, Utheim TP. Corneal Collagen Cross-Linking with and without Epithelial Removal: A Contralateral Study with 0.5% Hypotonic Riboflavin Solution. *Biomed Res Int.* 2014;619398.

[33] Elsheikh A, Anderson K. Comparative study of corneal strip extensometry and inflation tests. *J R Soc Interface.* 2005;2:177-85.

[34] Sánchez P, Moutsouris K, Pandolfi A. Biomechanical and optical behavior of human corneas before and after photorefractive keratectomy. *J Cataract Refract Surg.* 2014;40:905-17.

[35] A. John Kanellopoulos, Mark Alan Kontos, Shihao Chen, and George Asimellis. Corneal collagen cross-linking (CXL) combined with simulation of femtosecond laser assisted refractive lens extraction: an ex-vivo biomechanical effect evaluation. *Cornea* 2014; under review.