Keratoconus and cross-linking
Wednesday, May 07, 2014 8:30 AM–10:15 AM
Exhibit/Poster Hall SA Poster Session
Program #/Board # Range: A0228–A0260
Organizing Section: Cornea
Contributing Section(s): Multidisciplinary Ophthalmic Imaging

Program Number: 4200 Poster Board Number: A0228
Presentation Time: 8:30 AM–10:15 AM

Growth factor and interleukin secretion of human keratoconus keratocytes after crosslinking/riboflavin-UVA photodynamic therapy (PDT)

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Purpose: Crosslinking (CXL)/riboflavin-UVA photodynamic therapy (PDT) is a treatment option to stop the progression of keratoconus. Growth factors and interleukins have the function to regulate proliferation and motility of the cells and even wound healing. The purpose of this study was to determine the impact of crosslinking on growth factor and interleukin secretion of human keratoconus keratocytes, in vitro.

Methods: Primary human keratoconus keratocytes were isolated by digestion in collagenase (1 mg/ml) from human corneal buttons, and cultured in DMEM/Ham’s F12 medium supplemented with 10% FCS. Keratocyte cell cultures underwent UVA illumination using light (370 nm) for 4.10 minutes during exposure to 0.1% riboflavin and 20% dextran containing PBS. Five and twenty-four hours after CXL, secretion of FGFβ1, HGF, TGFβ1, VEGF, KGF, IL-1β, IL-6, and IL-8 was measured by enzyme-linked-immunoabsorbent assay (ELISA).

Results: KGF and IL-1β secretion of keratocytes was below the measurement limit for all time points. Using riboflavin or UVA light illumination separately, growth factor and interleukin secretion of keratocytes remained unchanged for both time points (p>0.35). Five hours after crosslinking, FGFβ secretion of keratoconus keratocytes increased significantly (p = 0.037) compared to untreated controls, whereas HGF, TGFβ1, VEGF, IL-6, and IL-8 secretion remained unchanged. Twenty-four hours following CXL, none of the growth factor and interleukin concentrations differed significantly from untreated controls (p>0.15).

Conclusions: Crosslinking triggers FGFβ secretion of keratoconus keratocytes transiently (five hours), which normalizes after 24 hours. Crosslinking does not have an impact on HGF, TGFβ1, VEGF, KGF, IL-1β, IL-6, and IL-8 secretion of keratoconus keratocytes in the short-term.

Commercial Relationships: Xuefei Song, None; Tanja Stachon, None; Jiong Wang, None; Achim Langenbucher, None; Berthold Seitz, None; Nora Szentmary, None

Keratoconus and Multi-Disciplinary Imaging

Program Number: 4201 Poster Board Number: A0229
Presentation Time: 8:30 AM–10:15 AM

Derivation and Characterization of Human Induced Pluripotent Stem Cells from Stromal Keratocytes of Patients with Keratoconus

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Purpose: Keratoconus (KC) is a frequent cause of visual abnormalities due to corneal thinning and loss of function. It causes a significant health burden due to donor organ shortage and high cost. It is characterized by a complex inheritance of multiple genetic defects with several gene candidates (Lu et al., 2012). Disease progression is poorly understood due to a lack of cellular and animal models. A new paradigm in disease modeling is the creation of human induced pluripotent stem cells (hiPSCs). We took advantage of the fact that hiPSCs can maintain residual epigenetic memory of the original cell that they are derived from. Here we show corneal keratocyte-derived hiPSCs from KC patients and healthy individuals.

Methods: Primary keratocytes were isolated from freshly isolated corneas by collagenase digestion at 37°C, rinsed in keratocyte media (DMEM/F12, N2, B27, bFGF) and plated. To induced proliferation, isolated keratocytes were then “activated” by addition of serum and reprogramming was performed using episomal plasmid transfection expressing pluripotency factors. Cultures were plated on Matrigel and fed a non-serum containing medium supplemented with reprogramming small molecules for 18 days. Colonies resembling PSC morphology were isolated and plated on Matrigel coated plates. To induce re-differentiation to keratocytes, hiPSCs from a GFP expressing reporter line were plated on isolated Descemet’s membrane using 12 well Cell Crown.

Results: Human IPSCs were successfully generated from primary keratocytes isolated from fresh human corneas of healthy individuals and KC patients after serum activation. Twelve clones were selected for further expansion and 3 clones were characterized using battery of pluripotency assays. Further, we demonstrated that iPSCs expanded and survived during differentiation in keratocyte conditioned medium after direct plating on Descemet’s membrane isolated from donor corneas.

Conclusions: In this study we demonstrate for the first time efficient generation of KC-specific human iPSCs from stromal keratocytes. These keratocyte-derived iPSCs exhibited all properties of human pluripotent stem cells, including morphology and expression of genes and surface markers indicative of pluripotency. These cells will provide a valuable “disease-in-a-dish” model to study KC pathogenesis and progression, and aid in the development of novel therapies.

Commercial Relationships: Loren A. Ornelas, None; Yelena Bykhovskaya, None; Dhruv Sareen, None; Yaron S. Rabinowitz, None

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Program Number: 4202 Poster Board Number: A0230
Presentation Time: 8:30 AM–10:15 AM

3-D Imaging of Epithelial-Stromal Interactions Found in Keratoconus

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Purpose: Keratoconus (Kc) is a disease where the cornea becomes thinner, ectatic, and scarred. The basement membrane (BM), anterior limiting lamina (ALL), and anterior stromal lamellae are altered or lost throughout the course of the disease. While epithelial and stromal involvement is thought to contribute to disease initiation and progression, the nature of this interaction is unclear. The purpose of this study was to use serial block face scanning electron microscope (SEM) imaging and computerized 3-D image reconstruction to evaluate the pathophysiology of Kc.

Methods: A penetrating keratoplasty was performed on a 47 year old white male diagnosed with Kc four years prior. The surgically removed Kc corneal button was fixed, processed, and embedded in
resin blocks for transverse serial block-face imaging using a Gatan 3 View system mounted in an FEI Quanta FEG 200 SEM, using an established protocol. A stack of 165 serial images separated by 100 nm increments and measuring 49 μm X 49μm in XY was obtained from the central anterior cornea. Amira 5.4.3 software was used to segment the anterior cornea for 3-D image reconstruction.

**Results:** Following 3-D reconstruction, epithelial and stromal interactions were evident. Two keratocytes were observed to project anteriorly and engage with epithelial cells located in the basal epithelium. Likewise, complementary basal epithelial cell protrusions were seen to extend toward the keratocytes.

**Conclusions:** The novel Gatan 3 technology can provide unique and useful data on Kc pathophysiology. The 3-D reconstructions obtained by serial block-face imaging demonstrate for the first time in Kc that there are interactions between the stroma and epithelium. This observation warrants further study as it will be important to establish the extent and consequence of this pathological tissue interaction.

Segmented anterior cornea. The anteriorly placed keratocytes (purple, blue, red) are located very close to the epithelium (yellow). Two cells located in the epithelium (orange and green) extend out and interact with the anteriorly placed keratocytes.

**Commercial Relationships:** Colton Heinrich, None; Ali Behzad, None; John D. Goosey, None; Alan R. Burns, None; Jan P. Bergmannson, None

**Program Number:** 4203 Poster Board Number: A0231

**Presentation Time:** 8:30 AM–10:15 AM

**Detecting early keratoconus in Down’s syndrome**

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**Purpose:** Keratoconus is significantly more prevalent in young people with Down’s syndrome (DS) than in the typical age-matched population. However, patients who have DS are unlikely to report early changes in vision both because of their communicative limitations, and because the DS visual system is inherently less sensitive than that in typically developing young people. As a result, presenting disease is commonly moderate to advanced, creating challenges for contact lens fitting and corneal grafting. Early diagnosis is a priority for young people with DS, so that they can benefit from interventions such as corneal cross-linking, but this diagnosis is difficult owing to a naturally steeper, thinner cornea, which is present even in healthy individuals with DS. The current study evaluates corneal and visual norms of young people with DS and suggests indicators which should raise suspicion of keratoconus during screening in this population.

**Methods:** A case-control study was conducted involving 56 eyes of 31 subjects (17 male, 14 female) with DS. The age range was 7 to 26 years (mean±SD 18.19±5.12). The subjects underwent corneal examination including slit lamp biomicroscopy, corneal tomography analysis using Pentacam (Oculus GmbH, Wetzlar, Germany) and biomechanical evaluation using Oculus Response Analyzer (Reichert, Buffalo, NY). Optometric data including best corrected spectacle visual acuity, contrast sensitivity, binocular status and spectacle refraction were obtained. Subjects were classified using a modified Amsler-Krumeich approach prior to statistical analysis.

**Results:** The current cohort of young people with DS have a lower corneal resistance factor (mean±SD 8.7±2.2) than has been noted in typical subjects of a similar age in other published studies. In the non-keratoconic eyes examined, a minimum corneal thickness of as little as 378μm was noted (range 378μm to 575μm), whilst the maximum corneal power was 47.7D (range 42.6D to 27.7D). The range of regular corneal astigmatism was 0.1D to 1.6D. Corneal astigmatism >1.60D and corneal power >50D are each significant risk factors for keratoconus in DS.

**Conclusions:** Young people with DS, even without keratoconus, display an altered biomechanical status. Standard topographical and pachymetric indices for the identification of keratoconus in DS, are on their own, inappropriate, and this carries implications for monitoring and diagnosis in primary and secondary care.

**Commercial Relationships:** Stephanie Campbell, None; J. M. Woodhouse, None, Keith M. Meek, None

**Program Number:** 4204 Poster Board Number: A0232

**Presentation Time:** 8:30 AM–10:15 AM

**Keratoconus detection by corneal asymmetry analysis with Pentacam Scheimpflug tomography**

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**Purpose:** To evaluate the diagnostic performance of intereye asymmetry analysis in corneal indices provided by the Pentacam tomograph for detecting keratoconus.

**Methods:** Observational case series of 35 healthy subjects in group 1 and 40 keratoconic patients in group 2, evaluated with Scheimpflug tomography (Pentacam HR, Oculus Optikgerate GmbH, Germany). For data analysis, Student’s t test was used to compare means and the area under the receiver operating characteristic curve (AROC) was used to compare the diagnostic performance of the intereye asymmetry in corneal descriptors for keratoconus detection. The statistical significance criteria used was p <0.05.

**Results:** In group 1 vs group 2, mean age (33.5±11.4 vs 36.5±15.2 yrs) and gender distribution (60% vs 52.5% males) did not differ significantly. Intereye asymmetry for all variables in group 1 was not correlated to anisometropia. Compared with group 1, intereye asymmetry was significantly greater in group 2 for all variables except for Dy, ARTMin and ARTMax. No patients in group 1 had...
an intereye difference in anterior keratometry > 0.5 D or in thinnest or apex pachymetry > 25 μm. Eight of the 19 variables had AROC > 0.93 for their asymmetry. The best 8 indices (AROC, cutoff, sensitivity and specificity) were D (0.99, >1.24, 95%, 97.1%), AvgPPI (0.97, >0.2, 92.5%, 97.1%), MaxPPI (0.97, >0.33, 92.5%, 94.3%), Dp (0.97, >1.37, 92.5%, 94.3%), anterior keratometry (0.97, >0.5 D, 90%, 100%), posterior keratometry (0.94, >0.1 D, 85%, 97.1%), MinPPI (0.94, >0.13, 87.5%, 91.4%) and Df (0.93, >1.17, 87.5%, 91.4%), respectively. Combined metrics of intereye asymmetry in 4 and 5 corneal descriptors had 100% sensitivity and 93% and 98% specificity, respectively.

**Conclusions:** Intereye asymmetry is rare in normal corneas and very common in keratoconus patients. Almost half of the corneal descriptors analyzed had very high specificity and sensitivity when considered alone. A combined approach showed remarkable performance and might serve to discriminate true keratoconic patients from false positive cases that arise in clinical practice when examining single corneal indices.

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**Presentation Time:** 8:30 AM–10:15 AM

**Between-eye Asymmetry Detected by Scheimpflug Imaging in Subjects With Normal Corneas and Keratoconus**


**Purpose:** To evaluate the diagnostic accuracy of measuring between-eye asymmetry with regard to pachymetry and corneal elevation in the prediction of keratoconus.

**Methods:** This study included 115 patients: 68 subjects with bilateral normal corneas (NC) and 47 with bilateral keratoconus (KC) including 10 forme fruste corneas. Central corneal thickness (CCT), pachymetry at the thinnest point (ThCT), posterior elevation at the thinnest point of the cornea (PE) was measured in both eyes using Scheimpflug imaging. Between-eye asymmetry was determined by subtracting the lower value from the higher value for each variable. Covariate adjustment for area under the receiver operating characteristic curve (AUROC) was used to determine predictive accuracy of different variables to identify keratoconic corneas.

**Results:** In normal subjects, the mean between-eye asymmetry in CCT, ThCT and PE were 5.59±4.90 μm, 6.57±5.30 μm and 3.13±3.71 μm, respectively. In keratoconus patients, the mean asymmetry in CCT, ThCT and PE were 30.13±35.80 μm, 39.70±36.42 μm and 35.40±37.31 μm. In normal eyes, the mean between-eye asymmetry were significantly lower compared with the keratoconic eyes (p<0.001 for CCT, ThCT and PE). There was significant correlation between ThCT and asymmetry of ThCT (KC: r=-0.40, p=0.003), between CCT and asymmetry of CCT (KC: r=-0.72, p=0.002) and between PE and asymmetry of PE (KC: r=0.82, p<0.001) in the keratoconus group, but not in the control group (p=0.05 for all of the variables). After adjustment for these correlations, between-eye asymmetry of CCT, ThCT and PE showed high accuracy to predict keratoconus with an AUROC value of 0.99 for ThCT, 0.94 for CCT and 0.96 for PE.

**Conclusions:** There is a greater between-eye asymmetry in pachymetry and posterior corneal elevation values in keratoconic patients than in subjects with normal corneas. After adjustment for the significant effect of keratoconus progression measured by increase in posterior elevation or by decrease in pachymetry on between-eye asymmetry keratoconic eyes can be identify with high accuracy. This method may help to identify corneas with risk of developing corneal ectasia with simple pachymetry measurements.

**Commercial Relationships:** Lorant Dienes, None; Kinga Kranitz, None; Andrea Gyenes, None; Eva Juhasz, None; Janos Nemeth, None; Illes Kovacs, None; Zsolt Z. Nagy, None

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**Presentation Time:** 8:30 AM–10:15 AM

**Comparison of Machine Learning Methods to Automatically Classify Keratoconus**

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**Purpose:** Given the strong clinical similarities between normal eyes and eyes with subclinical or forme fruste keratoconus (KTC), the screening of patients for refractive surgery can sometimes be challenging. This problem could be solved by machine learning algorithms that automatically and objectively classify into different groups based on a combination of parameters. This work aims to compare the accuracies of 3 such machine learning algorithms to automatically classify corneas into normal, keratoconus and suspect.

**Methods:** This case-control study analyzes the basic biometric parameters provided by the Pentacam for 1379 eyes (924 subjects), of which 944 had keratoconus (671 of grade 1, 201 grade 2 and 72 grade 3 of the Krumeich scale), 85 KTC-suspect eyes, and 350 normal control eyes.

Within each group, 33% of the subjects were randomly selected for the test group and the remaining were used to train the classifiers, which were Naive Bayes (NB) with kernel density estimation, Discriminant Analysis (DA) and Support Vector Machine (SVM). The dimensionality of the data was reduced either performing Canonical Discriminant Analysis (CDA) or using a Sequential Feature Selection (SFS) algorithm with NB accuracy optimization. Finally, accuracy was estimated from the results when applying the classifier to the test group and also using a Cross-Validation (CV) method on the training set.

**Results:** The highest accuracy in classifying KTC 1 and normal (96.57%) was achieved by NB with SFS parameter reduction. Classifying KTC 1 to 3 and normal eyes, the highest accuracy was obtained by NB without parameter reduction (97.81%). KTC-suspect and normal eyes could be distinguished with 91.95% accuracy by da without parameter reduction (see figure 1).

**Conclusions:** The most accurate kFC detection method depends on the comparison at hand. Reducing the number of parameters does not impair the performance of the classification algorithm. Instead, it can even improve the accuracy in some cases. Furthermore, in the particular case of SFS, it is possible to derive the best discriminant features for KTC detection, obtaining a good accuracy with only 5 or 7 parameters.

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Commercial Relationships: Irene Ruiz Hidalgo, None; Pablo Rodriguez Perez, None; Jos J. Rozema, None; Marie-Jose B. Tassignon, None

Program Number: 4207 Poster Board Number: A0235
Presentation Time: 8:30 AM–10:15 AM
Comparison of Orbscan Indices with the ORA KMI for Predicting Keratoconus
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Purpose: Keratoconus (KCN), a corneal ectasia, is diagnosed by abnormal corneal topography or tomography (Orbscan). The Ocular Response Analyzer (ORA) is a newer tool that measures corneal biomechanics and uses its non-FDA approved KCN Match Index software (KMI) to compare 37 biomechanical variables to a patient database. This study aims to evaluate the agreement between Orbscan indices and KMI values.

Methods: This retrospective study included patients (>18y) who underwent both Orbscan and ORA KMI testing. The ORA KMI software assigns probability percentages in 5 classes from normal to severe KCN. The Orbscan measures properties of the anterior and posterior corneal surfaces. Using the KMI value as ‘truth’, we analyzed its agreement with Orbscan indices commonly used to diagnose KCN. Raw agreement scores were calculated, and those greater than .70 were considered significant. Cohen’s kappa agreement coefficients were determined for the significant indices, and those that were >.61 were considered to have substantial agreement with KMI scoring.

Results: Among 5 patients (10 eyes), the ORA KMI values ranged from .017 to 1.214. KMI software classified 4 eyes as normal, 3 as suspect, and 5 as mild KCN, and none as moderate or severe KCN. Indices with significant raw agreement rates (> .70) were normal band scale (NBS), thinnest corneal thickness (TCT), irregularity index (II) at 3mm (.8) and at 5mm (.9), posterior float elevation above BFS (.9), and astigmatism difference between eyes (.7). Of these positive raw agreement rates, the following kappa coefficients were calculated between KMI and each index: NBS (.83), TCT (.78), II at 3mm (.6) and at 5mm (.78), posterior float elevation above BFS (.78), and astigmatism difference between eyes (.39). Corneal steepness, inferior steepening at 3mm and at 5mm, posterior BFS, and ratio of BFS radii had raw agreement rates ≥ .7 and therefore they did not sufficiently agree with the KMI.

Conclusions: Compared to KMI, the NBS, TCT, II at 3mm and 5mm, posterior float elevation above BFS, and astigmatism difference between eyes had the highest agreement rates (>70%). Of these, NBS, TCT, II at 5mm, and posterior float elevation above BFS had “substantial” agreement by kappa scoring and thus may be more sensitive for diagnosing KCN than other indices. By measuring corneal biomechanics the ORA KMI may be a useful adjunct in diagnosing KCN, especially in early subtle stages of the disease.

Commercial Relationships: Kelley Bohm, None; Ryan A. Vasan, None; Christopher E. Starr, None

Program Number: 4208 Poster Board Number: A0236
Presentation Time: 8:30 AM–10:15 AM
Analysis of Predictors of Keratoconus Progression
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Purpose: Keratoconus is a congenital, progressive, ecstatic disease of the cornea. However, the mechanism of exacerbation is unclear. We retrospectively analyzed the characteristics of keratoconus patients to identify predictors of disease progression.

Methods: This study retrospectively analyzed 55 eyes of 35 patients (10 females and 25 males; age, 25.3±8.0 years) with keratoconus, who were followed for at least 6 months at our institute. In addition to standard ophthalmic examinations, we investigated the change in manifest refraction (spherical equivalents [SE] and cylinder power [Cyl]), steepest keratometric value (Ks), and thinnest corneal thickness (TCT). We also asked the age at which the patients were diagnosed with keratoconus. We defined progression as a decrease of more than 0.5 diopters per year (D/Y) for SE and increases of 0.5 D/Y for Cyl and Ks. We assigned progression scores from 0 to 3 as follows: 0, no progression observed for SE, Cyl, and Ks; 1, one of the factors progressed; 2, two factors progressed; and 3, all three factors progressed.

Results: The age at diagnosis ranged from 14 to 41 years. At the first visit, SE, Cyl, Ks, and TCT were –4.41±4.24 D, –3.05±2.01 D, 50.42±7.25 D, and 451.7±62.1 μm, respectively. SE progressed –0.95 ± 0.53 D/Y, Cyl increased 0.44 ± 0.37 D/Y, the steepest K-value increased 0.44 ± 0.37 D/Y, and the TCT decreased 4.45 ± 18.08 μm per year. Spearman’s rank correlation test showed that the rates of change of SE, Cyl, and the progression score were correlated with the change in TCT (ρ = 0.3375, 0.3816, and –0.331; p = 0.0165, –0.063, and 0.0189, respectively). Ks was correlated with the rate of change of Cyl (ρ = 0.3012, p = 0.0269). The rate of change of Ks progressed with patient age at the first visit (ρ = –0.2845, p = 0.039).

Conclusions: A thin, steep cornea may help to predict the progression of keratoconus. Particularly, the decrease in corneal thickness is an important indicator of progression; however, patient age could be a secondary factor.

Commercial Relationships: Naoko Katō, None; Kazuo Negishi, None; Megumi Saiki, None; Kazuo Tsubota, None

Program Number: 4209 Poster Board Number: A0237
Presentation Time: 8:30 AM–10:15 AM
SF-36 as a quantitative assessment of the quality of life in patients with keratoconus and its relationship to severity of the disease
Virgilio Galvis1, Arantxa Acera1, Alejandro Tello1, Jesus Meryayo-Looves1, Tatiana M. Suarez-Cortes1, 1Ophthalmology, Centro Oftalmologico Virgilio Galvis, Florida, Colombia; 2Ophthalmology, Centro Oftalmologico Virgilio Galvis, Florida, Colombia; 3Oftalmik SL, Derio, Spain; 4Instituto Oftalmologico Fernandez Vega and University of Oviedo, Oviedo, Spain.

Purpose: Keratoconus (KC) is a corneal disease associated with a cone-shaped protrusion and progressive corneal thinning. Correct the optical error induced by an irregular corneal surface is of paramount importance with respect to quality of life and ability to work. Our purpose was to determine if the Impact of SF36-Item Health Survey is a valid questionnaire to measure the vision-related quality of life in bilateral keratoconus patients and to relate it to the severity of the disease.

Methods: 115 keratoconus patients and 32 healthy subjects (control group) were included in the study. The stage of keratoconus was graded as mild when the steepest keratometric reading (K2) was < 45 diopters (D), moderate if K2 was between 45 and 52 D and
severe with K2 >52 D. We considered K2 as the quantitative clinical variable to assess the severity of keratoconus. The parameters studied included patients demographics, clinical features, topography and visual acuity and SF-36 questionnaire.

**Results:** Both groups were demographically similar and comparable. Mean age was 33.14 ± 7.54 years (40% women and 60% men) in KC group and 34.19 ± 7.98 years (42% women and 58% men) in control group. The general health status of patients with keratoconus was good (76.71 ± 5.13). However, SF-36 scores showed that patients with severe degree of keratoconus had lowest scores regarding the social and emotional status component (83.66 ± 6.80) as well as the job component (80.04 ± 9.5) (p=0.002).

**Conclusions:** The SF-36 questionnaire seems to be a useful tool for measuring health-related quality of life in KC patients given its ability to measure health in physical, psychological and social health scales.

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**Program Number:** 4210 **Poster Board Number:** A0238 **Presentation Time:** 8:30 AM–10:15 AM

Native enzymatic collagen cross-linking in human cornea as determined by simultaneous fluorescence and mass spectrometry (LC/MS)

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**Purpose:** Even while corneal tissue cross-linking using riboflavin photochemistry (CXL) is gaining widespread clinical use, we still know very little regarding the native enzymatic cross-links that "pre-exist" in the human cornea. Thus, the present study was undertaken in order to examine levels of enzymatic collagen cross-links in human corneal tissue by traditional (HPLC/DAD/FLD) and modern methods (LC/MS).

**Methods:** Human corneas (n=11) were obtained from the NY EyeBank from donor ages 18-58. The samples were pulverized, defatted (folch), reduced (NaBH4), hydrolyzed (6N HCl at 110°C 18hrs), and cellulose enriched prior to analysis by C8 LC/MS (Agilent 1100 system) equipped with DAD, FLD, and MSD in SIM mode (20mM heptafluorobutyric acid/Methanol 70:30 isocratic at 1mL/min). A post-column flow splitter allowed for simultaneous fluorescence and mass detection. Acetylated pyridinoline (AcPYD) was used as an internal standard. Nine different cross-links were measured and included enzymatic collagen di-[DHLNL (m/z=308.2), HLNL (m/z=292.2), LNL (m/z=276.1)] and tri-functional [HHL (m/z=445.2), PYD (m/z=429.2), DPYD (m/z=413)] cross-links, elastin [desmosines (m/z=526.3)] cross-links, and a non-enzymatic age-related glycation [pentosidine (m/z =379.2)] cross-link. Cross-link density (mol/mol) was expressed relative to collagen (MW=300kD) content (determined colorimetrically).

**Results:** Average cross-link levels (mol/mol collagen) were as follows: DHLNL=0.048±0.01, HLNL=0.043±0.002, LNL=0.19±0.02, and HHL=0.042±0.01. When stratified by age older (n=6) or younger (n=5) than 45y/o, the levels were DHLNL =young=0.033±0.01 vs. old=0.062±0.008 (p=0.045); HLNL =young=0.041±0.003 vs. old=0.045±0.001, (p=0.24); LNL =young=0.185±0.025 vs. old=0.194±0.024, (p=0.80); and HHL =young=0.054±0.016 vs. old=0.032±0.009 (p=0.264). PYD, DPYD, desmosines, and pentosidine were not detected. Collagen content (based on a 14% hydroxyproline calculation) was 56±2% in young vs. 50±2% in controls (p=0.35).

**Conclusions:** The human cornea contains both di- and tri-functional histidine (HHL) based collagen cross-links, with LNL being the major difunctional. In addition, no tri-functional PYDs and elastin were detected by cross-link analysis. These studies are relevant to our understanding of the human cornea in aging, disease, and therapy.

**Average cross-link levels (mol/mol collagen) in human corneas (n=11).**

**Commercial Relationships:** David C. Paik, None; Anna Takaoka, None; Stephen L. Trokel, None

**Support:** NCRR UL1RR024156, NEI P30EY019007, NEI R01EY020495 (dp), Research to Prevent Blindness

**Program Number:** 4211 **Poster Board Number:** A0239 **Presentation Time:** 8:30 AM–10:15 AM

Ultrasound-assist penetration of riboflavin with soluble collagen as regulating reagent during UVA crosslinking

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**Purpose:** The aim of this study is to protect collagen microstructure damage and to enhance riboflavin penetration by inducing the combination of collagen solution and ultrasonic treatment during crosslinking.

**Methods:** Fresh rabbit corneas were treated with riboflavin isotonic solution with or without soluble collagen. The tissues were treated by ultrasound for 0–10 min before UVA crosslinking was performed. During UVA crosslinking, hydration was maintained by adding crosslinking solution periodically. Samples were analyzed by confocal microscope, differential scanning calorimetry and transmission electron microscopy.

**Results:** Ultrasound treatment was able to promote riboflavin and collagen solution absorption compared to the non-ultrasound treated group. UVA crosslinking increased transition temperature from 72.5±1.52 to 73.4±1.77 after 30 min of UVA irradiation. TEM images showed significant collagen layer condensing and fibril thickening, as well as the formation of inter-fibrillar residues and the damage of collagen banding pattern after crosslinking. The inducing of soluble collagen was able to partially protect the condensing of collagen fibril layers and fibril thickening.

**Conclusions:** This study shows ultrasound treatment promotes riboflavin, as well as the collagen regulating agent penetration.
Collagen fibrils and banding patterns are damaged by UVA irradiation, and soluble collagen regulating agent partially protects the collagen fibril damages and prevent the trend in cornea tissue thinning during crosslinking. This study provides the potential clinical relevance of damage and prevention strategies of collagen crosslinking therapy for keratoconus.

**Commercial Relationships:** Xiaokun Wang, None; Shoumyo Majumdar, None; Jennifer Elisceff, None

**Support:** APL/DOD Eye Patch grant, Research to Prevent Blindness

**Program Number:** 4212 Poster Board Number: A0240
**Presentation Time:** 8:30 AM–10:15 AM

**Iontophoresis Corneal Collagen Cross-Linking for Progressive Keratoconus**

**Riccardo Vinciguerra**, 1, 2, Emanuela F. Legrottaglie, 1 Pietro Rosetta, 1 Claudia Azzolini, 1 Paolo Vinciguerra, 1 Ophthalmology, Humanitas Clinical and Research Center, Milan, Italy; 2Department of Surgical and Morphological Sciences, Circolo Hospital, University of Insubria, Varese, Italy.

**Purpose:** The aim of this study is to report the preliminary clinical results of transepithelial corneal collagen cross-linking (CXL) with Iontophoresis.

**Methods:** Ten eyes of ten patients with progressive keratoconus were included in this prospective clinical study. Best corrected visual acuity (BCVA), sphere and cylinder refraction, corneal topography, Scheimpflug tomography, aberometry, anterior segment optical coherence tomography (AS-OCT) and endothelial cell count were assessed at baseline and at 1, 3 and 6 months of follow up. The parameters considered to establish keratoconus progression always were proved with differential maps as change in curvature in the cone area of at least 1 diopters obtained with an instantaneous map.

**Results:** Functional parameters showed a significant improvement (p<0.05) of BCVA after 6 months of follow up together with a significant decrease of sphere. Conversely morphological parameters showed a non significant reduction of topographic parameters and a significant increase in minimum pachymetry after 6 months of follow up. None of the patients had continuous progression of keratoconus or had to repeat crosslinking procedures. Endothelial cell counts did not changed significantly (p>0.05).

**Conclusions:** Despite the low number of patients and a short term follow up our preliminary results on transepithelial corneal collagen cross-linking with iontophoresis indicate a stabilization and a significant improvement of functional parameters till 6 months of follow up after the treatment.

**Commercial Relationships:** Riccardo Vinciguerra, None; Emanuela F. Legrottaglie, None; Pietro Rosetta, None; Claudia Azzolini, None; Paolo Vinciguerra, NIDEK (C), OCULUS (C), SOOFT (C)

**Program Number:** 4213 Poster Board Number: A0241
**Presentation Time:** 8:30 AM–10:15 AM

**Mechanical and thermal characterization of corneas submitted to crosslinking by Açai fruit (Euterpe oleracea) extract**

**Paulo Schor**, 1 Regina F. Nogueira, 1 Patrícia A. Bersanetti, 1, 2 Ophthalmology, Humanitas Clinical and Research Center, Milan, Italy; 2Department of Health Informatics, UNIFESP, Sao Paulo, Brazil.

**Purpose:** To characterize rabbit corneas subjected to crosslinking by Açai extract (Euterpe oleracea) in comparision to riboflavin/UVA treatment by analyzing its Young modulus of elasticity (YM) and thermal denaturation (DSC) profile.

**Methods:** Ten rabbit eyes obtained from a local slaughterhouse (Granja dos Ipês, Salto de Pirapora, Brazil) were divided in three groups: 1. eyes treated with 0.1% comercial riboflavin (Ophthalmos, Sao Paulo, Brazil) and ultraviolet-A irradiation (365 nm) for 30 min, using a solid-state commercial device (X-Link; Opto Eletronica, Sao Carlos, Brazil); 2. corneas treated with 4% Açai extract by 2 hours; 3. control corneas (without treatment). After treatment corneas were deepethelialized and maintained in Optisol GS (Bausch&Lomb, New York, USA) or balanced saline solution (BSS) until characterization. YM was determined by the three point bend method by compressing corneal strips (2.0 mm x 7.0 mm) in an Universal Tester Shimadzu EZTest-SX (Kyoto, Japan) with a 1 N load cell at crosshead speed of 0.5 mm.min^-1. Denaturation temperature was measured by differential scanning calorimetry (DSC). The DSC curves were obtained from 25 to 100°C using heating rate of 10°C.min^-1 in a calorimeter (Shimadzu DSC-60, Kyoto, Japan).

**Results:** YM value at 3% strain of group 1 was 0.118±0.001 MPa and 0.35±0.03 in group 2 (P<0.01). Control corneas (group 3) showed a YM of 0.038±0.008 MPa (p>0.01). DSC curves of control and treated corneas showed endothermic events that correspond to denaturation process of collagen. The results showed that the temperature of denaturation increased in the groups treated with agents that promote crosslinking (group 1= 64.5±0.9°C and group 2= 63.4±0.7°C) compared to control samples (60.8±2.2°C) (p<0.05).

**Conclusions:** The Açai extract was more effective in promoting the increase in mechanical resistance in rabbit corneas than the traditional treatment. This behaviour was confirmed by mechanical characterization that demonstrated a higher increase in YM in corneas treated with Açai extract than in riboflavin/UVA group. The thermal characterization by DSC analysis showed that crosslink leads to an increase in denaturation temperature of collagen that was similar for the two crosslink methodologies. The increase in denaturation temperature can be attributed to the denaturation of collagen fibrils that occurs during the crosslinking process.

**Commercial Relationships:** Paulo Schor, Patent Requested - INPI (P); Regina F. Nogueira, None; Patricia A. Bersanetti, None

**Support:** Fapesp Grant 2012/07343-8

**Clinical Trial:** 0580/10

**Program Number:** 4214 Poster Board Number: A0242
**Presentation Time:** 8:30 AM–10:15 AM

**Cosmetic preservatives as therapeutic corneoscleral tissue cross-linking (XL) agents**

**Natasha Babur**, 1 Jessica R. Cohen, 2 Michael J. Fernandez, 2 Anna Takaoka, 1 Xia Li, 1 Mi Jung Kim, 1 Kristin M. Myers, 2 Stephen L. Trokel, 1 David C. Paik. 2 Ophthalmology, Columbia University College of Physicians and Surgeons, New York, NY; 2Mechanical Engineering, Columbia University, New York, NY.

**Purpose:** The growing clinical success of riboflavin photochemical corneal cross-linking (CXL) suggests that inducing mechanical tissue strength in vivo can be beneficial. Alternatively, tissue cross-linking (XL) can be induced by aliphatic β-nitroalcohols, which deliver formaldehyde via a base-catalyzed reverse Henry reaction. There are a number of related agents, known as “formaldehyde releasers” (FARs), that are in commercial use as preservatives in cosmetics and other personal care products. Thus, the present study was undertaken in order to screen such compounds for potential clinical utility.

**Methods:** A chemical registry was created from a literature review and included characteristics relevant to XL such as molecular weight, carcinogenicity/mutagenicity, toxicity, hydrophobicity, and commercial availability. From this registry, compounds were then selected for efficacy screening using an ex vivo rabbit corneal cross-linking simulation set up. XL solution was administered via a corneal reservoir for 30 min in 0.1M NaHCO3 at either pH 7.4 or 8.5. The epithelium was left intact and 0.5% proparacaine was applied prior to XL. The control contralateral eye was treated identically with vehicle.

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Effectiveness of XL was based on shifts in thermal denaturation temperature ($T_m$) as measured by differential scanning calorimetry (Perkin-Elmer DSC 6000). Favorable DSC results were validated using biomechanical inflation tests with digital image correlation (DIC) as previously described by Myers et al.

**Results:** Sixty-four FARs (regularly found in cosmetics) were identified from the literature. Three compounds have been tested thus far [diazolidinyl urea (DAU), imidazolidinyl urea, and 5-Ethyl-3,7-dioxa-1-azabicyclo [3.3.0] octane]. Of the three, DAU has showed effective XL at pH 8.5. Compared to the controls, the $T_m$ using DAU was shifted 1.92°C ± 0.14°C ($n=2$). Mechanical inflation testing confirmed increased tissue stiffness in pressure ranges mimicking physiological pressures (1.875-45mmHg). Tissue creep was also diminished under the current loading protocol (Fig. 1).

**Conclusions:** DAU is a potentially useful corneal XL agent as indicated by thermal denaturation and biomechanical inflation testing. Continued screening of compounds from the compiled chemical registry should lead to identification of additional potent agents.

Net apical displacement response over time for control and DAU cross-linked cornea at pH 8.5.

**Commercial Relationships:** Natasha Babar, None; Jessica R. Cohen, None; Michael J. Fernandez, None; Anna Takaoka, None; Xia Li, None; Mi Jung Kim, None; Kristin M. Myers, None; Stephen L. Trokel, US,8,466,203 (P); David C. Paik, US,8,466,203 (P)

**Support:** NCRR UL1RR024156, NEI P30 EY019007, and NEI R01EY020495 (dcp), Research To Prevent Blindness

**Program Number:** 4215 **Poster Board Number:** A0243

**Presentation Time:** 8:30 AM–10:15 AM

Two-photon fluorescence (TPF) microscopy of corneal riboflavin absorption

**Purpose:** To correct for attenuation in TPF measurements of riboflavin absorption in porcine corneas

**Methods:** A 300μl reservoir of 0.1% riboflavin was placed (air-bubble free) on top of epithelial-debrided porcine corneas (globe intact). TPF imaging was performed through this reservoir with 10μm z-stacks across the cornea repeated every 2.5 minutes (maximum 60 minutes). Riboflavin was excited by two-photon light of 890nm wavelength, with fluorescence signal detected between 525-650nm.

TPF signal attenuation was corrected with reference to a uniformly soaked plot from each eye towards the end of the 60 minute soak. This protocol was repeated in 4 eyes. In selected eyes, z-stacks were additionally performed through uniformly-soaked excised corneal buttons.

**Results:** Significant TPF signal attenuation was observed in all eyes, with the signal decreasing linearly with depth in uniformly soaked tissue. This signal loss was observed for intact globes and when imaging through excised corneal buttons (epithelial-debrided surface up or down). Cross-sectional TPF images taken of additional excised corneal strips confirmed the tissue was uniformly soaked so that the decrease in signal was not due to variations in riboflavin concentration.

After correcting for this signal attenuation, we observed TPF signals increased with longer riboflavin soak duration, with the maximum tissue concentration recorded at 0.06%. Uniform riboflavin absorption was achieved after a minimum 50 minutes. Following a standard corneal cross-linking soak of 30 minutes, a mean stromal concentration of 0.053% (0.002, standard deviation) was achieved at a depth of 300μm. Half this concentration (0.026%) was observed within the first 5 minutes.

**Conclusions:** TPF microscopy has previously been applied to study corneal riboflavin absorption without accounting for depth-dependent signal attenuation (Cui et al. IOVS 2011;52:2556–64). Our data suggest this signal loss needs to be taken into account and we report a method of correcting the TPF measurements to more accurately quantify the concentration of riboflavin throughout the cornea. The minimum concentration for effective cross-linking is unknown: Our data suggest this may be effected with a tissue concentration of approximately 0.05% within the 250-350μm demarcation zone commonly seen after treatment.

SD error bars
Post-operative pains were less severe in the I-CXL patients group. At 1 month and 3 months, keratometry were stable in both groups. The OCT showed inconstantly a less pronounced and a less deep demarcation line in the I-CXL treated patients group. No complications were observed in the two groups.

Conclusions: These preliminary results suggest a less riboflavin penetration in the stroma but a similar effect on keratoconus stabilization 3 months after after I-CXL compared to C-CXL. I-CXL seems to be a promising alternative methodology for riboflavin delivery in crosslinking treatment, preserving the epithelium. However, a longer follow up on a larger number of patients is necessary. We will be able to present our following results at the ARVO annual meeting.

Commercial Relationships: myriam Cassagne, None; Ivan Delayo, None; Nicolas Mesplié, None; Pierre Fournié, None; Béatrice Cochener, None; Francois Malecaze, None

Clinical Trial: 13 030 03

Program Number: 4216 Poster Board Number: A0244
Presentation Time: 8:30 AM–10:15 AM
Transepithelial corneal collagen crosslinking using iontophoresis: preliminary clinical results

myriam Cassagne1, Ivan Delafoy2, Nicolas Mesplié1, Pierre Fournié1, Béatrice Cochener1, Francois Malecaze1, 1:ophthalmology, Purpan Hospital, TOULOUSE, France; 2:Ophthalmology, Morvan Hospital, BREST, France; 3:Hôpital Saint Jean de Luz, France.

Purpose: The conventional corneal collagen crosslinking (CXL), according to the Dresden protocol, have proved is efficiency on the stabilization of progressive keratoconus. However, this procedure presents various complications, due to the epithelial removal that is essential for riboflavin penetration into the corneal stroma. In this context, we wanted to evaluate a new riboflavin penetration procedure, the iontophoresis, in order to perform a CXL preserving the epithelium.

Methods: Our randomized prospective multicentric clinical trial aims at compare a CXL using a riboflavin application by iontophoresis (I-CXL) to a CXL using a conventional de-epithelialized riboflavin application (C-CXL).

Fifty patients, aged over 18 years, with a progressive keratoconus have been included between April and November 2013, in accordance with the Declaration of Helsinki. The I-CXL involved the administration of a new formulation of charged riboflavin (Ricrolin+)® into cornea by applying a constant current of 1mA for 5 min. After this application, the cornea was irradiated with UVA light at 10mW/cm2 for 9 minutes. The C-CXL was performed in accordance with the Dresden protocol.

The investigation included: per-operative ocular fluorophotometry measurement, pain evaluation, visual acuity, topographic keratometry, counting endothelial cells, Ocular Coherence Tomography (OCT) of cornea, corneal confocal microscopy and ocular complications.

Results: The ocular fluorophotometry showed a riboflavin corneal concentration 2.5 times lower after I-CXL (752.8 ± 274.5 ng/ml) than after C-CXL (1902 ± 539.4 ng/ml) (p<0.05).

No complications were observed in the two groups. At 1 month and 3 months, keratometry were stable in both groups. The OCT showed inconstantly a less pronounced and a less deep demarcation line in the I-CXL treated patients group. No complications were observed in the two groups.

Conclusions: These preliminary results suggest a less riboflavin penetration in the stroma but a similar effect on keratoconus stabilization 3 months after after I-CXL compared to C-CXL. I-CXL seems to be a promising alternative methodology for riboflavin delivery in crosslinking treatment, preserving the epithelium. However, a longer follow up on a larger number of patients is necessary. We will be able to present our following results at the ARVO annual meeting.

Commercial Relationships: myriam Cassagne, None; Ivan Delayo, None; Nicolas Mesplié, None; Pierre Fournié, None; Béatrice Cochener, None; Francois Malecaze, None

Clinical Trial: 13 030 03

Program Number: 4217 Poster Board Number: A0245
Presentation Time: 8:30 AM–10:15 AM
Evaluation of a new Riboflavin Formulation (MDV1224) for Transepithelial Corneal Collagen Cross-linking

Danilo Aleo1, Myriam Cassagne2,3, Anne Galinier4, Barbara Melilli5, Sergio Mangiafico6, Carole Gard7, Francois Malecaze1,8, 1:R&D, Medivis, Catania, Italy; 2:Department of Ophthalmology, CHU Purpan Toulouse, Toulouse, France; 3:EA 4555, Paul Sabatier Université de Toulouse III, Toulouse, France; 4:Department of Biochemistry, CHU Rangueil, Toulouse, France; 8:R&D, Horus Pharma, Saint Laurent du Var, France.

Purpose: The conventional corneal collagen crosslinking (CXL), according to the Dresden protocol, have proved is efficiency for the stabilization of progressive keratoconus. This procedure presents various complications due to the epithelial removal that is indispensable for the riboflavin penetration into the corneal stroma. We wanted to evaluate a new riboflavin formulation allowing to perform a CXL preserving the epithelium. Room temperature stability as well as in vitro corneal penetration of this new riboflavin–base formulation (MDV1224) has been reported (ARVO 2013, e-abstract C0178). MDV1224 has been obtained from lipophilic riboflavin-base and sodium tetraborate which results in an aqueous solution of various complexes between the borate ion and the ribitol moiety of riboflavin-base. The objective of our study was to evaluate the toxicity and the in vitro stromal penetration of MDV1224 in comparison to a commercial riboflavin phosphate formulation (Ricrolin) used in accordance with the Dresden protocol.

Methods: The assessment of the potential irritant and cytotoxic effects of MDV1224 has been carried out through the model of the reconstituted human corneal epithelium (HCE) provided by SkinEthic Laboratories (Nice, France). MDV1224, its positive control (1% Sodium Dodecyl Sulphate) and its negative control (saline) were placed on the surface of the corneal tissue and left in contact for 10min (irritation test) or 24hr (cytotoxic assay). The results are expressed as percentage of viability (MTT assay) compared to negative control. The transepithelial penetration of MDV1224 after instillation for 30 min on epithelialized rabbit corneas was evaluated in comparison to Ricrolin after instillation on de-epithelialized corneas. Riboflavin diffusion in the cornea was analyzed by measuring riboflavin concentration in corneas using HPLC method.

Results: MDV1224 was not irritant (viability 95%–100%) or cytotoxic (viability 92%–98%). Stromal riboflavin penetration of MDV1224 was 215±1247 ng/g and 1608±235 ng/g for Ricrolin.

Conclusions: These preliminary experimental studies suggest that MDV1224 is a promising formulation for riboflavin delivery in
CXL treatment, preserving the epithelium. These studies have to be completed by a largest sample and the evaluation of the corneal structural modifications and biomechanics after UVA application.

**Commercial Relationships:** Danilo Aleo, Medivis (E); Myriam Cassagne, None; Anne Galinier, None; Barbara Melilli, Medivis (E); Sergio Mangialfico, Medivis (E); Carole Gard, Horus Pharma (E); Francois Malecave, None

**Program Number:** 4218 Poster Board Number: A0246
**Presentation Time:** 8:30 AM–10:15 AM

Label free LC-MS/MS quantitative analysis of aqueous humor proteome from keratoconus and myopic controls patients Arantxa Ageretxe1, Javier Sorria1, Alberto Villarrubia2, Felix Elortza3, Mikel Azkargorta3, Juan Alvarez de Toledo4, Ignacio Rodriguez-Ageretxe5, Jesus Merayo-Lloves6, Tatiana M. Suarez-Cortes1.

1Research, Bioftalmik, Derio, Spain; 2Instituto La Arruzafa, Cordoba, Spain; 3Proteomics Platform, CIC bioGUNE, Derio, Spain; 4Instituto Barraquer, Barcelona, Spain; 5Instituto Clinico Quirurgico de Oftalmologia, Bilbao, Spain; 6Instituto Oftalmologico Fernandez Vega and University of Oviedo, Oviedo, Spain.

**Purpose:** The etiology and the factors governing keratoconus (KC) progression remain to be elucidated. It has been reported to arise as a consequence of biochemical alterations in the cornea. It is known that the expression profile for some proteins in the aqueous humor (hAH) changes in some diseases. Our purpose was to identify the possible implication of hAH in the development of KC disease based on hAH protein expression differences among patients with KC and control subjects by a high-throughput mass spectrometry approach.

**Methods:** Aqueous humor samples were acquired from 5 patients with keratoconus and 5 myopic control subjects. Spectral counting mass spectrometry analysis was performed to determine the relative amounts of hAH proteins in keratoconus and controls patients.

**Results:** All patients included in the study presented severe keratoconus (K2>52D), and slit lamp examination revealed microfolds in Descemets membrane without clinical signs of hydrops. As a result of mass spectrometry analysis a total of 242 distinct proteins were significantly identified. Eleven proteins exhibited inter-group significant protein expression differences, seven of which were overexpressed whereas four exhibited decreased expression levels. Gene ontology analyses revealed that deregulated proteins are implicated in biological processes such as regulation of proteolysis, response to hypoxia, response to hydrogen peroxide and regulation of collagen biosynthesis, among others.

**Conclusions:** The protein expression profile in hAH from KC patients varies from that of myopic control subjects evidencing the implication, direct or indirect, of hAH in keratoconus disease. In consequence, this study demonstrates that in-deep analysis of hAH proteome will lead to a greater understanding of the pathophysiology of keratoconus disease.

**Commercial Relationships:** Arantxa Ageretxe, None; Javier Sorria, None; Alberto Villarrubia, None; Felix Elortza, None; Mikel Azkargorta, None; Juan Alvarez de Toledo, None; Ignacio Rodriguez-Ageretxe, None; Jesus Merayo-Lloves, None; Tatiana M. Suarez-Cortes, None

**Support:** CEN-20091021

**Program Number:** 4219 Poster Board Number: A0247
**Presentation Time:** 8:30 AM–10:15 AM

Accelerate transepithelial corneal collagen cross-linking for progressive keratoconus

Mauricio Rodriguez Diaz, Maria A. Henriquez, Luis Izquierdo. Ophthalmoogy, Instituto Oftalmosalud, Lima, Peru.

**Purpose:** To evaluate the safety, efficacy, and stability of accelerate transepithelial corneal collagen crosslinking in progressive keratoconus.

**Methods:** This prospective study included 27 eyes of 27 patients with progressive keratoconus diagnosis whom underwent corneal collagen crosslinking between January 2013 and September 2013. The solution used was TE-riboflavin (Peschke Ltd, Germany) and the Ultraviolet-A treatment was performed with CCL-VARIO (Peschke Ltd, Germany) with 5 minutes of irradiation (18mW) and 30 minutes of impregnation. Uncorrected visual acuity (UCVA), best corrected visual acuity (BCVA), manifest refraction, demarcation line using anterior segment OCT (optical coherence tomography), and Scheimpflug imaging parameters where evaluated at pre and postoperatively day one, and 1, 3, 6 and 9 months.

**Results:** The mean crosslinking deep was 179.16 um at 1.5 mm from the center of the cornea, compared with 255.45 um at the center of the cornea. The mean diameter of the crosslinking was 3.63 mm visualized by AS-OCT. There was a not statistically significant difference between pre and postoperative pachymetry at the center and the thinnest point of the cornea (p > 0.05 in all visits). Mean maximum posterior elevation decrease from 36 um to 33.11 um (p = 0.09). Mean maximum keratometry at preoperative and postoperative follow up (3 month) was 50.21 and 50.75 diopeters (D) respectively (p = 0.45). There was not complications during the follow up.

**Conclusions:** Accelerate transepithelial crosslinking is safe and efficacy to stop the progression of the keratoconos. AS-OCT images showed that the intended depth of crosslinking is achieved within the central area of the cornea.

**Commercial Relationships:** Mauricio Rodriguez Diaz, None; Maria A. Henriquez, None; Luis Izquierdo, None

**Program Number:** 4220 Poster Board Number: A0248
**Presentation Time:** 8:30 AM–10:15 AM

Evaluation of Transepithelial Corneal Collagen Cross-Linking (CXL) at 6 months and 1 year Follow Up in Patients with Different Proprietary Transepithelial Riboflavin Solutions


1South Florida Eye Associates, Boca Raton, FL; 2Center for Excellence in Eye Care, Miami, FL; 3Washington Eye Physicians and Surgeons, Chevy Chase, MD.

**Purpose:** Transepithelial corneal cross linking has been reported to be ineffective. The purpose of this analysis was to demonstrate efficacy and compare operative procedure time relative to standard crosslinking in a clinical setting.

**Methods:** Patients with a diagnosis of keratoconus or post-Lasik ectasia who had undergone Transepithelial CXL in one or both eyes were included in this analysis. Patients with previous RK, INTACS, repeat CXL procedures, and/or patients who were pseudo-phakic or had a diagnosis of cataract were excluded from this analysis. Outcome measures included intraoperative transepithelial corneal stromal riboflavin loading times, and UCVA, BSCVA, and K Max at 6 months and 1 year follow up visits. Corneal stromal loading was assessed by slit lamp examination.

**Results:** 199 eyes were treated with proprietary riboflavin formulation 1. Loading time ranged from 30 to 100 minutes (average 56.3 minutes). 70% of these eyes were male and the average age was 35.7 years old. 84% of eyes had a pre-Op diagnosis of keratoconus. 127 eyes were treated with proprietary riboflavin formulation 2. Loading time ranged from 8 to 60 minutes (average 22.17 minutes). 70% of these eyes were male and the average age was 36 years old. 88% of eyes had a pre-Op diagnosis of keratoconus.
At 6 months post-CXL, 52% and 52% of eyes (formulation 1) and 45% and 47% of eyes (formulation 2) improved 1 or more lines in UCVA and BSCVA from pre-Op. Patients treated with formulation 1 and formulation 2 experienced 0.24D flattening and 1.36D in K Max, respectively, at 6 months follow-up. At 1 year follow up, 59% and 55% of eyes (formulation 1) and 63% and 50% of eyes (formulation 2) experienced 1 or more lines of improvement in UCVA and BSCVA. Patients treated with formulation 1 and formulation 2 experienced 0.52D flattening and 2.00D flattening, respectively, in K max from Pre-Op.

**Conclusions:** The efficacy of trans-epithelium corneal cross linking depends on the formulation and the application of the solution. The presented results demonstrate that trans-epithelium cross linking with an appropriate formulation can provide an efficacy that is comparable to the epi-off Dresden protocol without substantial increase in the loading time.

**Commercial Relationships:** Jennifer Loh, None; William Trattler, CXLO (C); Roy Rubinfeld, CXLO (C); Gabriela Perez, None; Eduardo J. Polания, None; Rosane Correa, None

Clinical Trial: NCT1726283

Program Number: 4221 Poster Board Number: A0249
Presentation Time: 8:30 AM–10:15 AM

**An evaluation of enzymatic collagen cross-linking in keratoconus using LC/MS**

Anna Taoka1, Julia Hogan1, Natasha Babar1, Mi Jung Kim1, Marianne O. Price1, Francis W. Price1, Stephen L. Trokel1, David C. Paik1. 1Ophthalmology, Columbia University, College of Physicians and Surgeons, New York, NY; 2Cornea Research Foundation of America, Indianapolis, IN, 3Price Vision Group, Indianapolis, IN.

**Purpose:** Riboflavin photochemical corneal cross-linking is gaining widespread clinical acceptance. Recent genetic and immunohistochemical studies suggest that an enzymatic collagen cross-linking defect may underlie keratoconus (KC) pathogenesis. Current literature contains a paucity of information regarding enzymatic collagen cross-linking in the KC cornea. Thus, the present study was undertaken in order to examine levels of enzymatic cross-links in KC tissue by LC/MS, correlating levels with differences in fibril stability as determined by thermal denaturation temperature or melting temperature (Tm).

**Methods:** Surgical KC samples (n=9) and eyebank control (n=4) corneas of age<45y/o were analyzed. The samples were defatted (folch), reduced (NaBH4), hydrolyzed (6N HCl at 110°C 18hrs), and cellulose enriched prior to analysis by C8 HPLC (Agilent1100) equipped with FLD/MSD in SIM mode (20mM heptafluorobutyric acid/MeOH 70:30 isocratic at 1ml/min). Nine different cross-links were measured and included enzymatic collagen di- [DHLNL (m/z=308.2), HLNL (m/z=292.2), LNL (m/z=276.1)] and tri-functional [HHL (m/z=445.2), PYD (m/z=429.2), DPYD (m/z=413)] cross-links, elastin [desmosines (m/z=526.3)] cross-links, and a non-enzymatic age-related glycation [pentosidine (m/z=379.2)] cross-link. Cross-link density (mol/mol) was expressed relative to collagen content (determined colorimetrically). Tm was determined by differential scanning calorimetry (Perkin Elmer DSC-6000).

**Results:** KC corneas underwent thermal denaturation at Tm=58.9±0.23°C vs. controls=61.4±0.28°C (p<0.001), indicating greater fibril instability. Collagen cross-link levels (mol/mol collagen) were as follows: DHLNL [KC=0.066±0.01 vs. controls=0.033±0.01, (p=0.092)]; HLNL [KC=0.043±0.01 vs. controls=0.044±0.004, (p=0.736)]; LNL [KC: 0.12 ± 0.02 vs. controls: 0.19 ± 0.03, (p=0.121)]; and HHL [KC=0.052±0.014 vs. controls=0.062±0.019 (p=0.693)]. PYD, DPYD, desmosines, and pentosidine were not detected. Collagen content (based on a 14% hydroxyproline calculation) was 48±2% in KC vs. 56±5% in controls (p=0.213).

**Conclusions:** Alterations in difunctional enzymatic collagen cross-links may contribute to mechanical instability in KC. These studies have relevance to both KC pathogenesis and therapy.

Average cross-link levels (mol/mol collagen) detected in control and KC corneas.

![Average cross-link levels (mol/mol collagen) detected in control and KC corneas.](image)

Program Number: 4222 Poster Board Number: A0250
Presentation Time: 8:30 AM–10:15 AM

**Demarcation line after cross-linking using two different treatment protocols**

Sara Brtingham, Christoph Tappeiner, Beatrice E. Frueh. Department of Ophthalmology, Inselspital, University Hospital of Bern, Bern, Switzerland.

**Purpose:** To compare the occurrence of a demarcation line after corneal cross-linking (CXL) for keratoconus using two different protocols (standard versus rapid protocol with higher intensity and shorter irradiation time).

**Methods:** A retrospective analysis on an interventional case series of 119 progressive keratoconus eyes treated with CXL using the standard Dresden protocol (30min irradiation, 3mW/cm², UV-XTM 1000) or a rapid protocol (10min irradiation, 9 mW/cm², UV-XTM 2000) was performed. The presence of a corneal demarcation line was assessed with anterior segment OCT (Heidelberg Spectralis) one month after treatment by a masked observer.

**Results:** In the standard protocol group, 65% (58/89) of treated corneas revealed a demarcation line one month after cross-linking, whereas such a demarcation line was observed in only 20% (6/30) of eyes treated with rapid protocol.

**Conclusions:** Increasing UV intensity and reducing irradiation time for CXL has a negative effect on the occurrence of the demarcation line one month after the procedure. Further studies are needed to evaluate the effect on keratoconus progression.

**Commercial Relationships:** Sara Brtingham, None; Christoph Tappeiner, None; Beatrice E. Frueh, None
Program Number: 4223 Poster Board Number: A0251
Presentation Time: 8:30 AM–10:15 AM
Corneal Thickness Changes and Results of Collagen Crosslinking using Riboflavin/Dextran or Hypotonic Riboflavin
Elan A. Rosenblat, Steven A. Greenstein, Peter S. Hersh. Department of Ophthalmology, Inst of Ophthalmology and Visual Science Rutgers University, Newark, NY.
Purpose: This study was designed to compare the use of two riboflavin formulations, riboflavin 0.1% in 20% dextran solution and its hypotonic formulation without dextran with regard to intraoperative corneal thinning and clinical results.
Methods: 63 eyes with keratoconus and corneal ectasia were analyzed in a prospective randomized control clinical trial. Both arms of the study received pretreatment with riboflavin 0.1% in 20% dextran solution; one drop was administered every 2 minutes for 30 minutes after the corneal epithelium was removed. If the corneal thickness was less than 400μm hypotonic riboflavin was administered until the cornea swelled beyond 400μm. Both study groups then received 30 minutes exposure to UV light (365 micron wavelength, irradiance 3mW/cm2). During UV exposure, eyes received continued Riboflavin administration. The formulation of which was assigned by 2 randomized study arms: 35 patients received riboflavin/dextran and 28 patients received hypotonic riboflavin every 2 minutes for the duration of UV exposure. Corneal thickness measurements were then analyzed between both arms of the study. Pachymetry was measured by ultrasound before the corneal epithelium was removed, after initial riboflavin loading prior to, and after UV light exposure. At least 5 pachymetry measurements were taken at each time point and the lowest used for analysis. Patients were then followed at 1, 3, 6 and 12 months postoperatively. If any patient missed an appointment they were removed from the study. Thirty nine patients with either keratoconus (29) or post lasik ectasia (10) who underwent corneal cross-linking remained in the study. 21 patients (15 keratoconus, 6 ectasia) received standard riboflavin dextran solution during UVA light administration and 18 patients (14 keratoconus, 4 ectasia) received a hypotonic riboflavin solution during UVA light administration.
Results: Please see attached Figures.
Conclusions: The cornea thins substantially during the collagen crosslinking procedure. The use of hypotonic riboflavin rather than riboflavin/dextran during UV administration decreases the amount of corneal thinning during the procedure 24%, from thinning of 135 microns to 102 microns. These intraoperative changes were transient and there were no statistically significant difference in outcomes at 1, 3, 6, or 12 month followup.

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Commercial Relationships: Elan A. Rosenblat, None; Steven A. Greenstein, None; Peter S. Hersh, Avedro, Inc (C)
Support: Research to Prevent Blindness
Clinical Trial: NCT01152541

Program Number: 4224 Poster Board Number: A0252
Presentation Time: 8:30 AM–10:15 AM
Topographic, Aberrometric, and Refractive Outcomes 12 Months after Combined Transepithelial Phototherapeutic Keratectomy and Corneal Collagen Crosslinking
Ganjezer Develi Can, Nurullah Cagil, Ozge Sarac Ilhan, Eminike Kalkan Akay. Ophthalmology, Yildirim Beyazit University Ataturk Training and Research Hospital, Ankara, Turkey.
Purpose: To evaluate the visual, topographic, and aberrometric outcomes 12 months after combined transepithelial phototherapeutic keratectomy (t-PTK) and corneal collagen crosslinking (CXL) in eyes with progressive keratoconus.
Methods: This is a retrospective case series involving 18 eyes of 15 patients with progressive keratoconus treated with combined t-PTK and CXL. The main outcomes measured at baseline and 12 months after treatment were; uncorrected distance visual acuity (UDVA), corrected distance visual acuity (CDVA), spherical equivalent, cylindrical refraction, corneal topographic parameters including Simulated (Sim) K1, K2, average K, Kmax, steepest point of the anterior surface (AKf), corneal aberrations including secondary astigmatism, trefoil, coma, and spherical aberration.
Results: The mean age of the patients was 26.7±7.5 (13-43) years. The mean baseline UDVA was 0.7±0.4 logMar, while it was improved to 0.5±0.4 logMar after 12 months (p=0.007). The mean CDVA was improved from 0.2±0.2 to 0.1±0.1 logMar (p=0.05). The mean cylindrical refraction and spherical equivalent were decreased from -3.3±1.2 D, -2.6±1.5 D to 2.4±1.6 D, -2.0±1.6 D respectively (p=0.03, p=0.05 respectively). The mean Sim-K1, K2, average K, apical K, Kmax, and AKf were 46.52±2.25 D, 49.91±1.89 D, 48.14±1.89 D, 55.72±3.65 D, 55.80±3.90 D, and 54.87±2.99 D respectively. They were decreased to 45.64±2.04 D, 49.18±1.99 D, 47.33±1.82 D, 54.60±3.82 D, 53.89±3.39 D, and 53.74±2.27 D respectively (all p<0.05). The mean baseline coma was significantly decreased from -1.2±0.4 μm to -1.0±0.4 μm 12 months after treatment (p<0.004). The mean secondary astigmatism, trefoil, and spherical aberration did not show any significant difference after treatment (all p>0.05). No complications were seen intra and post operatively.
Conclusions: This study found that combined t-PTK and CXL is effective and safe in the treatment of progressive keratoconus through one year of follow up. Combined t-PTK and CXL not only improves the topographic and refractive parameters but also improves the optical quality of the eye in progressive keratoconus.
Commercial Relationships: Gamze Dereli Can, None; Nurullah Cagil, None; Ozge Sarac Ilhan, None; Emine Kalkan Akcay, None

Program Number: 4225 Poster Board Number: A0253
Presentation Time: 8:30 AM–10:15 AM
Software-based assessment of intrastromal rings surgical plan for keratoconus

Roberto Albertazzi1, Leonardo Ferlini1, Guillermo Rao1, Jesus Merayo-Lloves2,3, Jose Alfonso1,3. 1Cornea, Centro de Ojos Quilmes, Quilmes, Argentina; 2Fundación de Investigacion Oftalmologica, Oviedo, Spain; 3University of Oviedo, Oviedo, Spain.

Purpose: To develop and validate a novel automated software, Keratoconus Easy Fix®, to assess intrastromal rings (ICRS) surgical plans for keratoconus.

Methods: We developed a novel software that uses the data inserted by the surgeon and correlates this information with normograms, provided by manufacturer and modified by Dr. Albertazzi, giving as result a surgical plan indicating which ICRS should be used, ring position, and incision location.

A Single-center, retrospective, noncomparative series of 62 eyes (45 patients) was conducted using the surgical plans provided by the software. Patients name, clinical ID, keratoconus topography type, subjective refraction with sphere, astigmatism with axis or theoric refraction with Kf and Ks with respective axis must be inserted for software analysis and surgical plan result.

All patients had at least 6-month follow-up. Main outcome measures were evaluated as a reduction of the keratometric (K) values: K minimal (Kmin) and K maximal (Kmax)). Visual outcome were measured as improve in uncorrected distance visual acuity (UDVA), best corrected visual acuity (CDVA), and refractive error (myopia and astigmatism) reduction (decimal scale). Data were processed with R-programing language for descriptive and inference statistical analysis.

All patients were informed and consented use of the software.

Results: There were a significant reduction of keratometric values Kmax (Kmax-pre 49.72 ± 4.36 to Kmax-post 46.84 ± 3.59). Regarding VA there were a significative (p<0.01) reduction: Mean UDVA change from 0.31 ± 0.22 to 0.56 ± 0.31: Mean DCVA from 0.73 ± 0.32 to 0.81 ± 0.21. Myopic and astigmatism components were highly reduced after ICRS implantation (P < 0.01). Myopia was reduced from -2.64 ± 3.34 to -1.81 ± 2.78. Astigmatism was reduced from -2.99 ± 1.63 to -1.01 ± 1.36. No intraoperative and postoperative complications occurred in these eyes.

Conclusions: Keratoconus Easy Fix®, is an effective, user-friendly software that provides ICRS surgical plans with good postsurgical result. This program should be used to assess keratoconus surgeons but mustn’t be replaced by a good and detail clinical evaluation, the final decision is always a surgeons responsibility.

Commercial Relationships: Roberto Albertazzi, Keratoconus Easy Fix (I); Leonardo Ferlini, None; Guillermo Rao, None; Jesus Merayo-Lloves, None; Jose Alfonso, None

Program Number: 4226 Poster Board Number: A0254
Presentation Time: 8:30 AM–10:15 AM
Retrospective Evaluation of Epithelial-On Cross-linking with Two Formulations of Riboflavin in Advanced Keratoconus

Eduardo J. Polania1, William Trattler2, Roy Rubinfeld2, Gabriela Perez1, Jennifer Loh1, Rosane Correa1. 1Facultad de Medicina, Universidad de La Sabana, Bogota, Colombia; 2Florida International University, MIAMI, FL.

Purpose: The Purpose of this analysis was to evaluate the safety and efficacy of two formulations of riboflavin, Formulation 1 and Formulation 2, for their use in Trans-epithelial corneal collagen cross-linking. In addition, a different light intensity was used with each formulation for 30 minutes.

Methods: Patients with keratoconus who had undergone trans-epithelial CXL in one or both eyes were included in this analysis. Outcome measures included UCVA, BSCVA, and K Max at 6 months and 1 year follow up.

Results: 345 eyes were assessed. For formulation 1, 177 eyes were evaluated (69.5% male) the average age was 32.3 with an average corneal loading time of 59.3 minutes, pre-op Kmax of 60.0 D and pre-op thinnest point of 447 microns. 125 eyes went through a 6
Purpose: To determine the effect of symmetric vs asymmetric intracorneal ring segment (Intacs®) placement, with corneal collagen crosslinking (CXL), on visual and topographic outcomes in patients with keratoconus (kc) and corneal ectasia (ec).

Methods: 61 eyes (46 KC and 15EC) were analyzed in a prospective randomized control clinical trial. Eyes were divided into three groups: those with symmetric Intacs® placed (either two 450 μm or two 350 μm segments), those with asymmetric Intacs® placed (450 μm/210 μm segments), and those with a single Intacs® placed (450 μm or 400 μm segments). Statistical analysis was performed on those eyes with two symmetric or asymmetric Intacs placed. The six month outcomes analyzed included uncorrected (UCVA) and best corrected (BCVA) visual acuity, maximum (Kmax), flat (Kf), steep (Ks), and average (Kavg) keratometry, and the point of maximum flattening (Pmax) as measured by the Pentacam.

Results: Overall (n=61), at 6 months, UCVA significantly improved from logMAR 0.94 ± 0.32 to 0.77 ± 0.30 (P<0.01), and BCVA changed from 0.39 ± 0.26 to 0.26 ± 0.17 (P<0.01). Topographically, Kmax, Kavg, Kf, Ks, changed from 59.8 ± 7.7D to 59.3 ± 8.5D (p=0.2), 49.8 ± 5.6D to 48.2 ± 5.7D (p<0.01), 47.8 ±5.3D to 46.6 ± 5.5D (p<0.01), 52.9 ± 6.2D to 49.9 ± 6.1D (p<0.01), respectively. On average Pmax flattened by 6.5 ± 2.0D, 6 months after Intacs®/CXL . When the symmetric (n=33) vs asymmetric (n=22) groups were compared, there was no statistical difference between the visual or topographic outcomes, 6 months after Intacs®/CXL. Additionally, similar visual and topographic improvement was seen in a limited number of single Intacs® eyes (n=6).

Conclusions: Overall, eyes treated with symmetric or asymmetric Intacs®, and adjunctive CXL therapy experienced an improvement in corneal topography, UCVA, and BCVA, 6 months after therapy. There was no difference between symmetric vs asymmetric Intacs® treatment 6 months after therapy. Future studies will be preformed to analyze the placement of a single Intacs with adjunctive CXL therapy.

Commercial Relationships: Steven A. Greenstein, None; Peter S. Hersh, Avedro Inc (C)
Support: Avedro Inc, Addition Technology
Clinical Trial: NCT01112072

Clinical Trial: nct01726283

Program Number: 4227 Poster Board Number: A0255
Presentation Time: 8:30 AM–10:15 AM

Symmetric vs. Asymmetric Intracorneal Ring Segment placement with Adjunctive Corneal Collagen Crosslinking for Keratoconus and Corneal Ectasia

Steven A. Greenstein1,2, Peter S. Hersh1, 2, 1Ophthalmology, Rutgers University - New Jersey Medical School, Newark, NJ; 2Cornea and Laser Eye Inst- Hersh Vision, Rutgers University - New Jersey Medical School, Teaneck, NJ.

Purpose: To determine the effect of symmetric vs asymmetric intracorneal ring segment (Intacs®) placement, with corneal collagen crosslinking (CXL), on visual and topographic outcomes in patients with keratoconus (kc) and corneal ectasia (ec).

Methods: 61 eyes (46 KC and 15EC) were analyzed in a prospective randomized control clinical trial. Eyes were divided into three groups: those with symmetric Intacs® placed (either two 450 μm or two 350 μm segments), those with asymmetric Intacs® placed (450 μm/210 μm segments), and those with a single Intacs® placed (450 μm or 400 μm segments). Statistical analysis was performed on those eyes with two symmetric or asymmetric Intacs placed. The six month outcomes analyzed included uncorrected (UCVA) and best corrected (BCVA) visual acuity, maximum (Kmax), flat (Kf), steep (Ks), and average (Kavg) keratometry, and the point of maximum flattening (Pmax) as measured by the Pentacam.

Results: Overall (n=61), at 6 months, UCVA significantly improved from logMAR 0.94 ± 0.32 to 0.77 ± 0.30 (p<0.01), and BCVA changed from 0.39 ± 0.26 to 0.26 ± 0.17 (P<0.01). Topographically, Kmax, Kavg, Kf, Ks, changed from 59.8 ± 7.7D to 59.3 ± 8.5D (p=0.2), 49.8 ± 5.6D to 48.2 ± 5.7D (p<0.01), 47.8 ±5.3D to 46.6 ± 5.5D (p<0.01), 52.9 ± 6.2D to 49.9 ± 6.1D (p<0.01), respectively. On average Pmax flattened by 6.5 ± 2.0D, 6 months after Intacs®/CXL . When the symmetric (n=33) vs asymmetric (n=22) groups were compared, there was no statistical difference between the visual or topographic outcomes, 6 months after Intacs®/CXL. Additionally, similar visual and topographic improvement was seen in a limited number of single Intacs® eyes (n=6).

Conclusions: Overall, eyes treated with symmetric or asymmetric Intacs®, and adjunctive CXL therapy experienced an improvement in corneal topography, UCVA, and BCVA, 6 months after therapy. There was no difference between symmetric vs asymmetric Intacs® treatment 6 months after therapy. Future studies will be preformed to analyze the placement of a single Intacs with adjunctive CXL therapy.

Commercial Relationships: Steven A. Greenstein, None; Peter S. Hersh, Avedro Inc (C)
Support: Avedro Inc, Addition Technology
Clinical Trial: NCT01112072

Program Number: 4228 Poster Board Number: A0256
Presentation Time: 8:30 AM–10:15 AM

Intrastromal Corneal Rings Segments for the Treatment of Keratoconus


Purpose: To report the visual, keratometric and safety outcomes of intrastromal corneal rings segments placed in patients diagnosed with keratoconus.

Methods: An observational, retrospective, longitudinal, uncontrolled study was conducted. Patients diagnosed with keratoconus that were treated with intrastromal rings between 2010 and 2012 with a minimum of 1 year follow up were included in the study. Uncorrected visual acuity, best corrected visual acuity, spherical equivalent,
keratometry, pachymetry, degree of incision, depth of the segments, follow up and complications were assessed. **Results:** A total of 197 eyes were included in the study with an average follow up of 23.82 ± 14.07 months. Overall, the uncorrected visual acuity and the best corrected visual acuity improved by 0.36 logMAR ± 0.47 (p<0.001) and 0.16 logMAR ± 0.28 (p<0.001) respectively. The spherical equivalent also improved with a mean of -2.03 ± 0.63 D (p<0.001). Mean keratometric values decreased by 2.66 ± 3.62 D (p<0.001). A total of 8 (4.06%) patients had surgical complications. The most common complication was extrusion of the segment. **Conclusions:** Intrastromal corneal ring segments appear to improve visual acuity and corneal topography.  

**Commercial Relationships:** Erick Hernandez-Bogantes, None; Franklin Rechnitzer, None  

**Program Number:** 4229 **Poster Board Number:** A0257 **Presentation Time:** 8:30 AM–10:15 AM **Study of the ocular surface quality in patients with keratoconus before and after intrastromal rings surgery**  

Gonzalo Carracedo1, Alberto Recchioni2, Nicolas Alejandre1, Alba Martin-Gil1, Almudena Crooke1, Íñigo Jimeneza-Alfaro2, Jesus Pintor1.  
1Faculty of Optics and Optometry, Universidad Complutense de Madrid, MADRID, Spain; 2Ophthalmology, Fundacion Jimenez Diaz, Madrid, Saint Barthelemy.  

**Purpose:** To determine if there are differences in dry eye signs and symptoms among healthy patients group (control) and an affected keratoconus group (KC), before surgery (pre). Also, to identify the surgery effects on dry eye signs and symptoms, assessing the symptoms before (pre) and after (post) the intrastromal corneal ring segments (ICRS) insertion.  

**Methods:** We included 15 patients (15 eyes) with KC and Kerarings® intrastromal rings implanted, while the control group consisted of 16 healthy subjects (16 eyes). Schirmer test without anesthesia, BUT, corneal staining, OSDI questionnaire and the concentrations of diadenosine tetraphosphate (Ap4A) from samples collected through Schirmer tear strips were evaluated. Goblet cells density, mucin cloud height (MCH) and thickness of the cell (CLT) were measure using impression cytology by means of the Eye Prim medical device. All measurements were performed before (pre) surgery, one month after (post) and at 6 months (post6m) to assess the evolution of the changes in time.  

**Results:** The values obtained from Schirmer test indicate that patients with KC show statistically lower tear volume, 5.70 ± 3.74 mm, than healthy patients, 12.44 ± 8.83 mm, whereas no statistical differences were found when compared before and after implementing the ICRS group in KC. We found no differences in BUT, or in the evaluation between control groups and KC group before or after ICRS surgery. At OSDI, KC patients showed scores 44.96 ± 8.65 before surgery, decreasing to 19.31 ± 4.28 value, 6 months after surgery. KC patients presented statistically significant more corneal staining than subjects in the control group. In addition, KC patients presented very high concentrations of Ap4A, 2.56 ± 1.10 mM, compared to the control group 0.15 ± 0.12 μM, while at 6 months the concentration decreased to 1.02 ± 0.65 μM. Finally, in KC group the goblet cells cell density was 21.2 ± 8.02 cells/mm² compared to the control 32.22 ± 12.59 cells/mm², and further decreasing after ICRS surgery. On the contrary, the mch increased at 6 months after surgery, with no statistically significant differences between KC and control group.  

**Conclusions:** Keratoconus patients present more symptoms and signs of dry eye than healthy subjects. It seems that ICRS surgery induces changes improving dry eye symptoms in KC patients.  

**Commercial Relationships:** Gonzalo Carracedo, None; Alberto Recchioni, None; Nicolas Alejandre, None; Alba Martin-Gil, None; Almudena Crooke, None; Íñigo Jimeneza-Alfaro, None; Jesus Pintor, None  

**Program Number:** 4230 **Poster Board Number:** A0258 **Presentation Time:** 8:30 AM–10:15 AM **Intra stromal corneal rings segments assessment in corneal ectasias**  

Cristina Pacheco Del Valle, Alejandro Babayan, Regina Velasco, Oscar Baca. Cornea, Hospital de la Luz, Mexico, Mexico.  

**Purpose:** To asses clinical features after intra stromal corneal rings segments (ICRS) in corneal ectasias.  

**Methods:** An historic cohort was studied. Descriptive variables were: age, gender and visual acuity; analized variables were: stromal depth planed to implant corneal rings vs measured by OCT visante (chi square) and complications in and after surgery (CI to binomial proportions) and follow up.  

**Results:** Twentythree eyes (18 patients) were analized, of them 10 were male, median age was 28 years (range 18-40), visual acuity of 3 logMAR or worst. All ISCRS were INTACS(TM), 0.45mm segment thickness. Complications (6 cases) were: neovascularization (2) and superficial placement (4) CI=0.12-0.51. Two patients underwent removal of ISCRS due to superficial placement. Stromal depth compared p<0.001  

**Conclusions:** In conventional technique the depth predicted is hard to achieve and complications more than expected.  

**Commercial Relationships:** Cristina Pacheco Del Valle, None; Alejandro Babayan, None; Regina Velasco, None; Oscar Baca, None  

**Program Number:** 4231 **Poster Board Number:** A0259  

**Presentation Time:** 8:30 AM–10:15 AM  

**Outcomes of intrastromal rings combination results for treatment of central ectasias**  

Leonardo Ferlini2, Roberto Albertazzi1, Guillermo Rao1, Jesus Merayo-Lloves3, Jose Alfonso3, 1Residency, Centro de Ojos Quilmes, Buenos Aires, Argentina; 2Fundación de Investigacion Oftalmologica - Instituto Oftalmologico Fernández-Vega, Oviedo, Spain; 3University of Oviedo, Oviedo, Spain.  

**Purpose:** Outcome Analysis of a combination of Intracorneal ring segment (ICRS) both Ferrara and Intacs, for correction of central keratoconus.  

**Methods:** 13 eyes of 8 patients with contact lens intolerance or progressive central keratoconus underwent ICRS surgery and were followed for at least 6 months. Tectonic outcome were evaluated as a reduction of the keratometric (K) values: K minimal (Kmin) and K maximal (Kmax)).Visual outcome were measured as improve in uncorrected distance visual acuity (UDVA), best corrected visual acuity (CDVA), and refractive error (myopia and astigmatism) reduction (decimal scale). Data were processed with R-programing language for descriptive and inference statistical analysis.  

**Results:** There was a reduction of keratometric values Kmax-pre 45.91 ± 3.50 to Kmax-post 43.41 ± 3.03. Regarding VA(Decimal Scale) Mean UDVA change in from 0.33 ± 0.19 to 0.66 ± 0.26. Mean DCVA change from 0.86 ± 0.20 to 0.94 ± 0.18. Myopic and astigmatism components were highly reduced after ICRS implantation. Myopia was reduced from -2.34 ± 2.31 to -1.13 ± 0.99. Astigmatism was reduced from -2.00 ± 1.39 to -0.56 ± 0.82. No intraoperative and postoperative complications occurred in these eyes.  

**Conclusions:** The combination of Ferrara and Intacs ICRS implantation archive good tectonic and refractive outcome in the care
of patients with keratoconus. Nomograms for ICRS implantation should be changed and customized in order to archive standart refractive outcome.

Commercial Relationships: Leonardo Ferlini, None; Roberto Albertazzi, Keratoconus Easy Fix (I); Guillermo Rao, None; Jesus Merayo-Lloves, None; Jose Alfonso, None

Program Number: 4232 Poster Board Number: A0260
Presentation Time: 8:30 AM–10:15 AM
INTRASTROMAL CORNEAL RING SEGMENT IMPLANTATION FOR ECTASIA AFTER REFRACTIVE SURGERY
Larissa Rossana Stival1, Belquiz A. Nassaralla1, Marisa Figueiredo1, Frederico Bicalho2, Joao J. Nassaralla1. 1Department of Cornea and Refractive Surgery, Goiânia Eye Institute, Goiânia, Brazil; 2São Geraldo Hospital, Belo Horizonte, Brazil.

Purpose: To evaluate the clinical outcomes of intrastromal corneal ring segment (ICRS) implantation to correct keratoconus on eyes with prior refractive surgery.

Methods: Forty-two eyes of 26 patients, 14 men and 12 women, with postoperative keratectasia after PRK (8 eyes, Group A) and LASIK (34 eyes, Group B) were included in a nonrandomized, retrospective, observational case series. Mean age at the time of ICRS implantation was 30.5 years. Mean follow-up was 23.5 months (range, 1 to 86 months). Corneal tunnels were created by means of mechanical dissection in all eyes. Main outcome measures included UCVA, BCVA, refraction, keratometry and computerized analysis of corneal topography.

Results: Mean preoperative refractive astigmatism decreased from -2.58 to -1.66D in Group A and -4.73 to -2.92D in Group B (p=0.0253). Mean keratometric astigmatism decreased from 44.05 to 42.92D in Group A and 47.42 to 46.06 D in Group B (p=0.2975). The mean spherical component was reduced from -2.97 to -2.05D in Group A and -3.32 to -2.11D in Group B (p= 0.4692). There were no intraoperative or postoperative complications.

Conclusions: ICRS implantation is an useful option for the correction of iatrogenic keratectasia following refractive surgery.