Proteomic comparison of keratoconus with and without vernal keratoconjunctivitis in individuals from Saudi Arabia

Shukti Chakravarti1, Alka Mahale1, Gajanan Sathe1, James W. Foster1, Vishal Shinde1, Akhilesh Pandey1, Charles Eberhart1, Albert S. Jun2, Samar A Al-Swailem1, Donald U. Stone1, Azza Maktabi1.

1Ophthalmology, Pathology, Johns Hopkins Sch of Medicine, Baltimore, MD; 2Wilmer Eye Institute, Baltimore, MD; 3Medicine, Johns Hopkins School of Medicine, Baltimore, MD; 4King Khaled Eye Specialist Hospital, Riyadh City, Saudi Arabia.

Purpose: Keratoconus (KC) is a vision-debilitating disease where the cornea undergoes progressive, degenerative thinning and steepening, with total loss of vision in 20% of severe cases. In Saudi Arabia there is a high incidence of keratoconus (KC), often associated with vernal keratoconjunctivitis (VKC). To elucidate pathogenic events that distinguish VKC from KC alone we performed highly sensitive multiplex mass spectrometry on individual cornea tissues from these two groups.

Methods: Three DN samples deemed unsuitable for transplantation (Tissue Bank International, Baltimore) and patient corneas from 3KC and 3 VKC (consented under an approved IRB, King Khalid Eye Specialist Hospital) were extracted using a Barocycler. Equal amounts of protein were reduced, alkylated and subjected to trypsin digestion. The resulting peptides were labeled with Tandem Mass Tag™ reagents as per the manufacturer’s instructions. The peptides were resolved by basic pH reverse phase chromatography into 24 fractions. These fractions were analyzed on an Orbitrap Fusion Lumos mass spectrometer coupled with Proxeon EASY-nLC 1000 liquid chromatography. The Proteome Discoverer software suite was used for MS/MS searches and protein quantification.

Results: A total of 3624 proteins were identified at high protein FDR confidence. We detected 33 different collagen polypeptides; basement membrane Col4A2-A6 chains were present at similar levels in DN and both KC types. Epithelial KRT3 was similar in DN and VKC, but increased in KC, while KRT12 was similar across all samples. Fibrillar collagen types, COL1A1, COL1A2, COL3A1 COL5A1, COL5A2 and COL5A3 were all elevated in KC, but not in VKC compared to DN samples. Whereas, other collagens like COL6A1-3 and COL8A1 were present at similar levels in all samples. The collagen associated proteoglycans; KERA and DCN were lower in the VKC samples.

Conclusions: The epithelial ECM proteins show fewer differences between KC and VKC. However, changes in stromal collagens and proteoglycans speak of loss in stromal ECM functions. Additional analyses of the entire dataset are underway to understand cellular and matrix pathogenesis underlying keratoconus and those associated with vernal keratoconjunctivitis.
AKT and mTOR differential signaling in keratoconus compared to donor stromal cells

Vishal Shinde1, James W. Foster1, Yassine J. Daoud2, Uri Soiberman1, Shukti Chakravarti1. 1Gastroenterology, Johns Hopkins School of Medicine, Baltimore, MD; 2Johns Hopkins School of Medicine, Baltimore, MD.

Purpose: Keratoconus (KC), a leading cause for cornea transplantation, is a progressive corneal thinning disease where the underlying molecular events are poorly understood. Our ongoing RNA seq and proteomics studies of keratoconus corneas and cultured stromal cells suggest changes in AKT/mTOR signaling. Here we tested this signaling mechanism in cultured KC and donor (DN) stromal cells.

Methods: DN (n = 5) and KC (n = 5) stromal fibroblast cultures were subjected to the following stresses, 1) ER stress with tunicamycin (2ug), 2) calcium ion overload using calcium ionophore A23187 (2uM) and 3) oxidative stress using H2O2 (20 uM), and cell viability measured by the MTT assay. The above concentrations were used after determining the LC50 for each drug by monitoring cell death at increasing concentrations of the drugs. Phosphorylated intermediates of AKT and mTOR signals were analyzed in cell lysates by immunoblotting.

Results: The expression of mTOR pathway associated proteins were significantly altered in untreated and treated KC cells as compared to DN cells. Basal levels of pmTOR (active form) was 5-fold higher in KC cells, and after subjecting the cells to cellular stresses, pmTOR decreased by 3-fold in KC cells as compared to DN. Consistent with this finding mTOR pathway regulators PI3K (2.6 fold) and P62 (3.4 fold) were increased in KC cells as compared to DN. At stressed condition, there was 2.5-fold increase in PI3K expression in KC cells whereas there was no change in P62 expression. We examined the expression of downstream effectors of both mTORC1 and mTORC2 complexes and only the downstream effector of the mTORC1 complex, 4EBP1 was 3-fold in KC cells at basal level and 6-fold after subjecting to cellular stress in KC compared to DN cells.

Conclusions: MTORC1 signaling allows cells to regulate protein synthesis, proliferate, grow or undergo autophagy or apoptosis in response to nutrient levels, energy, growth factors and stress. Therefore, MTORC1 signaling may be central to stromal cell homeostasis in the avascular nutrient-restricted cornea. Our keratoconus cell culture model suggests major changes in this signaling mechanism in keratoconus pathogenesis.

Commercial Relationships: Vishal Shinde, None; James W. Foster, None; Yassine J. Daoud, None; Uri Soiberman, None; Shukti Chakravarti, None

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Presentation Time: 8:30 AM–10:15 AM

Molecular insights into ECM and collagen assembly disruptions in keratoconus

James W. Foster1, Vishal Shinde1, Uri Soiberman2, Yassine J. Daoud2, Fares Alsaleh2, Gajanan Sathe1, Jun Wan1, Jiang Qian2, Albert S. Jun2, Akhilesh Pandey1, Shukti Chakravarti1. 1Johns Hopkins School of Medicine, Baltimore, MD; 2Wilmer Eye Institute, Baltimore, MD; 3Institute of Genetic Medicine, Baltimore, MD.

Purpose: The underlying mechanisms of Keratoconus (KC) progression is poorly understood. Collagen fibril assembly at points of nucleation on the cell surface is a highly regulated, complex multistep process requiring cell matrix interactions, removal of the terminal non-collagenous globular domains and stacking of fibrils.

We used a monolayer cell culture system to investigate if specific steps in this process are compromised in KC stromal cells.

Methods: Human unaffected donor (DN) and keratoconus patient (KC) stromal keratocytes (n=7) were maintained for 14 days in serum free medium to induce a more keratocyte-like phenotype. Cell layer associated collagen (indication of matrix assembly) was assayed using 1) picro-sirius red staining, 2) hydroxyproline content, 3) Western blotting and 4) MALDI-TOF mass spectrometry. Collagen production, modification and breakdown genes were assayed by qRTPCR. Gelatin zymography was used to quantify MMP activity in the cell culture media. Two-tailed Student’s t-test was used for statistical analysis.

Results: KC cultures harbor less cell layer associated collagen matrix in our in vitro KC model (Hydroxyproline p=0.0449, Sirius red p=0.0195). This difference is not attributed to cell number (p=0.93), density (p=0.32), or activation state (THY-1, α-SMA). Transcriptional levels of COL1A1 are markedly increased in KC cultures. Yet, Type I collagen within the media of KC samples was increased (p<0.01). Collagen processing proteins were examined, HSP47 was unchanged as quantified by western blot, and qPCR. The N-terminal collagen processing enzyme ADAMTS2 followed expression increases of COL1A1 (p=0.0074 and p= 0.0009 respectively), The C-terminal enzyme BMP-1 was found to be unchanged. Type V Collagen, was not found to be changed by qPCR, MS, Immunohistochemical localization or western blotting. MMP2 activity was shown to be increased by 49% in KC media relative to DN cultures (p<0.04). Concordantly collagen lost to the media was higher in KC cultures.

Conclusions: Our results indicate that collagen assembly in KC stromal cell cultures is fundamentally perturbed. We hypothesize that this dysregulation is due to a combination of reduced cell-matrix interactions, insufficiency of BMP1 and the increase of MMPs, and may be related to changes in TGFβ signal as we reported earlier. As such it provides a novel model for investigation of disease progression and trial possible intervention strategies.

Commercial Relationships: James W. Foster, None; Vishal Shinde, None; Uri Soiberman, None; Yassine J. Daoud, None; Fares Alsaleh, None; Gajanan Sathe, None; Jun Wan, None; Jiang Qian, None; Albert S. Jun, None; Akhilesh Pandey, None; Shukti Chakravarti, None
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The generation and characterization of a novel mouse line, Cdhl2- rTα (Cdhl2−/−), for genetic manipulation of stem cell niche tissues

Yujin Zhang1, Lingling Zhang1, Diego O. Ogando2, Yuka Okada2,1, Yen-Chiao Wang1, Winston W. Kao1, Yueh-Chiang Hu1, Joseph A. Bonanno1, Cha-Yang Liu4, 1Optometry, Indiana University School of Optometry, Bloomington, IN; 2Wakayama Medical University, Wakayama, Japan; 3Ophthalmology, University of Cincinnati, Cincinnati, OH; 4Division of Developmental Biology, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH.

Purpose: Cell adhesion molecule such as N-cadherin (Cdhl2) that mediates stem cell-niche interactions is crucial for stem cell self-renewal and is dynamically regulated. To achieve specific genetic modifications in stem cell-niche interaction during embryonic development and adult homeostasis, a novel Cdhl2-rTα knock-in (Cdhl2−/−) mouse line was generated.

Methods: N-cadherin (Cdhl2) is expressed in multiple tissues including retina and corneal endothelium and functions to mediate cell-cell adhesion. A gene-targeting vector containing a self-cutting peptide (P2A)-reverse tetracycline transcription activator (P2A-rTα)
Purpose: Vogt striae are one of the clinical indicators of keratoconus, and consist of dark, vertically oriented lines crossing the depth of the cornea. We observed similar banding patterns in corneas with pathologies other than keratoconus, and normal corneas. We postulate that these bands form spring-like links between zones of collagen lamellae, and their visibility is enhanced in pathologies where corneal shape is under stress.

Methods: Images of 24 normal and 47 pathological human corneas along with macaque and mouse corneas, were captured with a combination of in vivo confocal microscopy (IVCM), in vivo spectral domain optical coherence tomography (SD-OCT), ex vivo full-field optical coherence microscopy (FFOCM), and histology. Collagen types were immuno-labeled, and electron microscopy was performed. Optical coherence tomography shear wave elastography (OCT-SWE) provided stiffness maps. Second harmonic generation (SHG) microscopy allowed mapping of fibrillar collagen distribution.

Results: Banding patterns were observed with all imaging modalities, in all species, with pathology-specific differences. In cross-sectional views of normal and non-keratoconic corneas, these dark bands depart from anchor points at Descemet’s membrane in the posterior stroma obliquely in a V-shape, whereas in keratoconus these bands depart vertically from posterior toward anterior stroma. In en face views the bands criss-crossed in the posterior stroma except in keratoconus where they tended to run parallel. OCT-SWE showed these bands are softer than surrounding stromal tissue, and their orientation was modified and visibility enhanced at non-physiological IOP. Immunohistology revealed these bands to predominantly contain collagen VI.

Conclusions: Banding patterns are visible in most corneas, not only in keratoconus. Their visibility may be indicative of stresses to the regular arrangement of collagen lamellae. The role of these soft, elastic regions of collagen 6A1 linking sets of collagen lamellae may be to maintain corneal shape under normal IOP conditions and absorb shocks.
Purpose: Keratocytes are neural crest-derived fibroblast cells in corneal stroma. Keratocytes are normally quiescent, but they can readily respond following injury. As the major neighbor cell to the corneal endothelium, we aim to evaluate keratocyte activation in vivo to illustrate the possible influence of endothelial cells and possible inflammatory pathogenesis in endothelial keratopathy.

Methods: We created a corneal endothelial dysfunction model in New Zealand White Rabbits (1.5-2 kg) by scraping corneal endothelial cells about 9 mm in diameter. Slit-lamp examination, anterior segment optical coherence tomography measuring central corneal thickness (CCT), confocal microscopy, proinflammatory cytokine transcript expressions and histology evaluation were performed before endothelial scraping and at 1, 3, 7, 14, 30 and 60 days after the surgery.

Results: All the corneas were opaque and moderately to severely edematous at day 1 (normal 385 ± 9.53 µm vs 1133 ± 55.71 µm, p<0.0001) and at day 3 (normal 385 ± 9.53 µm vs 741.9 ± 136.7 µm, p=0.0002); but regained transparency and normal thickness since day 7 (normal 385 ± 9.53 µm vs 370 ± 19.92 µm, p=0.46). In the posterior portion of corneal stroma, confocal microscopy detected many highly reflective activated keratocytes in a multiple dendritic cell-like shape with multiple long extensions. These activated keratocytes returned to their original quiescent form at 30-60 days after surgery, showing small oval nuclei without processes. During the same period, the endothelial cells are fully regenerated, but had lower endothelial cell counts at 60 days (normal 2587 ± 92.6 cells/mm² vs 1733 ± 106.61 cells/mm², p<0.0001). Interleukin-1 beta (IL1β), tumor necrosis factor alpha (TNFα), Il6 transcripts were upregulated in activated keratocytes vs quiescent ones (all p values<0.05). Historically, keratocytes with enlarged nuclei and long processes were noted microscopically at 1, 3, 7, 14 postoperative days, binucleated cells were also seen in the posterior stroma frequently; most keratocytes were quiescent at 30 and 60 days.

Conclusions: Our study showed keratocytes activation with proinflammatory cytokine upregulation in a rabbit model of endothelial keratopathy. Endothelial cell dysfunction might contribute to keratocyte activation, which in turn influences corneal homeostasis via inflammatory pathway in endothelial keratopathy.

Commercial Relationships: Yujuan Wang; Jiangna Chen, None; Zhijian Jiang, None

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Presentation Time: 8:30 AM–10:15 AM
Effects of the heat shock protein inhibitor, 17AAG, on corneal stromal haze using an in vivo rabbit phototherapeutic keratotomy model

Sydney G. Edwards,1 Philip Kass,2 Maryam Ali,3 Ariana Marangakis,1 Vijay K. Raghunathan,1 Christopher J. Murphy,2, 3, Sara M. Thomasy1
1Department of Surgical and Radiological Sciences, University of California, Davis, Davis, CA; 2Department of Population Health & Reproduction, School of Veterinary Medicine, University of California, Davis, Davis, CA; 3Department of Ophthalmology and Vision Science, School of Medicine, University of California, Davis, CA, Davis, CA.

Purpose: Corneal scarring remains a leading cause of blindness worldwide. 17-N-allylamino-17-demethoxygeldanamycin (17AAG), an anti-fibrotic heat shock protein 90 (HSP90) inhibitor decreases scar formation in tissues such as the liver and lung, and also stimulates reversion of myofibroblasts to keratocytes in vitro, even in the presence of transforming growth factor-b1 (TGF-b1). We hypothesized that 17AAG would reduce formation of corneal haze in an in vivo rabbit phototherapeutic keratotomy (PTK) model.

Methods: In vivo: 18 New Zealand White Rabbits received an axial 6 mm diameter, 100 mm depth PTK in the right eye (OD), and either 100 mM 17AAG at 6x (n=6), 3x daily (n=6) or topical placebo (vehicle control; 6x; n=6) for 28 days. Stromal haze scoring, slit lamp biomicroscopy, and Fourier-domain optical coherence tomography (FD-OCT) were performed at multiple time points. A mixed-effects ANOVA model was used for statistical analysis. In vitro: Primary rabbit corneal stromal cells were cultured on polyacrylamide gels of differing stiffness (5 or 25 kPa) or tissue culture plastic (TCP, >1 GPa), in the presence or absence of 10 ng/ml TGF-b1, and treated with 17AAG (500 nM) or vehicle control (DMSO), for 72 h. RNA was isolated and qPCR was performed for a-smooth muscle actin (a SMA) expression.

Results: In vivo: Topical 17AAG 6x, but not 3x had a greater corneal stromal haze score (p=0.02) and stromal haze thickness as measured by FD-OCT (p=0.03) versus placebo. In vitro: Treatment with 17AAG resulted in greater (P<0.05) a SMA expression in cells cultured on gels of lesser stiffnesses (5kPa) compared with 25kPa and TCP, in the presence of TGF-b1.

Conclusions: In vivo, 17AAG at high concentration (100 mM) and frequency (6x), when initiated at the time of wounding increases stromal haze in an in vivo rabbit PTK model. In vitro, 17AAG increases a SMA expression in cells cultured on soft substrates. Since it is known that stromal stiffness increases during stromal wound healing the effect of 17AAG treatment when initiated later in the wound healing process remains unknown.

Commercial Relationships: Sydney G. Edwards, None; Philip Kass, None; Maryam Ali, None; Ariana Marangakis, None; Vijay K. Raghunathan, None; Christopher J. Murphy, None; Sara M. Thomasy, None
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Gal-1 and Gal-3 represent new interesting biomarkers in keratoconus patients

Rosen M. Hazarbassanov, Frans Eberth Costa Andrade, Joyce Covre, Myrna Serapião dos Santos, Mauro S. Campos, Jose A. Gomes, Cristiane Damas Gil.

Purpose: To evaluate the expression of galectin-1 (Gal-1) and -3 (Gal-3) in the keratoconus (KC) diagnosed patients and riboflavin and ultraviolet light effect on human keratocytes cultivated in vitro.

Methods: Tear fluid and conjunctival impression cytology specimens from control and KC patients with and without atopy (n = 10/group) were used to evaluate Gal-1 and Gal-3 expression by immunofluorescence and ELISA. Primary human keratocytes were isolated by digestion in collagenase from surgically removed corneas of five normal human corneal buttons and cultured in DMEM/Ham's F12 medium supplemented with 2% fetal bovine serum. These cells were evaluated under two experimental conditions: control and submitted to the application of UVA light and riboflavin 0.1% for 30 minutes called cross-linking (CXL). After 24 hours of CXL, expression of Gal-1 and Gal-3 in the and their supernatants was performed using immunofluorescence and ELISA; multiplex assay was also done to detect inflammatory biomarkers in supernatants. Student’s t-test was used for statistical analysis.

Results: Atopic KC patients exhibited increased levels of Gal-1 in the tear fluids (14.5±3.3 ng/mL, p<0.05) and epithelial cells of bulbar conjunctiva (130±3 arbitrary units, p<0.001) compared to control (9.8±0.5 and 87±1.6, respectively). On the other hand, non-atopic KC patients exhibited increased levels of Gal-3 in the tear fluid (70±16 ng/mL, p<0.05), but decreased endogenous expression in conjunctival epithelial cells. In vitro experiments, keratocytes exhibited intense nuclear Gal-1 expression while Gal-3 was localized especially in the cytoplasm and both were strongly diminished after 24 hours of CXL. Further, CXL induced significant release of Gal-1 in the cell supernatants (116±18 ng/mL, p<0.05) and decreased inflammatory biomarkers as IL-6, IL-8, MMP-2 and MMP-9. Gal-3 levels was not detected in the keratocyte supernatants.

Conclusions: Gal-1 and Gal-3 represent new interesting biomarkers as revealed by their different expression patterns in atopic and non-atopic KC patients. CXL has immunosuppressive effect on keratocytes by reducing the release of cytokines and MMPs and increased anti-inflammatory protein Gal-1.

Commercial Relationships: Rosen M. Hazarbassanov, None; Frans Eberth Costa Andrade, None; Joyce Covre, None; Myrna Serapião dos Santos, None; Mauro S. Campos, None; Jose A. Gomes, None; Cristiane Damas Gil, None

Program Number: 3911 Poster Board Number: A0107
Presentation Time: 8:30 AM–10:15 AM
Engineering of a curved corneal stromal substitute
Noémie Parent1, Julie Bérubé2, Jean-Michel Bourget1, Maxence Mounier1, Teodor Veres3, Stephanie Proulx1,2, 1Department of Ophthalmology, Université Laval, Quebec, QC, Canada; 2Centre CUO-LOEX, Centre de recherche du CHU de Québec, Quebec, QC, Canada; 3Functional Nanomaterials Group Leader Life Sciences Division, National Research Council of Canada, Boucherville, QC, Canada.

Purpose: The self-assembly approach of tissue engineering allows to reconstruct 3D corneal stromal substitutes (Proulx et al., 2009; Bourget et al., 2016). Presently, these substitutes are flat. The purpose of this study was to engineer a stromal substitute with the curved shape of a native cornea.

Methods: Curved plastics were formed using a thermoplastic elastomer (TPE) and sterilized. Their size fitted into a well of 6-well plates. Keratocytes, previously isolated from a human cornea, were seeded on top of the TPE and cultured in the presence of serum and ascorbic acid. Flat 6-well culture plates were used as controls. Cells were cultured for 35 days and formed sheets of extracellular matrix. Two sheets were superposed to form a thicker stromal substitute, and cultured to allow for the sheets to adhere to each other (14 days for curved stromal substitutes; 8 days for flat stromal substitutes). The stromal substitutes were then characterized using microscopic images. They were then fixed in 3.7% formaldehyde for histology (Masson’s Trichrome staining).

Results: The activated keratocytes secreted extracellular matrix and formed sheets on both the curved TPE and the flat plastic controls. When removed from the curved TPE surface, the stromal substitute maintained a more pronounced curved form. Thickness of the stromal...
substitutes were calculated using histology cross-sections. The curved stromal substitutes had a mean thickness of 26.1±0.5µm (n=2 counts) and flat stromal substitutes 15.4±2.7µm (n=3 counts).

**Conclusions:** This study demonstrates that the self-assembly approach can be used to reconstruct curved stromal substitute. Therefore, this brings us closer to a reconstructed cornea whose properties are similar to the native tissue.

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

**Depth-dependent Corneal Suprastructure by Fourier-transformed Second Harmonic Generation Microscope**

Sheng-Lin Lee, YANG-FANG CHEN, Chen-Yuan Dong. Physics, National Taiwan University, Taipei, Taiwan.

**Purpose:** High tensile strength with optical clarity is the unique characteristic of cornea and the key role in corneal tissue development and wound healing. However, details on the suprastructure of corneal stroma are still not fully understood. We hypothesize that the depth-dependent structure of corneal lamella could be quantified non-invasively using SHG microscopy and optimized Fourier-transform techniques.

**Methods:** Specimens of adult avian cornea were used in this study. The fresh corneal samples were fixed with 10% formaldehyde in phosphate-buffered saline (PBS) solution. Prior to the imaging experiment, corneas were dissected as wide strips along the temporal-nasal direction and marked with scissors on the temporal side to identify the specimen's orientation. When doing experiments, the samples were enclosed in a confinement chamber attached to a cover slide and sealed with a cover glass and high-vacuum grease. Through the combination of the second harmonic generation (SHG) microscopy and post-processing of Fourier-transform images, mature avian corneas are investigated to observe and track the depth-dependent structure of the corneal stroma at different position.

**Results:** Our results show that the anterior stroma behaves like a fan-like distribution of successively and counterclockwise rotated fibrous lamellae for paired corneas from the same chicken. However, the posterior stroma maintains a non-rotating pattern while increasing in depth. The overall thickness of anterior stroma was 300 ± 14µm of the left and 279 ± 11µm of the right cornea. Results showed no significant difference between the total thickness of the left and right corneae at p < 0.05. But the thickness of anterior stroma was different between left and right cornea at p < 0.05 (T = 2.54, p = 3.5E-02).

**Conclusions:** Through quantitative analysis, we determine the natural transition between the anterior and posterior stroma. These findings enhance our understanding of biophysical behavior in the chicken cornea model. Moreover, the Fourier-transform-based modality, in combination with SHG microscopy, serves as a promising tool to determine collagen alignment in embryonic development, tissue engineering and corneal diseases.

**Commercial Relationships:** Sheng-Lin Lee; YANG-FANG CHEN, None; Chen-Yuan Dong, None

**Program Number:** 3913 **Poster Board Number:** A0109

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

**Assessing the Origin of Extracellular Matrix Proteins Released from the Cornea in Response to Injury**

SOMSHUVA BHATTACHARYA, Gudiseva Chandrasekher. PHARMACEUTICAL SCIENCES, SOUTH DAKOTA STATE UNIVERSITY, Brookings, SD.

**Purpose:** Turnover of corneal stroma extracellular matrix (ECM) protein is very slow, and the keratocytes metabolic activity is quiescent in physiological conditions as requirement for new extracellular protein matrix generation is limited. Our studies showed that the cornea releases different ECM proteins in response to injury (ARVO meeting 2016, abstract no. 4352). The currently undertaken study is to determine the origin of these ECM proteins.

**Methods:** De-epithelialized (injured) and intact porcine corneas were maintained in culture (serum free DMEM/F12) at 37°C and the cornea organ cultured medium (COCM) was collected at various times (4-24 hours). In separate experiments, corneal stroma extracellular matrix (CSEM) preparation was made by extracting de-epithelialized corneas in a buffer. The presence of multiple extracellular matrix components released in to the COCM and in the CSEM was identified by western immunoblotting. Further, the susceptibility of extracted proteins in CSEM to proteolytic cleavage by the enzymes possibly released into COCM was evaluated.

**Results:** We have identified in CSEM two different forms of peptides (250 and 140kDa) for both collagen I and collagen IV. The 140 kDa form of both collagen I and collagen IV was also found to be present in COCM collected from injured and uninjured corneas. Further, a low molecular weight (60-80kDa) form of collagen I but not collagen IV was also released from the corneas after injury. Interestingly, a 250 kDa fibronectin that was present in COCM is not identified in the CSEM, instead we found a 100 kDa form. Treatment of the native ECM proteins in CSEM with COCM did not result in the generation of the polypeptides that are identified in the COCM.

**Conclusions:** Presence of multiple types of ECM proteins in COCM from intact and de-epithelialized corneas suggests that the stroma component of the cornea is the major site from which they are released. Stromal keratocytes activation that occurs in response to injury leads to the process of healing and remodeling of stroma matrix through proteolytic degradation of damaged proteins and deposition of new ECM proteins. Proteases released from the cornea in response to injury may act on damaged ECM. It is possible that the multiple forms of ECM proteins which appeared in COCM are byproducts of stromal rebuilding process.

**Commercial Relationships:** SOMSHUVA BHATTACHARYA, None; Gudiseva Chandrasekher, None

**Support:** South Dakota State University Research Dissemination grant 2016 and Department of Pharmaceutical Sciences, SDSU
Methods: Mice were divided into groups of 16 mice each: normal untreated corneas (Group 1); UV-CXL without riboflavin or antibiotic (2); UV-CXL pretreated with moxifloxacin (3); gatifloxacin pretreatment (4); azithromycin pretreatment (5); riboflavin, no antibiotic pretreatment (6); riboflavin and moxifloxacin pretreatment (7). Topical riboflavin 0.1% was instilled. Antibiotic drops were instilled every 2 hrs for 12 hrs prior to 20 minutes of UV-light [1.4-7.3 J/cm² (0.7-4 mW/cm²)]. Animals were sacrificed at 24 hrs; corneas were excised and stained for apoptosis markers, matrix metalloproteinase 9 (MMP-9), and with terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). Polymerase chain reaction for apoptosis markers was performed, as was zymography for MMP-9. PCR for apoptosis markers and MMP-9 zymograms were quantified by scanning densitometry analysis. Comparisons were done between control and treatment groups. Spectrofluorometry was performed of dilute antibiotic concentrations (1.25 x 10⁻⁴ %).

Results: Expression of apoptosis markers BAX, caspases 3 and 9, and MMP-9, and TUNEL staining were higher in either FQ group, in riboflavin-pretreated, and riboflavin and moxifloxacin-pretreated corneas compared with the other groups: untreated, azithromycin, and UV-CXL (ANOVA, p < 0.001) (Figure 1). The highest expression of BAX, caspases 3 and 9, and MMP-9 was in the group pretreated with riboflavin and moxifloxacin. Excitation of both FQs occurred at the wavelength used for corneal cross-linking.

Conclusions: In murine corneas undergoing UV-CXL, pretreatment with FQ drops induced stromal apoptosis, inflammation, and MMP-9 production; combined riboflavin and moxifloxacin pretreatment (i.e., the clinical scenario) induced the highest expression of apoptosis markers and degradative MMP-9. Fluorescence likely increases when commercially available FQ is instilled prior to UV-CXL. These findings may result from photosensitization by riboflavin and phototoxicity of FQs.

* Denotes treatment with FQ ± riboflavin prior to UV-CXL increased expression of MMP-9, BAX, and caspases 3 and 9 compared with other groups.

Commercial Relationships: Irene C. Kuo, None; matias osaba, None; Victor E. Reviglio, None

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Presentation Time: 8:30 AM–10:15 AM
Clinicopathologic Correlation of Secondary Corneal Amyloid Deposition
Mark P. Ghassibi, Maria P. Fernandez, Eduardo C. Alfonso, Richard K. Forster, Sander R. Dubovy. Bascom Palmer Eye Institute, University of Miami, Miami, FL.

Purpose: To report the clinicopathologic findings of 26 cases with secondary amyloid deposition of the cornea.

Methods: In a non-comparative, consecutive case series, the database of the Florida Lions Ocular Pathology Laboratory was searched for surgical specimens diagnosed with secondary corneal amyloid deposition on light microscopic examination from the time period of 1998 to 2016. The specimens were reviewed and the clinicopathologic features were evaluated.

Results: The diagnosis of secondary amyloid deposition was established by light microscopic examination of formalin fixed, paraffin embedded tissue in the corneas of 26 eyes (Right = 13, Left = 13). From this group of 26 patients, 13 were female and 13 male. Mean age was 53±22 (range, 9-83) years. Findings on clinical presentation were keratoconus (7), keratoglobus (1), corneal opacification and/or scarring associated with trauma (5), Cogan syndrome (1), Peters anomaly (1), interstitial keratitis (2), Fuchs endothelial dystrophy (1), failed corneal graft (1), band keratopathy (1), bullous keratopathy (3), and congenital cataract/glaucoma (3). Twenty-one patients underwent penetrating keratoplasty (PK), 3 underwent enucleation, 1 underwent evisceration, and 1 underwent superficial anterior keratectomy. On histopathologic examination all eyes demonstrated a Periodic acid-Schiff (PAS)-positive acellular material within the corneal stroma that stained positively for Congo Red-stain and demonstrated apple-green birefringence under polarized light within the amorphous tissue.

Conclusions: We present the largest case series with clinicopathologic examination of secondary corneal amyloidosis. All cases demonstrated PAS- and Congo red-positive acellular material within the corneal stroma and exhibited apple-green birefringence under polarized light. All the cases of corneal amyloid deposition were secondary to chronic ocular surface inflammation, corneal degeneration, or trauma.


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* Denotes treatment with FQs ± riboflavin prior to UV-CXL increased expression of MMP-9, BAX, and caspases 3 and 9 compared with other groups.