Program #: A109

Presentation Time: 3:15 PM - 5:00 PM

Effect of the Synthetic NC-1059 Peptide on Diffusion of Riboflavin Across an intact Corneal Epithelium

Yuntao Zhang\textsuperscript{a}, Pinakin Sukthankar\textsuperscript{a}, John M. Tomich\textsuperscript{a}, Gary W. Conrad\textsuperscript{a,b}

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Purpose: To investigate the effect of NC-1059 peptide on riboflavin (RF) diffusion across an intact corneal epithelium into the stroma.

Methods: NC-1059 peptide was synthesized by solid-phase synthesis with 9-fluorenylmethoxycarbonyl chemistry, purified by reversed-phase HPLC and characterized with matrix-assisted laser desorption time-of-flight mass spectroscopy. The diffusion of RF across E18 chick corneal epithelium ex vivo was monitored using confocal microscopy. The depth distributions of RF in the cornea stroma were measured using a group of linear equations based on the relationship between RF fluorescence intensity and concentrations.

Results: Data presented in this study demonstrated that the NC-1059 peptide can open the epithelial barrier to allow the permeation of RF into the stroma. The effect of NC-1059 peptide on RF diffusion across an intact corneal epithelium appeared concentration-, and time-dependent. The concentration of RF at 30 µm depth of chick corneal stroma increased distinctly after exposure to NC-1059 for 10 minutes, reaching a much higher, stable level by 30 minutes. The concentrations of RF in the presence of NC-1059 at corneal stromal depths of 50, 100, and 150 µm were significantly higher than in the absence of the peptide. However, they were relatively lower when compared to corneas from which the epithelium first had been removed. In addition, a null assay indicated that the NC-1059 peptide did not kill corneal epithelial cells.

Conclusions: NC-1059 peptide can significantly enhance the diffusion of RF across intact corneal epithelium into the stroma before UVA corneal crosslinking. Commercial Relationships: None; Pinakin Sukthankar, None; John M. Tomich, None; Gary W. Conrad, None.

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Program Number: 1074 Poster Board Number: A110

Presentation Time: 3:15 PM - 5:00 PM

Evaluation Of Corneal Ultrastructure Following Microwave Keratoplasty

Craig Bootle\textsuperscript{a}, Stan R. Morgan\textsuperscript{a}, Sally Hayes\textsuperscript{a}, Jennifer Hiller\textsuperscript{a}, Nicholas J. Tertil\textsuperscript{a}, Yoshihori Nakai\textsuperscript{a}, Osamu Hieda\textsuperscript{b}, Shigeru Kinoshita\textsuperscript{b}, Andrew J. Quantock\textsuperscript{b}, Keith M. Meek\textsuperscript{b}, Optometry and Vision Sciences, Cardiff University, Cardiff, United Kingdom; 2Diamond Light Source Ltd, Didcot, United Kingdom; 3Ophthalmology, Kyoto Prefectural Univ of Med, Kyoto, Japan.

Purpose: Microwave keratoplasty is a new non-invasive therapy for the correction of myopia and treatment of corneal ectasia. An annular pulse of microwave energy is delivered to the corneal mid-periphery, which is intended to cause localised shrinkage of anterior stromal lamellae, thereby achieving flattening of the central cornea. However nothing is known of the potential effects of the therapy on stromal collagen at the sub-lamellar level. Here we present the first evaluation of ultrastructural changes in the stromal matrix following experimental microwave keratoplasty.

Methods: At all times animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Under anaesthesia, four adult New Zealand White rabbits received 915 MHz microwave treatment unilaterally as a 3.5mm (inner) to 4.3mm (outer) diameter annulus with a Vekera KXS machine (Avedro Inc. USA). This application should treat myopia of approximately -6D. Following treatment eyes received antibiotic eye drops. The untreated contralateral eyes acted as normal controls. After three weeks animals were euthanized by intravascular injection of pentobarbital sodium (100mg/kg) and the corneas harvested and fixed in 4% paraformaldehyde to preserve collagen ultrastructure. Small-angle x-ray scattering was performed using Beamline I22 at the Diamond Light Source, UK. Data analysis provided quantitative profiles of collagen fibril spacing, diameter and axial period at 0.25mm intervals across each specimen from limbus to limbus.

Results: Collagen fibril spacing, diameter and axial period, as an average through the stromal depth, were unaffected by the therapy. In contrast a highly significant (22%-50%) increase in local fibril disorder was measured in all treated corneas. This effect was restricted to within the microwave treatment region, with normal collagen architecture retained away from the treated tissue. The treatment area displayed a visible decrease in opacity compared to the untreated tissue.

Conclusions: Microwave keratoplasty causes localised corneal opacity within the treatment area that is likely due to spatial disruption of stromal collagen at the fibrillar level, with implications for peripheral vision. The stromal fibril changes induced by the therapy may also impact on corneal biomechanics.

Commercial Relationships: None; Stan R. Morgan, None; Sally Hayes, None; Jennifer Hiller, None; Nicholas J. Tertil, None; Yoshihori Nakai, None; Osamu Hieda, None; Shigeru Kinoshita, None; Andrew J. Quantock, None; Keith M. Meek, None.

Support: MRC Grant G0600755

Program Number: 1076 Poster Board Number: A112

Presentation Time: 3:15 PM - 5:00 PM

Using Collagen Density To Restore The Quiescent Phenotype Of Keratocytes

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Purpose: Keratocytes make up the bulk of the cellular component of the corneal stroma. They usually reside between the collagen fibrils and are critical for maintaining the clarity and function of the cornea. We hypothesise that by culturing keratocytes in a 3D environment that mimics a de novo or post-injury cornea a more quiescent phenotype will be maintained.

Methods: Both Compressed and Uncompressed Collagen gels were seeded with low passage human central keratocytes and to create a 3D artificial corneal stroma 1,2. These constructs were monitored over time for changes in hydration and contraction. Cell Phenotype was quantified by qPCR and protein analysis, alamar blue were used to examine proliferation. Rheology was used to monitor changes in stiffness and TEM was used to analyse fibril organisation.

Results: Keratocytes in compressed collagen gels expressed more of the quiescence markers Lumican and Keratan at levels that are comparable with that seen in native tissue, we have demonstrated that this is due to factors inherent to the compressed collagen gel as although the uncompressed gels did contract to a similar degree of Hydration and Structure they were unable to retain cells in an undifferentiated state.

Commercial Relationships: None; John M. Tomich, None; Brett Gudden, None; Sara Reggie, None; Tamika Y. Edwards, None; Jennifer Hobden, None; Jeffery Hobden, None; Jewel Germany, None.

Conclusions: We have demonstrated that encapsulating keratocytes in compressed collagen gels induces a de-differentiation from an aggressive myo-fibroblastic cell type to a more quiescent one. The improved mechanical properties make our constructs more suitable for tissue engineering applications and provide an improved model of the stroma that recreates the native environment of the stroma better than existing models such as contracted collagen gels. References: 1. Mi, S., Chen, B., Wright, B. and Connon, C. J. (2010) Plastic compression of a collagen gel forms a much improved scaffold for ocular surface tissue engineering over conventional collagen gels. Journal of Biomedical Materials Research Part A, 95A (2), pp. 447-453. ISSN 1549-3296 2: Ex Vivo Construction of an Artificial Ocular Surface by Combination of Corneal Limbal Epithelial Cells and a Compressed Collagen Scaffolding Containing Keratocytes. Shengli Mo, Bo Chen, Bernice Wright, Che J. Connon. Tissue Engineering Part A. June 2010, 16(6): 2091-2100.

Program Number: 1077 Poster Board Number: A113
Presentation Time: 3:15 PM - 5:00 PM
Molecular Signatures In Bulk Tissue Elucidated By X-ray Spectroscopy And X-ray Microscopy: Corneal Embryogenesis
Elena Koukouda1, Giulia Veronesi2, Imran I. Patel3, Marine Cotte4, Carlo Knupp1, Francis L. Martin2, Andrew J. Quantock1. 1School of Optometry and Vision Sciences, Cardiff University, Cardiff, United Kingdom; 2European Synchrotron Radiation Facility, X-ray Microscopy Beamline ID21, Grenoble Cedex, France; 3Centre for Biophotonics, Lancaster Environment Centre, Lancaster University, Bailrigg, Lancaster, United Kingdom.
Purpose: Uncovering the chemical and physiological changes that occur during corneal embryogenesis will lead to a heightened understanding of the tightly controlled developmental events that ultimately determine the requirements for stromal light transmission and corneal transparency. The current study aims to develop techniques for the analysis of bulk tissue, and to explore physico-chemical variations in corneal embryogenesis.
Methods: A combination of synchrotron scanning X-ray fluorescence microscopy and X-ray absorption near-edge structure spectroscopy were utilized for the investigation of, respectively, the chemical composition and sulfur speciation in the developing chick corneas at embryonic days 12, 14 and 16. Derived data was subjected to computational analysis in the form of principal component analysis followed by linear discriminant analysis.
Results: Observable differences in the elemental and sulfur species composition were observed over the developmental period, embryonic day 12 to embryonic day 16. The molecular signatures of chlorine, potassium, calcium, phosphorus and sulfate were found to be distinct with development. Notably, developmentally regulated alterations in thiols, organic monosulfides, ester sulfate and inorganic sulfate were observed in the developing chick.
Conclusions: Our findings establish the potential application of X-ray fluorescence microscopy and X-ray absorption near-edge structure spectroscopy for the analysis of bulk tissue and highlight their applicability in the life sciences. The chemical compositional and sulfur speciation alterations reported here provide a deeper understanding of the molecular basis of corneal embryogenesis during the transition from an immature opaque cornea to a mature transparent tissue. This study revealed transient molecular signatures and compositional changes which correlate with changes in corneal structure-function relationships.

Support: EPSCR Grant EP/F034970/1 and Cardiff University President's Research Scholarship

Program Number: 1078 Poster Board Number: A114
Presentation Time: 3:15 PM - 5:00 PM
Photodynamic Therapy Triggers Expression Of Haemopoietic Stem Cell Marker Cd34 Of Keratocytes
Nora Szentmary1, Tanja Stachon1, Jiong Wang2, Timo Eppig1, Achim Langenbucher2, Markus Bishoff1, Hans-Jochen Foth2, Berthold Seitz2. 1Department of Ophthalmology, Saarland University Hospital, Homburg/Saar, Germany; 2Department of Ophthalmology, Remun Hospital of Wuhan University, Wuhan, China; 3Experimental Ophthalmology, University of Saarland, Homburg, Germany; 4Institute of Medical Microbiology and Hygiene, University of Saarland, Homburg/Saar, Germany; 5Department of Physics, Technical University of Kaiserslautern, Kaiserslautern, Germany.
Purpose: Photodynamic therapy (PDT) may be a potential treatment alternative in therapy resistant infectious keratitis. PDT may eliminate the microorganisms from the infected cornea by damages caused through free oxygen radicals, or even by supporting different stages of activation of keratocytes and inflammatory cell response. The purpose of this study was to determine the impact of PDT on activation of human keratocytes in culture.
Methods: Primary human corneal keratocytes were isolated by digestion in collagenase A (1 mg/ml) from human corneal buttons, and cultured in DMEM/Ham’s culture medium supplemented with 10% FCS. Keratocytes underwent illumination (670 nm) for 13 minutes following exposure to 0, 50, 100, 150, 200 and 250 nMol/ml concentrations of photosensitizer chlorin e6 (Ce6) in the culture medium. The day after treatment α-smooth-actin and CD34 expression of the cells was analysed using flow-cytometry (FACS).
Results: Using Ce6 or illumination only, expression of α-smooth-actin or CD34 of the cells did not change significantly. Twenty-four hours after PDT the percentage of α-smooth-actin positive keratocytes decreased significantly (p<0.05) and the percentage of CD34 positive cells increased significantly (p<0.05) at concentrations higher than 50 nMol/ml of Ce6. Following PDT the percentage of α-smooth-actin/CD34 positive cells was 81/20% using 50 nMol/ml, and 40/71% using 250 nMol/ml concentrations of Ce6.
Conclusions: As a short term effect, photodynamic therapy seems to activate the dendritic interstitial cell system and inhibit myofibroblastic transformation of keratocytes in vitro.

Support: None
Purpose: Because most of GFP+ immunocompetent cells were depleted by the second x-ray-irradiation, GFP+ cells in the cornea are considered to be non-immunocompetent cells. Thus, it appears that BM-derived cells differentiate into non-immunocompetent cells in the cornea.

Conclusions: Bone marrow (BM) cells from C57BL/6 green fluorescent protein (GFP) transgenic +/− BM cells were intravenously injected into C57BL/6 wild type (WT) recipient mice that were x-ray-irradiated and received BM cells from C57BL/6 WT mice (2nd BMT mice). Three weeks later, the 2nd BMT mice were sacrificed and corneas and spleens were harvested for immunofluorescent microscopic and flow cytometric analyses, respectively.

Results: The percentage of GFP+ cells out of whole splenocytes was less than 1% in 2nd BMT mice. In contrast, many GFP+ cells were detected in the cornea of the 2nd BMT mice. These cells distributed abundantly in the limbus and less in the peripheral and central cornea. These cells existed in the stroma but not in the epithelial and endothelial layers.

Conclusions: Because most of GFP+ immunocompetent cells were depleted by the second x-ray-irradiation, GFP+ cells in the cornea are considered to be non-immunocompetent cells. Thus, it appears that BM-derived cells differentiate into non-immunocompetent cells in the cornea.

Materials and Methods: In order to test the hypothesis that corneal keratocytes express Rac1 and GTPases, rabbit corneal keratocytes were subjected to immunostaining for myofibroblast marker alpha-smooth muscle actin (αSMA), and Tunel assay for apoptosis.

Results: Myofibroblasts were induced in vitro in 3D collagen matrices in response to the Rac1 inhibitor NSC23766 (50 μM) and/or the Rho kinase inhibitor Y-27632 (10 μM). The density of SMA+ cells was significantly lower in the Sirolimus group compared to control and untreated tissues. The density of SMA+ cells out of whole splenocytes was less than 1% in 2nd BMT mice.

Conclusions: Rabbit corneal keratocytes were seeded within 3D collagen matrices that were compacted using external compression. Buttons (6 mm diameter) were subjected to immunostaining for myofibroblast marker alpha-smooth muscle actin (αSMA), and Tunel assay for apoptosis.

Results: Rabbin keratocytes were seeded within 3-D collagen matrices that were compacted using external compression. Buttons (6 mm diameter) were punched out and cultured in serum-free media, PDGF BB, IGF, TGFβ1 or TGFβ3, with or without the Rac1 inhibitor NSC23766 (50 μM) and/or the Rho kinase inhibitor Y-27632 (10 μM). After 1 to 4 days, cells were labeled for F-actin and α-SM-actin, and imaged using confocal microscopy.

Results: Corneal keratocytes in basal media within compressed matrices exhibited transplantation, the BMT mice were again lethally x-ray-irradiated and received BM cells from C57BL/6 WT mice (2nd BMT mice). Three weeks later, the 2nd BMT mice were sacrificed and corneas and spleens were harvested for immunofluorescent microscopic and flow cytometric analyses, respectively.
a broad, convoluted cell body and thin dendritic processes. Exposure to PDGF and IGF produced keratocyte elongation, without inducing stress fiber formation. In contrast, cells cultured in FGF2 lost their dendritic extensions and developed intracellular stress fibers. TGFβ induced formation of stress fibers expressing α-smooth muscle actin, suggesting a myofibroblastic phenotype. Addition of NSC23766 to basal media (Fig. 1), PDGF (Fig. 2) or IGF resulted in a loss of dendritic processes and formation of stress fibers at both 1 and 4 days of culture. NSC23766 also enhanced stress fiber formation in FGF, and increased the proportion of cells expressing α-SM-actin in TGFβ. Treatment with Y-27632 blocked the induction of stress fibers under all conditions studied.

Conclusions: Taken together, the results suggest that Rac1 plays a critical role in maintaining the quiescent corneal keratocyte phenotype, whereas Rho kinase is required for fibroblastic and myofibroblastic transformation of these cells.
Amniotic Membrane Inhibits The Expression Of TLR3, IFN-beta And IL-8 In Corneal Limbal Fibroblasts

Rodrigo Bolaños 1, Jessica Nieves 1, Alejandro Navas 2, Enrique O. Graue 2, Alfredo Domínguez 1, Yonathan Garfias 1. 1Research Unit, Institute of Ophthalmology Conde de Valenciana, Mexico City, Mexico; 2Cornea and Refractive Surgery, Conde de Valenciana, Mexico City, Mexico.

Purpose: The innate immune Toll Like Receptor-3 (TLR3) recognizes double-stranded RNA throughout a viral replication process. The synthesis of cytokines such as IFN-beta and IL-8 is induced by the TLR3-ligand poly I:C. It has also been demonstrated that TLR3 activation drives apoptosis on viral infected cells. The amniotic membrane (AM) exerts anti-inflammatory and immunomodulatory effects and promotes the re-epithelialization in corneal diseases. Although several anti-inflammatory and immunomodulatory mechanisms of the AM have been described, its role in the inhibition of innate immunity receptors such as TLR3 is unknown. The aim of this study was to analyze the effect of AM on corneal limbal fibroblasts (CLF) TLR3 expression and to determine whether AM is capable to inhibit the
Purpose: Transforming growth factor (TGF)-β1-induced increases in IL-6 release and 6-smooth muscle actin (SMA) expression in corneal fibroblasts require activation of the transcription factor Smad2/3 and the potential activation of Smad1/5/8 (Yao, Y et al, American Journal of Pathology 2011). This study examined if this dependence involves increases in reactive oxygen species (ROS) production and modulation of SMAD2 phosphorylation.

Methods: Stromal fibroblasts were isolated from fresh human cadaveric corneas (New York Upstate Transplant Service) and cultured following a described method. Flow cytometry assessed ROS production. TGF-β1 and H2O2-induced Ca2+ transients were determined by image analysis, α-SMA expression and SMAD2 phosphorylation by immunoblotting and the effect of TGF-β1 on IL-6 release by ELISA.

Results: TGF-β1 (1 ng/ml) treatment for 1 h increased ROS production by 1.5-fold. A ROS scavenger, N-acetyl-L-cysteine (10 mM), or the NADPH oxidase inhibitor, diphenylene iodonium (10 μM), abolished a TGF-β1-induced persistent SMAD2 phosphorylation (15 min and 16 h) as well as increases in α-SMA expression and IL-6 release. TGF-β1 and H2O2 (0.5 mM) induced Ca2+ transients, which were attenuated by capsaizine (CPZ, 10 μM), a selective TRPV1 antagonist. Similarly, either CPZ or withdrawal of extracellular Ca2+ inhibited TGF-β1-induced p-SMAD2 formation. TRPV1 siRNA gene silencing also suppressed TGF-β1-induced increases in SMAD2 phosphorylation, IL-6 release and α-SMA expression (vs. effects on cells transfected instead with scrambled siRNA).

Conclusions: SOF-induced increases in α-SMA expression and IL-6 release in human corneal fibroblasts depend on ROS formation and subsequent, TRPV1-dependent, Ca2+ spikes. Increases in ROS formation driven by TGF-β1 transactivate TRPV1, which in turn contributes to SMAD2 phosphorylation.

Commercial Relationships: Yuanquan Yang, None; Hua Yang, None; Zheng Wang, None; J Mario Wolosin, None; Peter S. Reinach, None

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Thrombin Increases Human Corneal Fibroblast Expression Of The Chemokines, CXCL1, CCL2, IL-6, IL-8, CXCL10 And CCL13
Sally S. Twining, Debra J. Warejcka. Biochemistry and Ophthalmology, Medical College of Wisconsin, Milwaukee, WI.

Purpose: Proteins of the coagulation cascade are synthesized by corneal cells and result in the activation of prothrombin to thrombin after injury to the epithelial layer or the stroma. Thrombin increases proinflammatory chemokines and cytokines such as CXCL1, CCL2, IL-6 and IL-8 in many tissues but has not been studied in the corneal fibroblasts. The purpose of this study was to determine whether thrombin increases human corneal stromal fibroblast expression of these chemokines plus CXCL10, an antiangiogenic chemokine, and CCL13, a proinflammatory chemokine which have not been previously studied in the presence of thrombin.

Methods: Stromal cells were isolated from three pairs of human donor corneas and were expanded in culture with treated with 1 ng/ml fibroblast growth factor (FGF). After seven days, cells were treated with 0.1 or 1 unit/ml thrombin for 1 hour (for mRNA) or 12 hours (for ELISA). RNA was purified and primers for CXCL1, CCL2, IL-6, IL-8, CXCL10, CCL13 and GAPDH were used in real time PCR. Twelve hour supernatants were assayed by ELISA for protein levels of the various chemokines.

Results: One unit/ml thrombin increased mRNA expression in stromal fibroblasts for IL-6 (15 fold), IL-8 (7 fold), CXCL1 (6 fold), and CCL2 (7 fold) over untreated cells. Expression of message for CXCL10 was increased from 10 fold and CCL13 mRNA increased only 2 fold when cells were treated with 1 unit/ml thrombin. Treatment of cells with thrombin increased protein expression of IL-6 (from 5 to 520 pg/ml), IL-8 (from 7 to 30 pg/ml), CXCL1 (from 17 to 100 pg/ml), CCL2 (from 1586 to 9888 pg/ml), CXCL10 (from 3 to 17 pg/ml) and CCL13 (from 0 to 14 pg/ml).

Conclusions: As expected, thrombin treatment of corneal fibroblasts increased expression of proinflammatory chemokines such as CXCL1, CCL2, IL-6 and IL-8. Expression of CXCL10 and CCL13 mRNA and proteins were also increased by thrombin treatment of stromal fibroblasts. Regulation of CXCL10 and CCL13 by thrombin has not been previously described. These results suggest that thrombin generated in response to corneal injury can regulate the immune response.

Commercial Relationships: Sally S. Twining, None; Debra J. Warejcka, None
Support: NIH Grant EY017855
Program Number: 1092 Poster Board Number: A128
Presentation Time: 3:15 PM - 5:00 PM
Impact Of Photodynamic Therapy (PDT) On Viability, Apoptosis And Proliferation Of Human Keratocytes In Vitro
Jiong Wang1, Tanja Stachon2, Markus Bischoff3, Hans-Joachim Foth1, Timo Eppig1, Achim Langenbucher1, Berthold Seitz4, Nöra Szentmáry1, Karl E. Kadler1, None; Andrew J. Quatrock, None
Support: EPSRC project grant EP/F034970 (AJQ); Wellcome Trust programme grant (KEK)

Program Number: 1093 Poster Board Number: A129
Presentation Time: 3:15 PM - 5:00 PM
The Impact Of Col6a2 Gene Mutations On Mouse Corneal Stromal Architecture
Frances E. Jones1, Huan Meng2, Robert D. Young1, Albert S. Jun2, Andrew J. Quatrock1, Optometry & Vision Sciences, Cardiff University, Cardiff, United Kingdom; Wilmer Eye Institute at Johns Hopkins, Baltimore, MD.
Purpose: Fuchs endothelial corneal dystrophy (FECD) is one of the leading causes of corneal vision loss. Missense mutations (L50W and Q455K) in the COL6A2 gene, which encodes the alpha-2 collagen VIII subunit of the procollagen molecule, are associated with early onset FECD in humans. The purpose of this study was to investigate how homologous mutations in the mouse Col6a2 gene might affect the architecture of the corneal extracellular matrix.
Methods: The corneas of 5-month-old mice with Col6a2*Q455K mice were stained with Cuprolinic blue and examined by transmission electron microscopy, along with those of age-matched wild type mice. Corneas were categorized into enzige groups: i) keratanase, ii) chondroitinase ABC, and iii) non-enzymite treated.
Post-operative human FEDC tissue, kindly provided by Peter Watson, FRCOphth, Cambridge, UK, was similarly examined.
Results: Electron micrographs of Col6a2 mutant mice revealed that the structural relationships between the water-binding proteoglycans (PGs) in the corneal stroma - keratan sulfate and chondroitin/dermatan sulfate - and the tissue hybrid collagen fibrils were unchanged by the mutation. The distribution of PGs was similar between the mutant and wild type mice.
Conclusions: The structural distribution of PGs in the mouse corneal stroma closely resembles that in human FECD stroma, although there were signs of mild basement edema in the posterior stroma of the mutant mice, which may be a characteristic of edema commonly observed in patients with late onset FECD. No widespread corneal edema was evident in Col6a2 mutant mice, although the occasional focal area of edema in Col6a2*Q455K was similar in the mutant mice to the condition in human FECD.

Program Number: 1094 Poster Board Number: A130
Presentation Time: 3:15 PM - 5:00 PM
The Role Of Cell Aggregation And Division And The Expression Of Extracellular Matrix During Human Keratocyte Sphere Formation
Purpose: Human keratocytes and progenitor cells can form spheres in serum-free culture conditions and maintain the expression of keratocyte-specific markers. The aim of this study was to determine the role of cell migration/aggregation and division in keratocyte sphere formation and the associated gene, protein and glycoprotein expression.
Methods: Stromal cells were isolated from human corneal rims and cultured in a serum-free sphere-forming culture system. The mechanisms and temporal sequence of sphere formation were investigated using live-cell, darkfield, scanning electron and confocal imaging. Live keratocytes were labelled and tracked using quantum dots, azito labelled sugars and nucleotides and investigated using immunocytometry to monitor collagen subtype and glycoprotein expression in conjunction with cell division during sphere formation. Isolated cells were sorted using fluorescence activated cell sorting and after sphere formation and gene expression was analysed using quantitative microfluidic arrays and real time PCR.
Results: Serum-free culture of keratocytes demonstrated early sphere formation by day 7. Spheres formed predominantly by cell migration and aggregation with less than 10% of cells dividing by day 7, although, as sphere formation progressed, cell proliferation played an increasing contribution in late sphere development with 30% of cells dividing by day 10. Primary spheroid cultures remained stable for up to 6 months and demonstrated up-regulated expression of extracellular matrix genes including the stromal collagen subtypes and proteoglycans including keratan. Immunohistochemistry confirmed expression of collagen subtypes and keratan in cultured spheres and deposition of azito labelled glycoproteins were detected during early and late sphere formation.
Conclusions: Human keratocytes can be isolated and cultured using a sphere-based
Chronic Keratitis With Intrastromal Epithelial Histiocytosis: A New Finding In Muckle-wells Syndrome

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Purpose: To report a new corneal finding in Muckle-Wells syndrome (MWS) and its subsequent treatment.

Methods: A 45 year-old woman with a history of MWS presented with progressive bilateral stromal scarring that had been worsening over the past year. The left eye, which had more severe stromal scarring and a Best Spectacle-Corrected Visual Acuity (BSCVA) of hand motion, had a history of cataract removal with lens implantation and trabeculectomy two years prior for open-angle glaucoma. The vision in the right eye was 20/40.

Results: The patient underwent penetrating keratoplasty in the more severely affected left eye. Six months postoperatively the patient had a BSCVA of 20/40. Pathologic specimen demonstrated a chronic keratitis with focal calcification of Bowman’s layer, localized Descemet’s membrane breaks, marked endothelial loss, and pronounced intrastromal epithelial histiocytoses without evidence of amyloidosis.

Conclusions: To the best of our knowledge, this case highlights a new association with MWS. Epitheloid histiocytosis has been demonstrated in other parts of the body in patients with MWS including the skin and joints, although it is generally accompanied by amyloidosis.

Commercial Relationships: Ian Gorovoy, None; Mark Gorovoy, None; Jaclyn Gorovoy, None

Support: None

Program Number: 1098 Poster Board Number: A134
Presentation Time: 3:15 PM - 5:00 PM

Mapping Collagen Orientation Changes In The Corneas Of Blindness-Enlarged Globe (beg) Chicken Mutants

Sian R. Morgan1, Craig Boote2, Erin P. Dooley1, Paul M. Hocking1, Chris F. Inglehearn1,2, Manir Ali1,2, Thomas Sorensen1, Keith M. Meek1.

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Purpose: The beg mutant chicken is a model system for studying retinal degeneration, and has been used extensively to study the pathogenesis of blindleness-enslarged globe (beg) phenotype, an inherited autosomal recessive condition in chickens. The beg phenotype is characterised by retinal dystrophy and blindness at hatch, with secondary globe-enlargement and loss of corneal curvature by age 3–4 months.

Methods: Wide-angle x-ray scattering (WAXS) data were collected from four homozygous beg chick corneas at 1, 3 and 9 months post-hatch, using station I02 at the Diamond Light Source synchrotron facility (Didcot, UK). Maps showing the orientation of corneal collagen and its relative mass distribution at 1, 3 and 9 months post-hatch were quantified from WAXS patterns collected at 0.5 mm intervals across each specimen. Correlation analysis was performed on the fibril orientation distribution functions in order to detect differences in collagen alignment in beg mutants compared to previously characterised age-matched controls.

Results: WAXS results disclosed alterations in the bulk alignment of collagen lamellae in the beg chick cornea, compared to age-matched controls. These changes accompanied eye globe enlargement and corneal flattening in affected birds, and were manifest as a progressive loss of circumferential collagen alignment in the peripheral cornea and limbus in birds older than 1 month.

Conclusions: Our data supports the hypothesis that the limbal fibril annulus is important in corneal shape preservation. We propose that corneal flattening in beg chickens is related to biomechanical changes brought about by an alteration in collagen arrangement at the corneal periphery. The beg chicken is a valuable animal model for investigating how pathological changes in collagen fibril arrangement impact on tissue form and function.

Commercial Relationships: Sian R. Morgan, None; Craig Boote, None; Erin P. Dooley, None; Paul M. Hocking, None; Chris F. Inglehearn, None; Manir Ali, None; Thomas Sorensen, None; Keith M. Meek, None

Support: MRC grant G0600755

Program Number: 1099 Poster Board Number: A135
Presentation Time: 3:15 PM - 5:00 PM

Penetration of the photosensitizer chlorin e6 into the cornea for Photodynamic Inactivation in infectious keratitis

Carole Simon1, Georg Wolf1, Jochen Foth1,2, Jochen Severins1,2, Valeria Castelletto3, Craig Boote1,2, Erin P. Dooley1,2, Paul M. Hocking1,2, Chris F. Inglehearn1,2, Manir Ali1,2, Thomas Sorensen2, Keith M. Meek2.

1Department of Physics, University of Kaiserslautern, Kaiserslautern, Germany; 2Department of Physics, University of Reading, Reading, United Kingdom; 3Department of Physics, University of Leeds, Leeds, United Kingdom.

Purpose: We explored the penetration of the photosensitizer chlorin e6 into the cornea for Photodynamic Inactivation in Infectious Keratitis (PAI).

Methods: Pictures were captured with an imaging system using a structured illumination microscope (SIM) and microscope (Olympus IX71) to visualize penetration of PA. A 195 nm excitation wavelength was used at 10 mW of power. The penetration and distribution of the photosensitizer chlorin e6 into the cornea for Photodynamic Inactivation in Infectious Keratitis (PAI).

Results: The penetration of the photosensitizer chlorin e6 into the cornea for Photodynamic Inactivation in Infectious Keratitis (PAI) was visualized. The penetration and distribution of the photosensitizer chlorin e6 into the cornea for Photodynamic Inactivation in Infectious Keratitis (PAI) was visualized. The penetration and distribution of the photosensitizer chlorin e6 into the cornea for Photodynamic Inactivation in Infectious Keratitis (PAI) was visualized.

Conclusions: PAI is a promising therapy for infectious keratitis.

Commercial Relationships: None

Support: None

Program Number: 1086 Poster Board Number: A132
Presentation Time: 3:15 PM - 5:00 PM

Program Number: 1096 Poster Board Number: A132
Presentation Time: 3:15 PM - 5:00 PM
Role of the JNK Signaling Pathway in Down-Regulation of Connexin43 by TNF-α in Human Corneal Fibroblasts

Takashi Toriyama, Kazuhito Kuroda, Koh-Hei Sonoda. Ophthalmology, Yamaguchi University, Ube, Japan.

Purpose: Corenlar fibroblasts are connected to each other via gap junctions and contribute to corneal neovascularization. We previously showed that Tumor necrosis factor (TNF)-α, a proinflammatory cytokine, down-regulates connexin43 (Cx43), gap junctional protein, and then inhibits gap-junctional intercellular communication (GJIC) in corneal fibroblasts. The authors examined the role of MAPK signaling pathway in the TNF-α-induced down-regulation of Cx43 in these cells.

Methods: Cultured corneal fibroblasts were exposed to TNF-α in the absence or presence of inhibitors of mitogen-activated protein kinase (MAPK) signaling pathways.

Results: The effects of TNF-α on Cx43 expression were attenuated by an inhibitor of c-Jun NH2-terminal kinase (JNK) inhibitor II but not by inhibitors of signaling by extracellular signal-regulated kinase (ERK) or p38 MAPK (PD90859). JNK inhibitor II also attenuated the inhibitory effect of TNF-α on GJIC and distribution of Cx43.

Conclusions: The inhibitory effects of TNF-α on Cx43 expression and GJIC are mediated, at least in part, by the JNK signaling pathway. JNK signaling pathway in corneal fibroblasts may play an important role in corneal inflammation.

Commercial Relationships: Takashi Toriyama, None; Kazuhito Kuroda, None

Support: None
**Purpose:** For a successful treatment of infectious keratitis Photodynamic Inactivation (PDI) of bacteria presents a novel alternative to conventional antibiotic therapy. To evaluate the possibility of PDI for bacterial pathogens in the cornea the diffusion of the photosensitizer (PS) chlorin e6 (C6e) was assessed in individual layers of corneal tissue in porcine eyes.

**Methods:** Chlorin e6, dissolved in sodium chloride, was evenly distributed on to porcine corneas using a gel forming agent (sodium polyacrylate). Series of measurements with different contact times and concentrations were carried out. After removal of the photosensitizer, tissue sections (8 μm) were prepared and investigated by fluorescence microscopy with an excitation wavelength of 405 nm. With this set-up, fluorescence intensity at 670 nm was spatially resolved whereas the penetration depth could be determined. To simulate the penetration in diseased or injured cornea tissue the epithelium was removed before application of chlorin e6. In addition, chemical and thermal modifications on the surface of the cornea were induced to analyze the impact on diffusion processes of the photosensitizer.

**Results:** After an exposure period of 15 min and concentrations in the region of 400 μm the photosensitizer was sufficiently absorbed for PDI of bacteria. Chemical injuries, such as acid and base, implied an increase of the penetration depth into the tissue. From the measured values of fluorescence intensity it was possible to determine the maximum concentration reached in the stroma. It turned out that this concentration almost reached the applied concentration. As a result of measurements on the diffusion of the PS in cornea the diffusion coefficients of chlorin e6 were calculated.

**Conclusions:** Fluorescence measurements were used to determine accurately the penetration depth and the concentration of the PS chlorin e6 into the cornea. In the real treatment situation, the epithelium has to be removed. Under those conditions concentrations > 1 mM of chlorin e6 were sufficient to reach a tissue penetration of the active ingredient to the depth of ~ 500 μm.

**Commercial Relationships:** Carole Simon, None; Georg Wolf, None; Dirk Hüttenberger, ApexCare Pharma (E); Hans-Jochen Foth, None; Berthold Seitz, None

**Support:** None

**Program Number:** A136
**Presentation Time:** 3:15 PM - 5:00 PM
**Distinctive Effects Of Tgfβ1, Fgf2, Igf1 And Igf2 On Growth And Extracellular Matrix Gene Expression By Primary Human Corneal Keratocytes**

**Sheri-Gae Scott, Shakti Chakravarti.** Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

**Purpose:** The keratocytes are specialized mesenchymal cells, central to corneal homeostasis. Their growth and abilities to express extracellular matrix (ECM) genes during corneal development and repair are regulated by growth factor signals. Here we tested the effects of TGFβ1, FGF2, IGF-I and IGF-II on primary human keratocytes with respect to growth and expression of collagens and proteoglycans.

**Methods:** Keratocytes, isolated by serial collagenase I (Sigma) digestion from transplant-unusable human corneas (Tissue Banks International) were cultured in DMEM/F12 with insulin, selenium, transferrin (ITS) and phosphoascorbic acid (2011, Exp. Eye Res.). After an initial 6 day period keratocytes were treated with 1) FGF2 10ng/ml, 2) TGFβ1 2ng/ml, 3) FGF2 and TGFβ1, 4) IGF (2011, Exp. Eye Res.). After ITS depletion the keratocytes were treated with 1) DMEM/F12 with insulin, selenium, transferrin (ITS) and phosphoascorbic acid superseded by 2) DMEM/F12+ITS and appeared fibroblastic in TGFβ1. The mesenchymal origin of keratocytes was confirmed by VIM expression and lack of epithelial KRT12. In ITS-DMEM/F12 cell growth increased by 3-fold after 24h of plating; growth was uninhibited by TGFβ1, but inhibited by FGF2. Cell growth was promoted by IGF-I and inhibited by IGF-II. Profibrogenic TGFβ1 promoted a 5-6 fold increase in COLS1A1 and BDNF transcripts relative to 18s RNA, while FGF2 counteracted these increases. FGF2 and TGFβ1, alone or together blocked COLS1A1 expression. TGFβ1 inhibited DCN expression by 3-fold while FGF2 had no detectable effect. Expression of LUM was decreased slightly by TGFβ1, unaffected by FGF2 and promoted by IGF-I.

**Conclusions:** The growth and profibrogenic effects of TGFβ1 were counteracted by FGF2. Insulin and IGF are known to promote somatic cell growth; the unexpected decrease in growth by IGF-II may be related to its higher affinity for IGF-IR/M6P and its pro-apoptotic functions.

**Commercial Relationships:** Sherrigae Scott, None; Shakti Chakravarti, None

**Support:** EY11654

**Program Number:** A137
**Presentation Time:** 3:15 PM - 5:00 PM
**Keratocyte Gene expression in the normal central cornea**

**Gao Scott, Yu Liu, Hirutu Goung, Sun Song, Guradam A. Luty, Jonathan Song, Deepak P. Edward.** Ophthalmology, Wilmer Eye Inst, Johns Hopkins Univ, Baltimore, MD; King Khaled Eye Specialists Hospital, Riyadh, Saudi Arabia.

**Purpose:** There is limited information on region specific gene expression in the human corneal stroma. In this study we used laser capture microdissection (LCM) to investigate the expression profile of extracellular matrix and adhesion molecules in the central human cornea.

**Methods:** Frozen sections of human cornea without ocular diseases preserved in Optisol-GS solution were used to isolate the central corneal stroma by LCM. RNA was extracted from LCM captured stroma tissues. The RT2 Profiler PCR Arrays (SA Biosciences) were used to examine the expression profile of extracellular matrix and adhesion molecules; Real-time quantitative (Q) PCR were used to quantify gene expression. The specificity of PCR products were determined by melting curve and agarose gel.

**Results:** An average 10798 +/- 6896 keratocytes were isolated from the central corneal stroma by LCM. The RNA and cDNA were successfully prepared from the LCM-isolated keratocytes, and the quality was validated by the housekeeping gene β-actin. The gene expression profiling demonstrated that normal keratocytes in the central stroma reliably expressed 37 out of the 84 extracellular matrix and adhesion molecules represented in the array. The six mostly abundantly expressed genes and their relative expression against the housekeeping gene GAPDH were: COL6A2 (105 ±1.2%±6.4%); COL2A1 (93.6 ±57.2%±6.5%); TIMP2 (24.5 ±275.8%±2.8%); TGFβ1 (456.6 ±139.6%±77.5%); TIMP1 (405.6 ±234.2%±34%); ECM1 (311.6 ±137.0%±4%)

Some of the more common ECM proteins in the stroma such as collagen I and other proteoglycans were not among the highly expressed genes.

**Conclusions:** LCM followed by analysis of gene expression profiles is an excellent approach to discover the regional gene expression in the corneal stroma. These six highly abundant expressed genes could potentially function as a network of genes involved maintenance of corneal matrix integrity and clarity and could be potentially altered in corneal diseases.

**Commercial Relationships:** Samuel C. Yu, None; Hu Huang, None; Guoying Sun, None; Gerard A. Luty, None; Jonathan Song, None; Deepak P. Edward, None

**Support:** In part by an unrestricted grant from Research to Prevent Blindness, New York, NY to the Wilmer Eye Institute.

**Program Number:** A138
**Presentation Time:** 3:15 PM - 5:00 PM
**IOP, CCT, corneal thinnest point and RNFL Thickness In Eyes With Keratocous before and after corneal transepithelial cross-linking**

**Pietro Biondi, Michele Reibaldi, Teresio Avitabile, Maurizio G. Uva, Cristina Cassar Scalia, Mario D. Toro, Silvio Zagarì, Marco Zagarì, Martina Battaggia, Livio M. Franco.** Ophthalmology, Policlinico G Rodolfo Hospital, Catania, Italy.

**Purpose:** To evaluate the intraocular pressure (IOP), the central corneal thickness and the papillary retinal nerve fiber layer (RNFL) obtained by Spectralis domain optical coherence tomography (OCT) in eyes with keratoconus, before and after transepithelial cross-linking.

**Methods:** In twenty-nine eyes of 29 patients (18 male, 11 female, mean age 30 ± 6.5) affected with stage I-II keratoconus (9 eyes stage I, 15 eyes stage II, 5 eyes stage III) that underwent transepithelial corneal cross-linking, IOP was measured by Goldmann application tonometer, CCT and corneal thinnest point were measured by Orbscan II (Bausch & Lomb, Salt Lake City, UT, USA) and peripapillary RNFL thickness was evaluated with Spectralis spectral domain OCT (Heidelberg Engineering, Heidelberg, Germany). Measurements were repeated before the treatment and after the treatment at the 1, 3, 6 months; for OCT measurements the follow-up function was used.

**Results:** Intraocular pressure increased at 6 months; value was at baseline at 11.9±2 mmHg, and at 6 months 13.1±1.7 mmHg (ANOVA p=0.004, Tukey Kramer p<0.01 vs baseline).

No significant change were found in Central Corneal Thickness (baseline 459±48 microns), in corneal thinnest point (baseline: 431±50 microns), and in all sectors of RNFL thickness (average value: 95±8 microns).

**Conclusions:** Transepithelial corneal cross-linking does not affect values of CCT, corneal thinnest point and RNFL thickness; increased IOP values were found after six months by Goldmann application tonometry.

**Commercial Relationships:** Pietro Biondi, None; Michele Reibaldi, None; Teresio Avitabile, None; Maurizio G. Uva, None; Cristina Cassar Scalia, None; Mario D. Toro, None; Silvio Zagarì, None; Marco Zagarì, None; Martina Battaggia, None; Livio M. Franco, None

**Support:** None

**Program Number:** A139
**Presentation Time:** 3:15 PM - 5:00 PM
**Life Outside Fleischer's Ring: Pathological Changes in Peripheral Keratonic Corneas**

**Deepak P. Edward, Samuel C. Yiu, Jonathan Song, Guradam A. Luty, Samuel C. Yiu, Guradam A. Luty.** Ophthalmology, Wilmer Eye Inst, Johns Hopkins Univ, Baltimore, MD; King Khaled Eye Specialists Hospital, Riyadh, Saudi Arabia.

**Purpose:** Life outside Fleischer’s ring in keratoconus has been studied with respect to its clinical significance. However, the histological and morphometric characteristics of the pathologic changes of the peripheral stroma are not well characterized.

**Methods:** To characterize the histological and morphometric changes of the peripheral stroma in keratoconus, we conducted an electron microscopic study of the peripheral stroma of the corneas from 20 keratoconus patients and 8 normal subjects.

**Results:** The epithelium and Bowman’s layer were preserved in all cases. The stromal kerocytes were smaller in keratoconus cases and the kerocyte-to-stroma ratio was higher than in normal subjects. The collagen fibrils in keratoconus were thinner and more disorganized than those in normal subjects.

**Conclusions:** The histological and morphometric changes of the peripheral stroma in keratoconus are characterized by smaller kerocytes, thinner collagen fibrils, and increased kerocyte-to-stroma ratio. These changes may contribute to the pathogenesis of keratoconus.

**Commercial Relationships:** Guradam A. Luty, None; Samuel C. Yiu, None

**Support:** None
**Purpose:** To quantify the histopathological changes to the anterior limiting lamina (ALL) and the epithelial basement membrane (BM) in keratoconic (Kc) corneas peripheral to the Fleischer ring, and to determine if a correlation exists between them. A second purpose was to search for histopathologic clues to prominent nerves in the Kc peripheral cornea.

**Methods:** Five surgically removed Kc corneal buttons (age: 16-71) and one control cornea (age: 43) were prepared for light and transmission electron microscopy (TEM) using a previously established protocol. Five pictures per cornea were taken of both ALL and the BM with TEM at 4200x and 16500x respectively. Two measurements were taken per micrograph using a millimeter rule and adjusted to scale. Average values were calculated and compared.

**Results:** Kc ALL thickness ranged from 5.68μm to 10.58μm (Mean 8.22μm) and BM thickness ranged from 0.19μm to 0.69μm (Mean 0.49μm). Mean control ALL thickness was 9.7μm and BM thickness was 0.16μm. No correlation was found between thinned ALL and thickened BM (R^2 = 0.0871). Nerve fibers with a thickened or incomplete BM and an abnormal Schwann cell and axon relationship were observed in the stroma.

**Conclusions:** The thickened BM and thinned ALL reported here supported an earlier report that these layers are involved in the progression of Kc in ALL in the peripheral cornea. However, the lack of correlation between changes in these two layers suggested independent pathological processes. Stromal nerve fibers observed were abnormal and could explain the reduced sensitivity in Kc patients.


**Support:** None. P30 EY007551; T35 EY007088

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**Purpose:** To investigate the influence of UVA-irradiance, dose and exposure time in eradication of bacteria by using collagen cross-linking method.

**Method:** Dilutions of a Staphylococcus epidermidis strain (ATCC12228) 2.5 x 10^7/ml was used in the experiments with riboflavin (SIGMA, R7649) and UVA (365 nm) using Collagen Cross-Linking lamps. The experiments showed that eradication of β-lactam-resistant Staphylococcus epidermidis with two different time of imbibition: 30 minutes and 2 hours. All samples were prepared for the detection of keratocyte apoptosis by TUNEL assay and for the morphological evaluation of epithelium and stroma by immunohistochemical analysis (β Catenin, Connexin 43, CD34, collagen I).

**Results:** Normal corneas exhibited no TUNEL positive keratocytes while keratoconus and transepithelial crosslinked corneas showed moderate apoptotic cell in the anterior part of the stroma. In cross-linked corneas CD34-positive keratocytes were regularly distributed throughout the whole cornea stroma as in the control, and keratocytes was associated with patchy loss of immunoreactivity. Moreover, the samples treated with transepithelial crosslinking 2 hours before PKP showed an almost completely deteriorated epithelium with TUNEL positive cells. The epithelial positivity for connexin 43 and β catenin was very similar in the control and in the corneas with crosslinking 3 months before PKP, while seemed more scattered in the keratoconus. In the samples treated with transepithelial crosslinking 2 hours before PKP the positivity was patchy in the few remained epithelial cells.

**Conclusions:** The treatment with transepithelial crosslinking leads to epithelial damage and a reduction of keratocytes in the sub-epithelial region in the corneas treated 2 hours before PKP. In the samples treated with transepithelial crosslinking 3 months before PKP the positivity of both CD34 keratocytes and βcatenin and connexin-43 epithelial cells is similar to control.


**Support:** None
Accumulation of Reactive Oxygen/Nitrogen Species is a Result of Decreased ROS/RNS Elimination, Including Superoxide Dismutase and Peroxiredoxin.

Results: Gene expression analyses were also performed for antioxidant enzymes responsible for ROS/RNS elimination, including Superoxide Dismutase and Peroxiredoxin.

Conclusions: Peroxiredoxin is one of the major antioxidant enzymes involved in the maintenance of cellular redox homeostasis. Reduced expression of Peroxiredoxin may contribute to the increased oxidative stress observed in KC corneal cells.

Purpose: The purpose of this study is to investigate the expression of Peroxiredoxin in keratoconus corneal cells.

Program Number: 1109 Poster Board Number: A145
Presentation Time: 3:15 PM - 5:00 PM
Correlation Between Corneal Topographic Parameters And Visual Outcomes
In Patients With Different Stages Of Keratoconus
Danielle L. Miura, Tiago S. Prata, Paulo Schor.

Purpose: We aimed to determine the correlation between corneal topographic parameters and visual outcomes in patients with different stages of keratoconus.

Methods: We reviewed all charts of keratoconus patients followed in a tertiary Eye Clinic between 01/2009 and 01/2011 (n=189 patients).

Results: A total of 164 patients were included. Mean age was 31.8±9.8 years, with 80% female patients. The dioptric power of the apex, in tangential algorithm, was higher in group I (p≤0.01; Mann-Whitney test). Multivariate logistic regression analysis revealed that Kmax was the only parameter significantly associated with final BCVA (OR: 1.11; 95% CI: 1.04-1.18; p=0.006) decreased by 11% for each increase of 1 diopter in the Kmax. The other topographic parameters evaluated were not significant in this model (p>0.05).

Conclusions: In this large series of patients with different stages of Keratoconus, Kmax seemed to be the best parameter to differentiate those with good visual outcomes from those with visual acuity deterioration.

Commercial Relationships: Danielle L. Miura, None; Tiago S. Prata, None; Paulo Schor, None
Support: None

Program Number: 1111 Poster Board Number: A147
Presentation Time: 3:15 PM - 5:00 PM

Correlation Between Corneal Topographic Parameters And Visual Outcomes
In Patients With Different Stages Of Keratoconus
Emilia Cantera, Magdalena Cortes, Silvia Conflitti, Ophthalmology, Villa Stuart - Rome, Rome, Italy.

Purpose: The purpose of this study is to investigate the correlation between corneal topographic parameters and visual outcomes in patients with different stages of keratoconus.

Methods: We reviewed all charts of keratoconus patients followed in a tertiary Eye Clinic between 01/2009 and 01/2011 (n=189 patients).

Results: A total of 164 patients were included. Mean age was 31.8±9.8 years, with 80% female patients. The dioptric power of the apex, in tangential algorithm, was higher in group I (p≤0.01; Mann-Whitney test). Multivariate logistic regression analysis revealed that Kmax was the only parameter significantly associated with final BCVA (OR: 1.11; 95% CI: 1.04-1.18; p=0.006) decreased by 11% for each increase of 1 diopter in the Kmax. The other topographic parameters evaluated were not significant in this model (p>0.05).

Conclusions: In this large series of patients with different stages of Keratoconus, Kmax seemed to be the best parameter to differentiate those with good visual outcomes from those with visual acuity deterioration.

Commercial Relationships: Emilia Cantera, None; Magdalena Cortes, None; Silvia Conflitti, None
Support: None

Program Number: A145
Presentation Time: 3:15 PM - 5:00 PM

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In Patients With Different Stages Of Keratoconus
Danielle L. Miura, Tiago S. Prata, Paulo Schor.

Purpose: We aimed to determine the correlation between corneal topographic parameters and visual outcomes in patients with different stages of keratoconus.

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Commercial Relationships: Danielle L. Miura, None; Tiago S. Prata, None; Paulo Schor, None
Support: None

Program Number: A147
Presentation Time: 3:15 PM - 5:00 PM

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In Patients With Different Stages Of Keratoconus
Emilia Cantera, Magdalena Cortes, Silvia Conflitti, Ophthalmology, Villa Stuart - Rome, Rome, Italy.

Purpose: The purpose of this study is to investigate the correlation between corneal topographic parameters and visual outcomes in patients with different stages of keratoconus.

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Conclusions: In this large series of patients with different stages of Keratoconus, Kmax seemed to be the best parameter to differentiate those with good visual outcomes from those with visual acuity deterioration.

Commercial Relationships: Emilia Cantera, None; Magdalena Cortes, None; Silvia Conflitti, None
Support: None
Presentation Time: 3:15 PM - 5:00 PM
Consequences of Progression in Keratoconus After Cross-Linking Therapy: A Multicenter Study

Purpose: To determine the most predictive parameters of corneal flattening after crosslinking treatment (CXL) in patients with keratoconus (KC).

Methods: A retrospective review of medical records and confocal microscopy was performed to evaluate 60 eyes of 30 patients with progressive KC treated with CXL at three centers. The main outcome was corneal flattening (CE) measured by anterior surface keratometry (K1, K2, Kmax) and pachymetry (ThCT) at baseline and at each follow-up visit. The primary predictor was defined as the parameter with the highest area under the receiver operating characteristic curve (AUROC).

Results: The mean CE at 1 year was 19.94±16.4 µm and the mean CE at 3 years was 35.9±24.4 µm. The AUROC values for CXL effect on CE were as follows: K1: 0.99 (p<0.001); K2: 0.98 (p<0.001); Kmax: 0.98 (p<0.001); ThCT: 0.99 (p<0.001). The most significant predictor of corneal flattening after CXL was the initial ThCT (p=0.005). CXL was performed in 25 eyes (CXL group) and 15 fellow eyes with stable keratoconus (Control group). The mean CE at 1 year was 28.2±16.3 µm in the CXL group and 5.3±4.8 µm in the Control group (p<0.001).

Conclusions: CE after CXL was significantly greater in eyes with higher initial ThCT, with a mean decrease of 22.1±14.4 µm in the CXL group and 4.2±3.8 µm in the Control group (p<0.001). CXL is the most effective treatment for progressive KC, with a significant improvement in CE, especially in eyes with a high initial ThCT.
Presentation Time: 3:15 PM - 5:00 PM

Vitamin D3 reduces TGFβIP in corneal fibroblasts derived from granular corneal dystrophy type 2 corneas

Eung Kweon Kim, Kyung Eun Han, Seung-il Choe, Tae-im Kim. Ophthalmology, Corneal Dystrophy Institute, Yonsei University College of Medicine, Seoul, Republic of Korea.

Purpose: To investigate the effect of 1, 25-Dihydroxyvitamin D3 (1,25(OH)2D3) on expression of transforming growth factor-beta induced gene protein (TGFβIP) in normal human corneal epithelial cells (HCE), normal human corneal fibroblasts (HCF) and homozygote granular corneal dystrophy type 2 (GCD2) corneal fibroblasts.

Methods: Cultured HCE, HCF and homozgygote GCD2 HCF were treated with various concentration and time of vitamin D3. The levels of expression of TGFβIP and Smad phosphorylation were analyzed by immunoblotting and the levels of TGFβIP mRNA was analyzed by RT-PCR.

Results: The expressions of TGFβIP and its mRNA of HCE, HCF and GCD2 homozgygote fibroblasts were decreased by vitamin D3 in dose- and time-dependent manner. Furthermore, vitamin D3 suppressed expression of TGFβIP and the Smad3 phosphorylation induced by TGF-β1. These effects of vitamin D3 were attenuated in the vitamin D receptor (VDR)-knock down corneal epithelial and fibroblasts. Cell viability analysis at different doses was greater than 90%, demonstrating that vitamin D3 did not inhibit proliferation of corneal epithelial and fibroblasts.

Conclusions: Vitamin D3 reduces the expression of TGFβIP mRNA and TGFβIP in HCE, HCF and homozgygote GCD2 HCF via VDR. These data suggest that vitamin D3 might be an ancillary treatment modality of GCD2 and other TGFβI-related corneal dystrophies.

Commercial Relationships: None.

Support: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. 2011-0028699)

Program Number: 1116 Poster Board Number: A152

Presentation Time: 3:15 PM - 5:00 PM

Non-Linear Optical (NLO) Collagen Crosslinking

Dongyul Chai, Tibor Juhasz, Donald J. Brown, James V. Jester, Gavin Herbert Eye Institute, University of California, Irvine, Irvine, CA.

Purpose: Non-linear optical, femtosecond laser-induced, two photon excitation provides for activation of photosensitizers within femtoliter volumes, thus allowing for precise control of photoactivated tissue reactions. The purpose of this study was to determine if NLO based excitation of riboflavin could induce crosslinking (CXL) and mechanical stiffening of collagen hydrogels.

Methods: Six compressed collagen hydrogels composed of rat-tail collagen type-1, averaging 179.33 ± 73.2 µm in thickness, were divided into two groups (3 gels each), NLO (group 1) and conventional UVA CXL (crosslinking group 2). NLO CXL of riboflavin soaked (0.5% w/v in PBS) hydrogels were performed by focusing 87 µm, 760 nm femtosecond light into the gels using a Zeiss 510 Meta CLSM and a 20x Zeiss Apochromat objective lens (NA=0.75). Gels were then scanned over a 5.2 mm x 5.2 mm area through the gel thickness at 2 um steps. Conventional CXL was achieved by irradiating a similar area with 3mW/cm2, 370 nm UVA light for 30 min. Mechanical stiffness was determined by measuring the indentation force through 1 mm displacement at 20 µm/sec velocity using a 250 µm diameter, round tip probe fixed to a force transducer after preloading. Hydrogel thickness was measured using second harmonic generation imaging. Hydrogel thickness and stiffness was measured at baseline and after crosslinking. Indentation modulus, E, was calculated using the following equation,

\[ E = f(v^2)/P \mu (d/h), f(v) = 1.040 - 0.146v - 0.158v^2 \]

E: indentation modulus, d: indentation displacement, v: 0.5 (Poisson’s ratio), a: radius of window holding gel, h: gel thickness, P: indenting force

Results: Mechanical stiffness of hydrogels before CXL averaged 0.45 ± 0.14 MPa. Following CXL there was a 3.35 (± 1.80) fold increase using NLO and a 4.38 (± 4.61) fold increase using UVA CXL. There was no difference between NLO and UVA CXL.

Conclusions: This is the first study to demonstrate that non-linear optical photodynamic therapy (NLO-PT) can be used to increase the mechanical stiffness of collagen hydrogels through crosslinking. Translation of this technology to the cornea may provide for a more precise, safe and effective therapeutic strategy for treating Keratoconus and other ectatic disorders compared to conventional UVA crosslinking.

Commercial Relationships: Dongyul Chai, None; Tibor Juhasz, None; Donald J. Brown, None; James V. Jester, None

Support: NIH EY016663, EY017959, EY019719 and Research to Prevent Blindness, Inc, the Discovery Eye Foundation, and the Skibb Program in Molecular Ophthalmology

Program Number: 1117 Poster Board Number: A153

Presentation Time: 3:15 PM - 5:00 PM

Subclinical Keratoconus Detection by Multiparametric Corneal Biomechanical Characterization with the Ocular Response Analyzer

Jeremias G. Galletti, Marisarina Delrivo, Tomas Pförtnert, Fernando Fuentes Bonthouse, Ophthalmology, Hospital de Clinicas, University of Buenos Aires, Buenos Aires, Argentina; ECOS (Clinical Ocular Studies) Laboratory, Buenos Aires, Argentina.

Purpose: To compare corneal wavefront descriptors of the Ocular Response Analyzer (ORA) in healthy and subclinical keratoconus eyes to assess their diagnostic capacity.

Methods: 95 eyes from 95 healthy subjects in group 1 and 70 fellow eyes from 70 unilateral manifest keratoconus patients were evaluated with ORA, corneal topography, abberometry and anterior segment optical coherence tomography. Corneal hysteresis (CH), corneal resistance factor (CRF) and other 41 parameters were obtained from the corneal waveform curves and compared between groups. Linear regression was used to correct for the effect of central corneal thickness (CCT) on correlated parameters and diagnostic performance was assessed by receiver-operating characteristic curve analysis.

Results: 29 of the 41 additional biomechanical parameters, along with corneal hysteresis (CH) and corneal resistance factor (CRF), showed statistically meaningful differences between the groups (p<0.05, Student’s t test). Six parameters, including CH and CRF, were significantly correlated with CCT in both groups. Six parameters, CRF, Timel, H2, H21 and Drive2 had the best performances (area under the curve, AUC), and only CRF’s was improved after correcting for CCT. The highest of these parameters had better diagnostic yields (error rate < 20%) than when considered individually, the highest being the combination of the 10 best parameters (AUC=0.82). These results applied also to eyes with normal higher-order 5-mm anterior corneal surface aberrations (>3.44 µm, taken from mean±1 SD of group 1), outperforming corneal topography.

Conclusions: Multiparametric biomechanical analysis with the ORA can detect a higher proportion of eyes with subclinical keratoconus than corneal topography, with a better diagnostic yield than CH and CRF when considered individually.

Commercial Relationships: Jeremias G. Galletti, None; Marisarina Delrivo, None; Tomas Pförtnert, None; Fernando Fuentes Bonthouse, None

Support: None

Program Number: 1118 Poster Board Number: A154

Presentation Time: 3:15 PM - 5:00 PM

Keratoconus Match Index of normal and keratoconus suspect eyes

Maxime Delbarre, Mikhaël Lussato, Catherine Verret, Bénédicte Blavier, Corinne Leduc, Pascale Crepy, Françoise Froussart-Maille, Jean Claude Rigal-Sastournet, 1,2. 1Department of Ophthalmology, Percy Military Hospital, Clamart, France; 2Military Department of Epidemiology and Public Health, Saint Mandé, France.

Purpose: To compare Keratoconus Match Index (KMI) of the Ocular Response Analyser between keratoconus, forme fruste keratoconus and normal corneas and estimate the sensitivity and the specificity of KMI in discriminating forme fruste keratoconus from healthy corneas.

Methods: The study population of this retrospective comparative case series was divided into 3 groups: 24 keratoconic (KCN) eyes (16 patients), 8 forme fruste keratoconic eyes (FFKCN, normal topography with controlateral KC), and 36 healthy eyes (18 patients). Every patient underwent a complete clinical eye examination, a corneal topography, and a biomechanical evaluation with the Ocular Response Analyser (ORA; Reichert Ophthalmic Instruments, Buffalo, NY, USA). Corneal hysteresis (CH), corneal resistance factor (CRF), and KMI were compared between the 3 groups. The receiver operating characteristic (ROC) curve was used to identify cutoff points that maximized sensitivity and specificity in discriminating FFKCN from normal corneas.

Results: The mean KMI was 0.20 for KCN, 0.67 for FFKCN and 0.928 for control eyes. There was a statistically significant difference between the 3 groups (P < 0.001) and also between the FFKCN group and the control group (P = 0.05). The mean CH was 7.31 mmHg for KCN, 9.21 mmHg for FFKCN and 10.8 mmHg for control eyes (P < 0.001). The mean CRF was 6.29 mmHg for KCN, 8.18 mmHg for FFKCN and 10.6 mmHg for control eyes (P < 0.001). For a threshold of 0.8, KMI had a sensitivity of 63% with a specificity of 72%. For a threshold of 10.7mmHg, the CH had a sensitivity of 75% and a specificity of 66.7%. The CH and a sensitivity of 75% and a specificity of 66.7%.

Conclusions: There is a significant difference in the mean KMI between normal and FFKCN corneas. Sensitivity and specificity of this index is not greater than those of CH and CRF. KMI seems to be useful for the detection of FFKCN but further studies with bigger samples are necessary.

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Cornea In Boney And Cartilaginous Fish: Comparative Anatomy And Phylogeny

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Purpose: To compare the corneal anatomy and physiology among 13 species of fish including members of Chondrichthyes (1), Sarcopterygii (1), Chondrostei (1), and Halecostomi (10, all teleosts). We intend to examine these and other fish from our collection to determine if the variations in corneal anatomy correlate with the phylogeny as fish evolved from basal to more derived species.

Methods: Fish eyes were fixed in formalin, decalcified, embedded in paraffin. Five micron sections were cut and stained with H & E.

Results: As lineages evolved, Gnathostomata (jawed vertebrates) radiated into the Chondrichthyes (sharks, rays and other elasmobranchs) and Teleostomi. Teleostomi radiated into the Osteichthyes which consists of two groups, Actinopterygii and Sarcopterygii. The Actinopterygii includes the Chondrostei and the Neopterygii. Sturgeons are Chondrostei, the next step after the elasmobranchs, the first bony ossification is observed in this lineage phylogenetically. The Sarcopterygii include Dipnoi (all lungfish) and all later tetrapods. The Chondrostei gave rise to the Neopterygii, which include the Holostei and the Teleostei (all teleosts). In the fish described, we attempted to cover a range of lineages. To date, we have observed a wide range of corneal structure that likely matured from the more basal to the more derived species. For example, some of the species have corneae in which the scleral and dermal cornea appear fused and indistinguishable (ie, more derived). Other specimens show a significant morphologic distinction between scleral and dermal layers, while still others show a complete separation. We also describe the unique epithelium covering the cornea in the mormyrid fishes, Stomatorhinus microops and the elephant-nosed fish, Gnathonemus petersii. Both species incorporate electric receptors in the epidermis. In the Southern Stingray, Dasyatis americana, the keratocytes are arranged in discrete lines perpendicular to the collagen fibers in the corneal stroma.

Conclusions: A wide range of corneal phenotypes exists in fish. These anatomical variants, likely, would have developed in response to environmental pressures and also represent phylogenic variations that may give us some additional details of the path traveled by the eye as it evolved.

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