ARVO 2016 Annual Meeting Abstracts

532 Corneal nerves, diabetes, tear film
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Exhibit/Poster Hall Poster Session
Program #/Board # Range: 6153–6194/A0056–A0097
Organizing Section: Cornea

Program Number: 6153 Poster Board Number: A0056
Presentation Time: 11:00 AM–12:45 PM
CORNEAL NERVE MORPHOLOGY AND TEAR FILM

SUBSTANCE P IN DIABETES
Maria Markoulli1, Edward Lum1, Jingjing You1, Carmen Duong1, Jonathan Tolenamento, Juno Kim2. 1School of Optometry and Vision Science, University of New South Wales, Sydney, NSW, Australia; 2Save Sight Institute, University of Sydney, Sydney, NSW, Australia.

Purpose: In vivo corneal confocal microscopy has been used to detect peripheral neuropathy by identifying morphological alterations to the sub-basal nerve plexus (SBNP). This work aims to characterise the relationship between changes in the tear film neuropeptide Substance P and the SBNP in diabetes.

Methods: Seventeen healthy control participants and twelve participants with diabetes were recruited in this cross-sectional study. Total protein content (TPC) and substance P (SP) concentrations were determined in the flush tears of participants. Corneal nerve morphology was assessed by capturing the SBNP using the Heidelberg Retinal Tomograph II with Rostock Corneal Module in the central cornea. Corneal nerve fiber density (CNFD) was measured using ACCMetrics on captured images. Hb1Ac levels and duration of diabetes were obtained through a questionnaire. Comparisons between groups were made using independent samples t-tests. Correlations between parameters were analysed using Pearson’s correlations.

Results: SP concentrations were significantly lower in the tears of participants with diabetes compared to the normal group (2.24 ± 0.87 mg/mL in healthy normals vs 1.87 mg/mL in participants with diabetes, respectively, p=0.04). There was a significant difference in total protein content between the groups (3.70 ± 2.21 vs 2.24 ± 1.87 mg/mL in healthy normals and participants with diabetes, respectively, p=0.04). CNFD was significantly lower in the participants with diabetes compared to the control group (21.46 ± 7.02 vs 16.09 ± 5.70 mm/mm2; p=0.05). There was a moderate correlation between SP and CNFD (r=0.62, p=0.02). There were no correlations between SP and Hb1Ac, SP and duration of diabetes, corneal NFD and Hb1Ac and CNFD and duration of diabetes.

Conclusions: Substance P is expressed at a significantly lower level in the tears of people with diabetes compared with healthy normals. The positive correlation between Substance P and corneal nerve density indicates that Substance P may be a potential biomarker for corneal nerve health.

Commercial Relationships: Maria Markoulli, None; Edward Lum, None; Jingjing You, None; Carmen Duong, None; Jonathan Tolenamento, None; Juno Kim, None

Program Number: 6154 Poster Board Number: A0057
Presentation Time: 11:00 AM–12:45 PM
The chemokine CXCL14 regulates neurovascular patterning during corneal development
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Purpose: Spatiotemporal signaling events of secreted molecules in the developing anterior eye guide intricate processes that result in the formation of a highly innervated but avascular cornea. Although there is increased interest in chemokines due to their involvement in cell proliferation, migration, and differentiation during embryogenesis, very little is known about their function in the eye. Based on the distinct expression pattern of CXCL14 chemokine in the anterior eye, we hypothesize that CXCL14 plays a role during the development of corneal innervation and avascularity.

Methods: Embryonic day (E)1 chick or Tg(tie1: H2b; eYFP) transgenic Japanese quail embryos were injected with RCAS-CXCL14-shRNA or RCAS control, and re-incubated for additional 9-11 days. Embryos were collected and examined for neurovascular defects in cornea development by immunofluorescent staining of whole-mount and corneal sections. The effect of CXCL14 on axon growth and cornea neovascularization was examined in vitro on isolated trigeminal sensory neurons and in vivo by bead implantation in developing cornea, respectively.

Results: Whole-mount analysis of ocular nerves in CXCL14 loss-of-function embryos indicated exacerbated projection of sensory nerves into the cornea. Cross-sections through E12 corneas revealed significantly increased stromal nerve density (P<0.001), stromal nerve occupancy (P<0.001) and innervation of the corneal epithelium (P<0.01) in CXCL14 knockout embryos compared to controls. In vitro analysis showed that CXCL14 inhibits CXCL12-mediated sensory axon growth. Furthermore, Knockdown of CXCL14 in transgenic quail embryos caused ectopic migration of YFP fluorescently labeled angioblasts into the cornea that resulted in cornea neovascularization, and bead implantation experiments revealed that CXCL14 inhibits VEGF-induced cornea neovascularization.

Conclusions: Collectively, these results demonstrate that CXCL14 plays a crucial role in the precise patterning of corneal innervation and maintenance of avascularity by limiting sensory nerve projection and preventing angioblast migration into the cornea. These findings identify CXCL14 as a novel regulator of the neurovascular patterning in the anterior eye during development.

Commercial Relationships: Ana Ojeda, None; Peter Y. Lwigale, None
Support: NH Grant EY022158

Program Number: 6155 Poster Board Number: A0058
Presentation Time: 11:00 AM–12:45 PM
Hyperglycemic condition altering corneal stroma environment and wound healing capacity
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Purpose: Prolonged hyperglycemic condition during diabetes mellitus (DM) often leads to various ophthalmic complications, such conditions seriously affects the cornea and various other regions of the eye. This often leads to impaired vision or blindness due to decreased wound healing capacity, corneal edema, and altered epithelial basement membrane. During DM, changes in bioenergetics which affects gene expression, protein metabolism, and various signaling pathways related to proliferation and differentiation are seen. In this study, our primary goal was to compare the metabolome and lipidome of normal, Type 1 and Type 2 diabetic corneas and identify similarities and differences. Identification of the key factors that are altered in the diabetic cornea compared to a normal cornea will unveil therapeutic targets for treatment of diabetes mellitus.

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Methods: Human corneas of healthy donors, Type I (T1DM), and Type 2 (T2DM) diabetic donors were obtained from NDRI and the Oklahoma Lions eye bank. Epithelium and endothelium layers were scrapped off and the corneal stroma was processed for mass spec-based metabolomics, lipidomics, western blot and TEM.

Results: Our study analysis showed increased expression of fibrotic markers α SMA (2 folds), Col I (3.5 folds) and Col III (6 folds) expressed in both T1DM and T2DM corneas when compared to the healthy controls. Metabolomics analysis showed upregulated tryptophan metabolites regulation in DM corneas in comparison to healthy corneas which leads to the significant upregulation of Kynurenine pathway. Increased lipids, such as sphingosine-1-phosphate, dihydro sphingosine, and dihydro sphingosine-1-phosphate were measured in diabetic corneas suggesting a link to altered lipid metabolism in DM. We also identified key metabolites like Aminoadipic acid, D.L-Pipecolic Acid and Dihydroorotate, which were significantly upregulated and could be possible novel bio-markers for identifying fibrosis caused in cornea due to DM.

Conclusions: We have clearly identified the multiple parameters that are altered during DM in the human cornea. Identification of key metabolites and lipids are crucial for maintaining a healthy cornea, and these findings pave the way for developing therapeutic measures in treating DM ocular complications.

Commercial Relationships: None; Tina B. McKay, None; John Asara, None; Jeremy Allegood, None; charles chalfant, None; Dimitrios Karamichos, None

Program Number: 6156 Poster Board Number: A0059
Presentation Time: 11:00 AM–12:45 PM
Role of Semaphorins in Impaired Sensory Nerve Regeneration and Wound Healing in the Diabetic mouse Cornea
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Purpose: Class 3 Semaphorins are secreted proteins that control axonal branching and pathfinding in the nerve system. This study sought to investigate the role of Sema3C, one member of the class 3 Semaphorins, in epithelium innervation in homeostatic corneas and re-innervation in post-wound corneas.

Methods: Corneas were wounded by debridement and allowed to heal in vivo. Fluorescein staining was used to assess wound size after debridement. siRNAs for gene knockdown and recombinant protein were subconjunctivally injected prior to epithelium-wounding. Sensory nerve fibers/endings were assessed by whole mount confocal microscopy.

Results: Corneal epithelial expression of Sema3C was found to be increased after wounding in normal B6 mice, as shown by real-time PCR, western blotting, and immunohistochemistry. This increase in expression was blunted in streptozotocin-induced diabetic B6 mice after wounding. Conversely, Sema3C knockdown by subconjunctival siRNA injections in wounded mice resulted in a decrease in regenerating nerve fibers as well as a decrease in the rate of wound healing. The presence of recombinant Sema3C in wounded mice led to an increase in regenerating nerve fibers. Finally, multiple injections of Sema3C siRNA every other day led to the degeneration of sensory nerves in unwounded corneas.

Conclusions: Sema3C expressed in corneal epithelial cells plays a role in the maintenance and post wound regeneration of sensory nerve fibers/endings. The hyperglycemia-suppressed expression of Sema3C may be responsible in part for diabetic neurotrophic keratopathy and delayed wound healing and sensory nerve regeneration.

Commercial Relationships: Patrick S. Lee; Fushin X. Yu, None

Support: EY010869, EY017960

Program Number: 6157 Poster Board Number: A0060
Presentation Time: 11:00 AM–12:45 PM
Corneal Sensitivity to Hyperosmolar Eyedrops: A New Behavioral Assay To Assess Diabetic Peripheral Neuropathy
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Purpose: Currently the diagnosis of peripheral neuropathy (PN), which affects about 50% of the diabetic population, is subjective with many patients receiving a diagnosis only after presenting with related symptoms, after significant nerve loss has occurred. Earlier diagnosis and treatment of PN is needed. Recently, in vivo confocal microscopy of sub-epithelial corneal nerve density has been promoted as a surrogate marker for early detection of PN, but imaging of corneal nerves can be challenging as a routine examination and requires sophisticated instrumentation and analysis tools. As an alternative, we have developed a novel and simple screening method that is based on sensitivity of corneal nerves for detecting PN.

Methods: Corneas of control and type 2 diabetic rats were given eyedrops of 290 mOsm, 375 mOsm and 900 mOsm solutions and the ocular response video-recorded over a 2.5 min period. In addition, other neuropathic endpoints including cornea sensitivity using Cochet-Bonnet filament test and sub-epithelial corneal nerve density using corneal confocal microscopy were determined.

Results: Diabetic rats had a significantly decreased motor and sensory nerve conduction velocity and were hypalgic. Corneal sensitivity as determined using Cochet-Bonnet filament esthesiometer was significantly decreased in diabetic rats compared to control rats. Total nerve fiber length of corneal nerves in the sub-epithelial layer was decreased by about 50% in diabetic rats. Applying the hyperosmotic solutions to the surface of the cornea caused an osmolality-dependent increase in squinting of the treated eye but not the untreated eye in control rats that was significantly suppressed in diabetic rats. The correlation coefficient for corneal nerves and corneal sensitivity vs. degree of squinting was r = -.64 (p < 0.005) and r = -.63 (p < 0.01). Pretreatment with proparacaine ophthalmic solution totally prevented the behavioral response to the 900 mOsm solution in control rats implicating a neural involvement in the hyperosmotic effect.

Conclusions: These results suggest that evaluation of corneal sensitivity utilizing the neural receptors for osmolality may be an alternative method to monofilament, thermal or vibration threshold tests for the early detection of PN.

Commercial Relationships: Mark Yorek, None; Eric Davidson, None; Matthew Yorek, None; Lawrence Coppey, None; Amey Holmes, None; Pieter Poolman, None; Randy H. Kardon, None

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Program Number: 6158 Poster Board Number: A0061
Presentation Time: 11:00 AM–12:45 PM
Analysis of corneal subbasal nerve plexus from wide-area mosaics in healthy subjects and in type 2 diabetics
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Purpose: Very little is known about the subbasal nerve plexus architecture and density over a wide field of view in type 2 diabetes. We therefore performed a cross-sectional observational study to examine qualitative plexus changes and subbasal nerve density in type 2 diabetes relative to an age- and sex-matched healthy control group.

Methods: Laser-scanning in vivo confocal microscopy (IVCM) with the HRT3-RCM system was performed in 82 subjects bilaterally (39 diabetics and 43 controls). Wide-area mosaics were reconstructed by an automated algorithm and subbasal nerves in all mosaics were traced manually and by a fully automated tracing algorithm. Subbasal nerve density was compared between diabetics and healthy groups and according to diabetes duration.

Results: 163 mosaics from 82 subjects were successfully reconstructed, representing a mean enhancement factor of 37 single confocal fields of view and mean area of 6 mm² of the central cornea. Patterns of innervation of the subbasal plexus could be studied qualitatively, and revealed regional variations in nerve density and tortuosity. Nerve density in the mosaic was highly correlated between manual and fully automated methods of nerve tracing (R² = 0.88, P < 0.001), while the automated technique gave a 100-fold improvement in the speed of analysis. The mosaic density in diabetics was significantly reduced relative to healthy subjects (P = 0.018), and mean mosaic density declined with progression of type 2 diabetes (ANOVA P = 0.036; Pearson P = 0.007).

Conclusions: A progressive degradation of the subbasal nerve plexus over a wide area is apparent in type 2 diabetes and this study establishes values for nerve density over a large area of the central cornea.

Commercial Relationships: Neil S. Lagali, None; Stephan Allgeier, None; Pedro Guimaraes, None; Reza A Badian, None; Alfredo Ruggeri, None; Bernd Koehler, None; Tor P. Utheim, None; Beatrice Bourghardi P Deo, None; Magnus Peterson, None; Lars Dahlin, None; Olov Rolandsson, None

Program Number: 6159 Poster Board Number: A0062
Presentation Time: 11:00 AM–12:45 PM
Pigment epithelium-derived factor (PEDF) combined with docosahexaenoic acid (DHA) promote nerve regeneration in diabetic and non-diabetic mouse corneas after injury
Jiucheng He, None; Thang L. PHAM, None; Azucena H. Kakazu, None; Haydee E. Bazan, None
Support: NEI grant R01 EY19465, NIH/NIGMS grant P30GM103340 and a grant from the Research Foundation to Prevent Blindness

Purpose: Diabetes damages corneal nerves, leading to diabetic keratopathy (He J, and Bazan HEP. Ophthalmology 2012;119:956-964). Studies done in our laboratory have shown that in rabbits, PEDF in combination with DHA, stimulates corneal nerve regeneration, restores sensitivity and increases epithelial wound healing after experimental refractive surgery (Cortina MS, et al. Arch Ophthalmol. 2012; 130: 76-83). In the current study, we tested the effect of this treatment on corneal nerve regeneration in diabetic and non-diabetic mice after corneal injury.

Methods: Normal and streptozotocin-induced diabetic mice (C57/ B6) were anesthetized and the right eye was injured by removing the epithelium and 1/3 of the anterior stroma of a central area of 2mm diameter, using a corneal rust ring remover. Afterwards, both the diabetic and non-diabetic mice were randomly divided into two groups: Treatment groups received PEDF (0.4ng/10µl) + DHA (80ng/10µl) eyedrops topically 3 times a day for 2 weeks, while the control groups received the vehicle in the same way. Mice were euthanized and the whole corneas were immediately fixed and stained with rabbit monoclonal anti-PGP9.5 antibody. Whole-mount images were acquired to build an entire view of the corneal nerve architecture. The nerve fiber densities within the injured area (about 3.14 mm² per cornea) were assessed on the basis of whole mount images by computer-assisted analysis.

Results: Strepotomycin-treated mice showed a loss of corneal sensitivity when measured with a Cochet-Bonnet esthesiometer. Immediately after injury, immunofluorescence showed that all the subbasal nerve bundles, together with the anterior stromal nerve branches, were ablated. After two weeks, the nerve densities were 12.54±1.32% in the treatment group and 4.47±0.39% in the vehicle group. In non-diabetic mice, the nerve densities were 14.5±3.5% in the treatment group and 5.2±2.1% in the vehicle group.

Conclusions: PEDF+DHA treatment promotes corneal nerve regeneration in both non-diabetic and diabetic mice. The mouse is an excellent model to study the mechanism of regeneration of corneal nerves affected by diabetes.

Program Number: 6160 Poster Board Number: A0063
Presentation Time: 11:00 AM–12:45 PM
Evaluating corneal sensation in patients with HIV Infection
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Purpose: Distal symmetric polyneuropathy (DSP) has been recognized as a common neurologic complication of Human Immunodeficiency Virus (HIV) infection. Currently, there is no gold standard for the diagnosis of HIV DSP. Its diagnosis remains based on a combination of clinical signs and symptoms, such as reduced ankle reflexes, reduced pinprick sensation, or reduced vibration sensation in the feet. The purpose of this study is to compare the corneal sensation in patients with and without HIV infection, and

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investigate the possibility of using corneal sensitivity as a new quantitative testing that allows objective assessment of HIV DSP.

**Methods:** This is a retrospective chart review of patients who had an eye exam at the eye clinic at a community hospital in the Bronx, NY. Adult patients who are HIV-positive and who have no history of ocular surgery or topical medication use were enrolled into the study group. The control group consisted of HIV-negative patients who had no history of ocular surgery or topical medication use. Corneal sensation assessment was performed with a Cochet-Bonnet aesthesiometer. The data was statistically analyzed using excel.

**Results:** 22 HIV patients and 22 control patients were included in the study. The average of the central corneal sensation in HIV group is 56.6 ± 1.0 mm, compared to 59.3 ± 0.4 mm in control group (P = 0.004). The average of the peripheral corneal sensation in HIV group is 57.6 ± 0.9 mm, compared to 59.4 ± 0.2 mm in control group (P = 0.019). Viral load of HIV patients has a weak positive correlation with the sensation of the central cornea, but this correlation is not statistically significant (correlation coefficient of +0.21, p = 0.321).

CD4 value of HIV patients has no correlation with the sensation of the central or peripheral cornea (correlation coefficient of -0.05 and -0.08, respectively).

**Conclusions:** Both central and peripheral corneal sensation measurements are statistically significantly decreased in the HIV group compared with that of the the control group. Our preliminary results suggest that corneal sensitivity may potentially be used as an additional clinical tool that allows for objective assessment of HIV distal symmetric polyneuropathy. The results also suggest that corneal sensory neurons may have relevance to the discrepancy between signs and symptoms in dry eye syndrome.

**Commercial Relationships:** Jing Grace Wang, None; Nathaniel Nataneli

**Program Number:** 6161 **Poster Board Number:** A0064 **Presentation Time:** 11:00 AM–12:45 PM

**The expression profile of neuropathic pain-related genes of trigeminal ganglion in the experimental dry eye model**

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1Department of Ophthalmology and Visual Science, Catholic University of Korea, College of Medicine, Seoul, Korea (the Republic of); 2Catholic Institute of Visual Science, Seoul, Korea (the Republic of).

**Purpose:** We evaluated the expression profile of neuropathic pain-related genes of trigeminal ganglion (TG), which give afferent nerves to ocular surface, in the experimental animal models with inflammatory dry eye.

**Methods:** Inflammatory dry eye was induced by treating 0.1% benzalkonium chloride (BAC) solution 2 times a day for 1 week on unilateral eyes of Sprague-Dawley rats. Some rats were administrated with artificial tear 4 times a day after 1 week BAC treatment. We extracted corneas and the ipsilateral TGs from controls, 1 week BAC treated group, and post-treatment 2 month group (n = 9 in all groups). All groups were assessed for corneal staining using surgical microscope before euthanasia. Whole mount corneas were stained with beta3 tubulin to measure the density of subbasal corneal nerves (μm/mm²). RT-PCR microarray (RT² Profiler PCR Array, Qiagen) from TG tissue homogenate was performed for screening the pain related 96 genes and PCR and immunohistochemistry were done for genes having over 2 fold-increase in post-treatment 2 month group versus controls and 1 week BAC treated group.

**Results:** One week 0.1% BAC treatment resulted in decrease in tear secretion and goblet cells density of treated eyes of rats. In 2 month recovery group, tear secretion and goblet cell density recovered to normal controls. The density of subbasal nerves (μm/mm²) was significantly lower in 1 week BAC group and post-treatment 2 month group than in controls. The density of subbasal nerves in 2 month recovery group increased compared to that in 1 week BAC group, although that in the former was lower than in controls. The expression of P2rx3, P2rx4, and P2rx7 were significantly upregulated in 2 month recovery group, while those expression in 1 week BAC group was not changed compared to controls. The others revealed lack of significant fold change or agreement between microarray and qPCR.

**Conclusions:** The experimental inflammatory dry eye rats showed recovery in corneal erosions, tear secretion, goblet cell density, and corneal nerve density over 2 months. Nevertheless, P2rx3, P2rx4, and P2rx7 among pain related genes in TG were upregulated over the period. These result suggested that purinergic receptors of corneal sensory neurons may have relevance to the discrepancy between signs and symptoms in dry eye syndrome.

**Commercial Relationships:** Yong Soo Byon; HeeJung Ju; Ji Young Kwon; Young-Sik Yoo; Jun-Sub Choi; Jeevon Mok; Choun-Ki Joo

**Program Number:** 6162 **Poster Board Number:** A0065 **Presentation Time:** 11:00 AM–12:45 PM

**Vitamin D and Neuropathy in Patients with Sjogren’s Syndrome**

Ryan O’Sullivan1, Fatymee Y. Bunya1, Maxwell Pistilli1, Gui-Shuang Jing2, Ilaria Macchi1, Frederick Vivino1, Mina Massaro-Giordano1.


**Purpose:** In Sjogren’s syndrome (SS), there is preliminary evidence that hypovitaminosis D is a risk factor for the development of peripheral neuropathy; however the relationship between serum vitamin D levels, corneal innervation, and ocular surface disease in these patients has not been examined. The current study will examine the relationship between vitamin D levels, corneal nerve morphology, and ocular signs and symptoms in patients with suspected or confirmed SS. We hypothesize that patients with hypovitaminosis will have abnormal corneal innervation, which will correlate with more severe ocular surface discomfort and signs.

**Methods:** Subjects included adult patients with suspected or confirmed diagnoses of SS. Corneal nerve morphology was assessed by confocal microscopy with the Heidelberg Retina Tomograph II (HRTII) and Rostock Cornea Module. Blood samples were collected at baseline and analyzed for serum levels of 25-hydroxy vitamin D. Images of the corneal nerves located at the sub epithelial plexus were analyzed with the NIH freeware ImageJ. Individual nerve fibers were traced and fiber density, length, branch density, and tortuosity were computed. Patients underwent an ocular surface exam (tear break-up time, ocular surface staining) and symptoms were assessed using the Ocular Surface Disease Index (OSDI).

**Results:** Preliminary data associates hypovitaminosis with abnormal corneal innervation and more severe ocular surface disease. After enrollment is complete, we will assess correlations among serum vitamin D levels, abnormal corneal innervation, ocular signs, and OSDI scores. Figure 1 displays confocal images obtained from one patient with Vitamin D deficiency, who presented with severe ocular surface pain and moderate signs of ocular surface disease.

**Conclusions:** Hypovitaminosis D may potentiate neuropathy and therefore play an important role in the corneal innervation and ocular surface disease in a subset of SS patients. This study represents the first effort to look at objective parameters of corneal nerve morphology alongside metrics of ocular surface disease in SS patients with variable levels of 25-hydroxy vitamin D. We hope to further investigate the possibilities of using corneal sensitivity as a new quantitative testing that allows objective assessment of HIV DSP.
characterize the importance of Vitamin D status in SS and ocular surface disease.

Figure 1: Image A is a representative control patient showing corneal nerves which are roughly linear and parallel. Images B and C were obtained from patient 1, and exhibit neuropathic branching and tortuosity.

Commercial Relationships: Ryan O’Sullivan, None; Vatinee Y. Bunya, None; Maxwell Pistilli, None; Gui-Shuang Ying, None; Ilaria Macchi, None; Frederick Vivino, None; Mina Massaro-Giordano, None

Program Number: 6163 Poster Board Number: A0066
Presentation Time: 11:00 AM–12:45 PM
The Effect of Intravitreal Injections on Corneal Esthesiometry
Yogin Patel, Therese Sassalos, Sachin Gandhi, Ankit Desai, Nitin Kumar, Uday Desai. Henry Ford Hospital, Southfield, MI.

Purpose: To determine the effect of intravitreal injections and associated anesthesia on corneal sensation.

Methods: We present a pilot study of an initial cohort of patients for whom corneal sensation was measured using the Cochet-Bonnet corneal esthesiometer. This measurement was repeated a total of two times on both the study as well as the control eye. Four quadrants were tested in each cornea. The results were correlated with the number of intravitreal injects each eye has had in order to determine their effect. Injections were limited to anti-VEGF agents and the fellow non-injected eyes were used as controls. Exclusion criteria included prior eye surgery, known corneal disease, and diabetes.

Results: 20 patients meeting the inclusion and exclusion criteria have been tested thus far. The most common diagnoses were exudative age related macular degeneration and retinal vein occlusion. All intravitreal injections were done after subconjunctival lidocaine injection in the inferotemporal quadrant 4 mm posterior to the corneal limbus. The esthesiometry measurements of specific quadrants were compared between injected and fellow eyes. When comparing the corneal esthesiometry measurements between injected and fellow eyes, there was a statistically significant difference in all quadrants except for superonasal. When stratifying the data between superior and inferior there was a statistically significant difference when comparing the superior (p = 0.189) and inferior (p < 0.0001) injected and fellow corneas. Finally an overall comparison between injected and fellow eye (taking into account all quadrants) showed a significant difference (p<0.0001).

Conclusions: The preliminary data and analysis shows a statistically significant difference in the corneal esthesiometry between eyes that have had intravitreal injections and those that have not.

Commercial Relationships: Yogin Patel, None; Therese Sassalos, None; Sachin Gandhi; Ankit Desai, None; Nitin Kumar, None; Uday Desai, None

Program Number: 6164 Poster Board Number: A0067
Presentation Time: 11:00 AM–12:45 PM
Developing an animal model of corneal neurotization as a means of restoring sensation and improving corneal epithelial maintenance and repair
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Purpose: Patients with corneal anesthesia are susceptible to corneal injury, scarring and progressive vision loss. Corneal neurotization is a surgical technique that restores corneal sensation using nerve grafts and donor sensory nerves from elsewhere on the face. Further study is needed to investigate factors that mediate nerve growth into the cornea and how nerves derived from other sensory nerves influence the maintenance and healing of the corneal epithelium. Here we present our progress in developing an animal model of corneal neurotization to further explore these questions.

Methods: Thy1-GFP+ Sprague-Dawley rats, which express green fluorescent protein (GFP) in axons, was used for all studies. Two techniques of corneal denervation were explored: i) transconjuctival ciliary nerve transection, and ii) stereotactic ablation of the ophthalmic nerve. Denervated cornea’s were then harvested and examined for nerve regrowth at 7, 14, and 28 days. In a separate group of animals, the ciliary nerves were exposed to 100% EtOH following transection to further damage axons and prevent regrowth.

Results: Corneal nerve imaging in the Thy1-GFP+ rat strain with confocal microscopy permitted accurate tracing of stromal and sub-basal corneal nerves (Figure 1A). Following transection of the ciliary nerves, denervation was complete 7 days following injury, however the corneal innervation began to regenerate by 14 days. Topical EtOH following ciliary transection delayed nerve regrowth, however regenerating corneal nerves could be visualized at 28 days (Figure 1B). There were no signs of corneal reinnervation at 14 days following stereotactic ablation of the ophthalmic nerve.

Conclusions: An animal model of corneal neurotization first necessitates a method of corneal denervation that does not permit nerve regeneration of the corneal innervation. Without such a method, any changes in corneal epithelium cannot be attributed to neurotization but to corneal nerve regrowth. Using stereotactic ablation of the ophthalmic nerve may further delay or prevent corneal nerve regrowth by injuring the corneal nerves more proximally, however longer term studies are required to ensure there is no corneal nerve regrowth past 28 days.
NEUROPATHIC OCULAR PAIN ASSESSMENT

Daniela Lazzarini, Alvise La Gloria Valerio, Iva A. Fregonà, Anna Mocellin, Edoardo Midena, Andrea Leonardi. Ophthalmology Unit, University of Padova, Padova, Italy.

Purpose: The cornea possesses the richest sensory innervation of the body to detect noxious stimuli: a clinical consequence of corneal damage to nociceptive pathways is itself a disease known as neuropathic pain. The aim of the present study is to investigate the prevalence of persistent ocular pain of suspected neuropathic origin, to determine its characteristics, location, and intensity according to a self-reported questionnaire and corneal confocal microscopy investigation.

Methods: A total of 196 patients referred to Ophthalmology Unit of University of Padova over a 4-months period were included in the study. All patients underwent slit lamp examination. Intensity of pain was assessed by Visual Analogue Scale (VAS) and FACES Pain Scale (FPS), while persistence was classified as chronic if its duration was more than one month. A modified version of the Self-Administered Leeds Assessment of Neuropathic Symptoms and Signs (S-LANSS) survey was completed by 54 subjects with chronic ocular pain. In 21 subjects with ocular pain and in 10 with no pain (control group) corneal confocal microscopy and esthesiometry was performed to study nerve morphology and function.

Results: The prevalence of chronic pain among this cohort was 28% with a VAS mean score of 54.7 ± 20.7. 27% of patients referred an acute ocular pain with a mean intensity pain of 46.6 ± 24.1 (p=0.062). 44% of the included subjects did not report any ocular pain. Chronic pain was more common in allergic keratoconjunctivitis, dry eye and blefaritis and pain experiences were endorsed as pricking, tingling, pins and needles sensations in 80% of patients. Painful area was reported to look like “more red” in 75.5% and to be abnormally sensitive/hypersensitive to touch in 51% of them. Corneal confocal microscopy performed in 11 patients with chronic pain without any corneal alteration by slit lamp reveals abnormalities at the subbasal nerve plexus levels with presence neuromas, sproutings and inflammatory cells; control group did not shown any corneal confocal microscopy alterations.

Conclusions: The assessment of ocular pain characteristics using questionnaire and corneal confocal microscopy allows the clinicians to recognize the differences between nociceptive and neuropathic pain. Understanding ocular pain may prevent acute patients to become long-standing symptomatic patients. Further study will evaluate potential mechanism-based treatment of neuropathic pain.

Commercial Relationships: Joseph Catapano, None; Michael P. Willand, None; Asim Ali, None; Gregory H. Borschel, None

Support: Canadian Institute of Health Research (CIHR)

Program Number: 6165 Poster Board Number: A0068
Presentation Time: 11:00 AM–12:45 PM

Rhode Island Department of Corrections (RIDOC), Providence, RI; 2Dartmouth College, Hanover, NH; 3University of Delaware, Newark, DE; 4College of Optometry, The Ohio State University, Columbus, OH; 5School of Optometry, Indiana University, Bloomington, IN.

Purpose: Posterior segment imaging with optical coherence tomography (OCT) has been facilitated by recent advancements in OCT technology allowing for high resolution imaging of the retina. However, the choroid has limited access limited to OCT in the posterior segment due to its anterior location in the eye. Intraocular pressure (IOP) is a measure of the pressure at a specific location within the anterior chamber of the eye. One of the challenges in measuring IOP is the potential for anterior-segment mechanical interferences such as conjunctival retraction and corneal deformation.

Methods: To determine the potential impact of anterior-segment mechanical interferences, we evaluated the impact of IOP measurement in the anterior chamber versus the potential impact of IOP measurement in the posterior chamber.

Results: The impact of anterior-segment mechanical interferences in 11 subjects who completed the IOP measurement at both the anterior and posterior chamber was demonstrated. The measurement at the anterior chamber was increased in the subjects with anterior-segment mechanical interferences.

Conclusions: The potential impact of anterior-segment mechanical interferences on IOP measurement in the anterior chamber is significant.

Commercial Relationships: Carolyn G. Begley, None; Jun Zhang, None; Richard J. Braun, None; Peter E. King-Smith, None

Support: The project was supported by Grant Number R01EY021794 (Dr. Begley) from the National Eye Institute.

Program Number: 6167 Poster Board Number: A0070
Presentation Time: 11:00 AM–12:45 PM

Modeling Evaporative Tear Break Up (TBU) with a Mobile Lipid Layer

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Purpose: To examine the formation of TBU and the pain response to determine whether pain responses follow these patterns.

Methods: After instillation of 2 microliters of 2% sodium fluorescein dye, 10 subjects were seated behind a slit lamp biomicroscope and kept one eye open as long as possible (maximum blink interval=MBI) while turning a “pain knob” (0-10 scale) to indicate the discomfort level during blink suppression (3 trials for each subject). Images of the tear film were converted to grayscale and analyzed by custom MATLAB programs that analyzed the rate of changes in pixel intensity over time and compared it to the slope and rate of change of the pain response.

Results: Eight subjects (average MBI= 15.26±10.93s) with relatively long trials showed gradual decreases in fluorescence within areas of TBU and gradual increases in discomfort (average slope = 2.157±2.447, 1.331±0.906, respectively) initially. Two subjects with short trials (average MBI= 7.48±1.528s) showed very rapid formation of the percentage of TBU area (slope = 4.043±3.700, with no discomfort for several seconds (average 4.634±1.528), followed by pain that induced rapid eye closure (average slope=4.742±4.502).

Conclusions: The relatively gradual increase in discomfort in the trials of 8 subjects is consistent with evaporation-dominated TBU, producing pain through increasing tear osmolarity, whereas fluid flow dominated TBU would not be expected to produce pain until the area of TBU exposes or dries the corneal surface. The purpose of this pilot study was to examine the formation of TBU and the pain response to determine whether pain responses follow these patterns.

Commercial Relationships: Carolyn G. Begley, None; Jun Zhang, None; Richard J. Braun, None; Peter E. King-Smith, None

Support: The project was supported by Grant Number R01EY021794 (Dr. Begley) from the National Eye Institute.

Program Number: 6166 Poster Board Number: A0069
Presentation Time: 11:00 AM–12:45 PM

Mechanisms involved in tear-break-up as revealed by pain response

Carolyn G. Begley1, Jun Zhang2, Richard J. Braun2, Peter E. King-Smith3. 1School of Optometry, Indiana University, Bloomington, IN; 2Dept of Mathematical Sciences, University of Delaware, Newark, DE; 3College of Optometry, The Ohio State University, Columbus, OH.

Purpose: We postulate that two basic mechanisms are primary in many cases of tear-break-up (TBU), evaporation and the lipid-driven Marangoni effect. Evaporation-dominated TBU would be expected to lead to a gradually gradual increase in the pain response due to increasing tear film osmolarity, whereas fluid flow dominated TBU would not be expected to produce pain until the area of TBU exposes or dries the corneal surface. The purpose of this pilot study was to examine the formation of TBU and the pain response to determine whether pain responses follow these patterns.

Methods: After instillation of 2 microliters of 2% sodium fluorescein dye, 10 subjects were seated behind a slit lamp biomicroscope and kept one eye open as long as possible (maximum blink interval=MBI) while turning a “pain knob” (0-10 scale) to indicate the discomfort level during blink suppression (3 trials for each subject). Images of the tear film were converted to grayscale and analyzed by custom MATLAB programs that analyzed the rate of changes in pixel intensity over time and compared it to the slope and rate of change of the pain response.

Results: Eight subjects (average MBI= 15.26±10.93s) with relatively long trials showed gradual decreases in fluorescence within areas of TBU and gradual increases in discomfort (average slope = 2.157±2.447, 1.331±0.906, respectively) initially. Two subjects with short trials (average MBI= 7.48±1.528s) showed very rapid formation of the percentage of TBU area (slope = 4.043±3.700, with no discomfort for several seconds (average 4.634±1.528), followed by pain that induced rapid eye closure (average slope=4.742±4.502).

Conclusions: The relatively gradual increase in discomfort in the trials of 8 subjects is consistent with evaporation-dominated TBU, producing pain through increasing tear osmolarity. In contrast, TBU formed by Marangoni-driven fluid flow that rapidly exposes the corneal surface, producing sudden sharp pain is a likely explanation for the formation of TBU in the remaining 2 subjects. The rate of change of fluorescence within areas of TBU or thinning, and the associated pain response, suggest that more than one mechanism may be operative in the formation of TBU.

Commercial Relationships: Carolyn G. Begley, None; Jun Zhang, None; Richard J. Braun, None; Peter E. King-Smith, None

Support: The project was supported by Grant Number R01EY021794 (Dr. Begley) from the National Eye Institute.

Program Number: 6167 Poster Board Number: A0070
Presentation Time: 11:00 AM–12:45 PM

Modeling Evaporative Tear Break Up (TBU) with a Mobile Lipid Layer

Michael Stapf1, Richard J. Braun1, Peter E. King-Smith1, Carolyn G. Begley1. 1Department of Mathematical Sciences, University of Delaware, Newark, DE; 2College of Optometry, The Ohio State University, Columbus, OH; 3School of Optometry, Indiana University, Bloomington, IN.
**Purpose:** Experiments recording tear film thickness reveal localized tear break up (TBU); however, there seems to be multiple causes of local TBU. Our goal is to better understand the causes of TBU by studying a mathematical model for tear film dynamics. To our knowledge, this is the first study of TBU using a mobile lipid layer with variable resistance to evaporation. We aim to reveal details of TBU from physically reasonable parameters and conditions caused by elevated evaporation due to lipid defects, and how this process can be aided by surfactant-driven Marangoni flow.

**Methods:** We study a math model for TBU including a mobile aqueous layer; a mobile lipid layer; tear viscosity; evaporation depending on lipid thickness and environmental humidity; osmosis from the epithelium; surface tension at the layer surfaces; and surface tension variation at the aqueous-lipid interface caused by polar lipid surfactants (Marangoni effect). We simulate this system for a variety of reasonable parameter values and analyze the results. We which effects contribute to localized TBU in some cases, then compare with in vivo experiments of King-Smith et al. (2013, IOVS 54:4900).

**Results:** Case A involves a lipid defect, such as a hole in the lipid layer with reduced resistance to evaporation. Results show the lipid defect causes elevated evaporation, resulting in local thinning. Evaporatively-driven thinning competes with surface-tension-driven aqueous flow toward the TBU region, however evaporation is dominant. This thinning can cause TBU and elevated osmolarity over several seconds, which is consistent with TBU seen in experiment. Case B considers simulations containing a region of significantly elevated surfactant concentration in the lipid layer. The surfactant drives flow in both layers, and rapidly creates a local thin region in the lipid layer (in well under a second) that changes little thereafter. The thin lipid results in increased thinning, and subsequent evaporative TBU over several seconds. Case B is remarkably similar to the results of Figure 6 in King-Smith et al (2013).

**Conclusions:** A mathematical model for tear film TBU with a dynamic lipid layer was studied. Evaporation through a thin local defect can lead to TBU in the model. The model can also capture Marangoni driven effects that cause local defects in the lipid layer leading to local TBU as seen experimentally.

**Commercial Relationships:** Michael Stapf, None; Richard J. Braun, None; Peter E. King-Smith, None; Carolyn G. Begley, None

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**Program Number:** 6168 **Poster Board Number:** A0071 **Presentation Time:** 11:00 AM–12:45 PM

**Human Tear-Film Evaporation Rates from Infrared Ocular-Surface Cooling and Fluorescein Breakup**

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**Purpose:** Tear-film evaporation is critical for maintaining anterior-eye health and comfortable contact-lens wear. For the first time, we devise a quantitative method for ascertaining in-vivo tear evaporation rates (TERs) from simultaneous measurement of transient corneal surface cooling and fluorescein breakup.

**Methods:** Ocular surface temperature (OST) and tear-film stability by fluorescein areal breakup were assessed for 20 subjects following Li et al. [1]. OST was obtained using a high-resolution infrared camera [1]. The dynamic area fraction of breakup after fluorescein instillation was evaluated through image analysis [1]. To interpret the in-vivo data (i.e., extract TERs from OST measurements), we developed a transient 1D heat-transfer model. The analysis accounts for evaporative cooling of the tear film, convective and radiative heat loss to the environment, and heat supply to the corneal surface by conduction from the anterior chamber. Based on the work of Peng et al [2], we propose that tear evaporation through black spots is close to that of pure water, whereas tear evaporation over the remainder of the cornea is through a lipid covering. Consequently, the area of black spots increases as tear breakup proceeds yielding locally-elevated TERs. The overall corneal TER is the area-weighted sum of evaporation through the lipid-free area and through the lipid-covered area.

**Results:** Figure 1 graphs dynamic OST for three human subjects (filled symbols). Shaded regions correspond to no tear-film breakup, with TERs that are 80-90% of that measured for pure water. In all cases, shaded regions overestimate OSTs. Solid lines account for increased tear evaporation through black-spot regions giving increased cooling of the eye. Reductions in evaporation rates through the lipid-covered regions are listed for each subject. They range from 62 to 95% reductions compared to that of pure water.

**Conclusions:** We developed a new procedure to extract human TERs from transient OST measurements. We establish that tear-film breakup must be accounted for when interpreting OST data. Our measured aqueous TERs through lipid layers range from to g/cm²/s (i.e., 62 to 95% that of pure water).


**Commercial Relationships:** Clayton J. Radke; Thomas Dursch, None; Wing Li, None; Baseem Taraz, None; Meng C. Lin, None

**Program Number:** 6169 **Poster Board Number:** A0072 **Presentation Time:** 11:00 AM–12:45 PM

**Clinical Research of Using Oculus Keratograph for Observing the Location of First Tear-Film Break-up Point in Healthy and Keratoconus Eyes**

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**Purpose:** To observe the location of first tear-film break-up point in keratoconus and healthy eyes with Oculus Keratograph.

**Methods:** This was a cross-sectional and single-masked study. 31 eyes of 31 patients(7 women, 24 men) comprised the healthy group, with a mean age of 24.50±6.46 years. 50 eyes of 42 patients(9 women, 41 men) comprised the keratoconus group with a mean age of 26.19±6.46 years. 51 eyes of 51 patients were recruited for the study, 25 keratoconus eyes and 26 healthy eyes.

**Results:** Our study used the first mode of Oculus Keratograph for observing the location of first tear-film break-up point on healthy and keratoconus eyes. The results showed that there is a significant difference in the distribution of the first tear-film break-up point in keratoconus and healthy eyes. The first tear-film break-up point in keratoconus eyes tended to be located further from the central cornea compared to healthy eyes.

**Conclusions:** The Oculus Keratograph is a valuable tool for observing the location of first tear-film break-up point in keratoconus and healthy eyes. This information can be used to understand the pathogenesis of keratoconus and to develop new treatments.

**Commercial Relationships:** None

**Program Number:** 6169 **Poster Board Number:** A0072 **Presentation Time:** 11:00 AM–12:45 PM

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Purpose: Keratoconus patients suffered worse dry eye symptoms than healthy person. Tear-film most frequently broke up in steepest corneal quadrant in keratoconus without dry eye patients (contingency coefficient = 0.47, P = 0.03). When the cone took place in inferonasal and infratemporal quadrant, tear-film most frequently broke up in the same area (inferonasal quadrant: OR = 12, 95% CI: 1.25-115.36, P = 0.031; infratemporal quadrant: OR = 3.00, 95% CI: 1.39-287.60, P = 0.02). There is no significant correlation between them in normal group (Figure 2, P = 0.23). Tear film more frequently broke up in periphery area in healthy group (P = 0.01).

Conclusions: Keratoconus patients suffered worse dry eye symptoms than healthy person. Tear-film most frequently broke up in steepest corneal quadrant in keratoconus eye. But for healthy person, tear film usually broke up in periphery cornea.

Results: 46%/23 eyes) of keratoconus patients had dry eye disorders. Keratoconus without dry eye patients had worse OSDI and fluorescein staining scores as well as Schirmer I test compared with healthy group (P < 0.05). The NIBUT values showed no significant difference (P > 0.05). The steepest corneal point mostly located in infratemporal quadrant of keratoconus group (23 eye, 46%), while in inferonasal quadrant of normal group (11 eye, 35.48%). The quadrant of steepest corneal point showed a significant positive correlation with first tear-film break-up point in keratoconus without dry eye patients (contingency coefficient = 0.47, P = 0.03). When the cone took place in inferonasal and infratemporal quadrant, tear-film most frequently broke up in the same area (inferonasal quadrant: OR = 12, 95% CI: 1.25-115.36, P = 0.031; infratemporal quadrant: OR = 3.00, 95% CI: 1.39-287.60, P = 0.02). There is no significant correlation between them in normal group (Figure 2, P = 0.23). Tear film more frequently broke up in periphery area in healthy group (P = 0.01).

Conclusions: Keratoconus patients suffered worse dry eye symptoms than healthy person. Tear-film most frequently broke up in steepest corneal quadrant in keratoconus eye. But for healthy person, tear film usually broke up in periphery cornea.

Program Number: 6171 Poster Board Number: A0073
Presentation Time: 11:00 AM–12:45 PM

How Do All Those Physical Effects Fit in with Tear Break Up (TBU)?

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Purpose: Models for TBU include many effects: surface tension of the tear/air interface; tear viscosity; evaporation of water to the environment; variation of surface tension from lipids and proteins at the aqueous/lipid interface (the Marangoni effect); osmotic flow from the epithelium; fluorescent intensity (to name just some). We hypothesize two main modes of TBU: Case I due primarily to evaporation and Case II due primarily to the lipid-driven Marangoni effect. The physical effects must combine differently in each case. We aim to clarify when the different effects are important in TBU.

Methods: Tear films of subjects were simultaneously recorded using either: (1) fluorescein (FL) and retroillumination (RI) methods or (2) FL and lipid microscopy (LM) for lipid layer thickness (Braun et al., 2015, PRER, 45, 132). Using these images for close comparison, math models were solved for the tear film thickness, insoluble surfactant concentration (representing the polar part of the lipid layer), as well as osmolarity and fluorescein concentrations inside the tear film. Fluorescein concentration was converted to fluorescent intensity as described by Nichols et al. (2012, IOVS, 53, 5426).

Results: In Case I, experimental FL+LM images show that the lipid layer is practically stationary, and TBU develops over a period of seconds or more through what are effectively holes in the lipid layer. Theory confirms that this TBU mode has localized evaporation competing with surface tension driven flow and diffusion of solutes; the details of the balance depend on TBU spot size. Osmolarity is steadily elevated in this case. In Case II, FL+RI or FL+LM experiments show sub-second spreading of the lipid layer and tear film and subsequent TBU. The spots tend to widen much more quickly and to a larger extent than for Case I. Theory confirms that Marangoni flow is dominant in the thinning here but it may cooperate with evaporation sometimes. Osmolarity does not immediately rise from the Marangoni flow as in Case I. Case II TBU can be driven by patches of lipid that are either mobile (bubbles bursting) or less mobile (globs). FL intensity results from the math models is consistent with, and helps interpret, the experiments.

Conclusions: The experimental data from subjects and mathematical models point to two modes of TBU, with Case I dominated by evaporation and Case II dominated by Marangoni effects. Subcases within the latter are possible.

Commercial Relationships: Richard J. Braun, None; Carolyn G. Begley, None; Peter E. King-Smith, None
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Program Number: 6171 Poster Board Number: A0074
Presentation Time: 11:00 AM–12:45 PM

Mathematical Modeling of Glob-Driven Tear Film Breakup

Lan Zhong1, Christiaan F. Ketelaar2, Richard J. Braun1, Tobin A. Driscoll3, Peter E. King-Smith1, Carolyn G. Begley4. 1Department of Mathematical Sciences, University of Delaware, Newark, DE; 2School of Optometry, Indiana University, Bloomington, IN; 3College of Optometry, The Ohio State University, Columbus, OH.

Purpose: Tear film thinning and breakup are often correlated with thin lipid due to higher evaporation rates in such areas. However, tear film break-up (TBU) has also been observed in the corresponding areas where lipid is more mobile. These models aim to clarify when the different effects are important in TBU.

Methods: Tear films of subjects were simultaneously recorded using either: (1) fluorescein (FL) and retroillumination (RI) methods or (2) FL and lipid microscopy (LM) for lipid layer thickness (Braun et al., 2015, PRER, 45, 132). Using these images for close comparison, math models were solved for the tear film thickness, insoluble surfactant concentration (representing the polar part of the lipid layer), as well as osmolarity and fluorescein concentrations inside the tear film. Fluorescein concentration was converted to fluorescent intensity as described by Nichols et al. (2012, IOVS, 53, 5426).

Results: In Case I, experimental FL+LM images show that the lipid layer is practically stationary, and TBU develops over a period of seconds or more through what are effectively holes in the lipid layer. Theory confirms that this TBU mode has localized evaporation competing with surface tension driven flow and diffusion of solutes; the details of the balance depend on TBU spot size. Osmolarity is steadily elevated in this case. In Case II, FL+RI or FL+LM experiments show sub-second spreading of the lipid layer and tear film and subsequent TBU. The spots tend to widen much more quickly and to a larger extent than for Case I. Theory confirms that Marangoni flow is dominant in the thinning here but it may cooperate with evaporation sometimes. Osmolarity does not immediately rise from the Marangoni flow as in Case I. Case II TBU can be driven by patches of lipid that are either mobile (bubbles bursting) or less mobile (globs). FL intensity results from the math models is consistent with, and helps interpret, the experiments.

Conclusions: The experimental data from subjects and mathematical models point to two modes of TBU, with Case I dominated by evaporation and Case II dominated by Marangoni effects. Subcases within the latter are possible.

Commercial Relationships: Richard J. Braun, None; Carolyn G. Begley, None; Peter E. King-Smith, None
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thicker lipid area that causes significant tangential flow and thinning. For the purposes of brevity, we call these thick lipid regions globs. This study used a mathematical model to test the hypothesis that lipid globs with different composition can cause tangential flow, which subsequently drives TBU near the glob.

**Methods:** We developed a mathematical model to simulate the glob’s different composition by assuming that the glob has a higher surfactant (polar lipid) concentration. Increased surfactant concentration was assumed to lower the tear film surface tension according to a linear equation of state; this is how the model incorporates the “Marangoni effect.” The corresponding math models were solved in the local region for the tear film thickness ($h$), the pressure inside the film and insoluble surfactant concentration ($\Gamma$) using a custom MATLAB program.

**Results:** When the glob has an elevated surfactant concentration, the surface tension is lower at the glob compared to the surrounding fluid; this leads to strong tangential flow away from the glob and may cause TBU. The figures show that TBU can be observed in about a second (0.63s). Flow away from the glob extracts fluid from under the glob. The model predicts that smaller globs (down to 45 µm radius) or thinner tear films will decrease TBU time (TBUT). For spots smaller than 45 µm in a 3.5 µm tear film, TBUT increases; where the increase begins depends on the tear film parameters. The model predicts increasing evaporation rate and stronger Marangoni effect decreases TBUT.

**Conclusions:** This model predicts that excess polar lipid can lead to TBU in appropriate time and length scales. The model predicts that TBUT decreases with decreasing glob size (up to a point) and tear film thickness, as well as increasing evaporation rate and surface tension difference (Marangoni effect).

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**Purpose:** To describe how, in addition to evaporation, divergent flow of tears can contribute to breakup.

**Methods:** Evidence for contributions of divergent flow of tears to tear film breakup was evaluated from fluorescein video recordings from over 100 subjects (method of King-Smith et al., 2013, IOVS 54, 6003). Two characteristics can be used to distinguish fluorescence dimming due to divergent flow, from that due to evaporation. First, evaporation is relatively slow, causing steady dimming over several seconds, whereas divergent flow can be much more rapid. Second, evaporation causes fluorescence dimming by quenching. Quenching does not occur when fluorescein concentration is much below a “critical concentration” of 0.2% (Nichols et al., 2012, IOVS, 53, 5426); thus dimming at low concentrations is probably due to divergent flow. Here, results for one subject – a 45 year old male with mild dry eye (OSDI score 19%) – illustrate characteristics associated with divergent tear flow.

**Results:** Fig. 1 shows images obtained after instillation of 1 µL of 0.1% fluorescein, yielding a concentration, after dilution by tears, much below the critical concentration. Fig. 1A, recorded just after a blink, shows dark patches which may be caused by spreading of thick “globs” (g) of lipid under an outward surface tension gradient. The dark spots appear too soon and the fluorescein concentration is too low for them to be explained by evaporation. Fig. 1B shows that,
later, globs may be stretched into “comets”, (c), by upward flow of the lipid layer. Fig. 1C indicates binding (b) of the lipid layer to the corneal surface; this binding effect is deduced from Fig. 1D, recorded after the next blink, which shows bright “afterimages” (a) interpreted as grooves generated in the corneal surface by shear stress from the lipid layer. Fig. 2 shows a lipid layer image (Braun et al., 2015, Prog Ret Eye Res, 45, 132) of comets and globs from another subject.

**Conclusions:** Divergent flow of tears can make important and characteristic contributions to tear film breakup.

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Fig. 1. A, B and C were recorded 0.3, 1.4 and 8.5 seconds after a blink. D (contrast increased) was recorded after the next blink.

- g, globs; c, comets; b, binding of lipid layer to corneal surface; a, afterimages; f, lens fluorescence; r, illumination reflection.

Fig. 2. Comets (c) and globs (g) in the lipid layer of a 41 year old white female (normal OSDI), recorded 0.2 seconds after a blink (contrast doubled).

**Commercial Relationships:** Peter E. King-Smith, None; Padmapriya Ramamooorthy, None; Carolyn G. Begley, None; Richard J. Braun, None

**Support:** NIH Grants EY017951 (PEK-S), EY021794 (CGB), NSF Grant 1412085 (RJB)
Purpose: The purpose of this study is to develop mathematical models to simulate the tear film dynamics on an eye-shaped domain during a realistic blink cycle. In the model we examine the influence of the blink on tear film formation. To our knowledge, this is the first mathematical model of tear film dynamics during blinking over the whole exposed ocular surface, and experimental methods do not yet have the capability to estimate the tear film thickness in the same detail. Therefore, the model is expected to improve understanding of tear film formation and to make predictions that may be experimentally tested in the future.

Methods: We formulate a mathematical model for the moving eye-shaped domain via a computational least-squares fit to the lid margins from a video recording of a blink. The result becomes the moving boundary for the simulation of tear film dynamics derived using a thin film approximation to the fluid flow inside that moving domain. The model includes surface tension, viscosity, evaporation and wetting of the ocular surface. We then implemented a moving overset grid method to numerically approximate the thin film equations for the tear film dynamics. The numerical approach is implemented in the Overture framework.

Results: The formation of the tear film over the eye during the upstroke is sensitive to the speed of lid motion, as well as to boundary fluxes from lacrimal supply and punctal drainage. A sufficiently large supply of aqueous tear fluid under the moving lids is required for adequately coating the ocular surface. Our results will be closely compared with prior modeling results as well as available experimental results.

Conclusions: A simulation of the tear film dynamics on a blinking eye-shaped domain was created and its influence on the tear film formation during the upstroke was studied. We proposed quantities that are candidates for experimental verification.

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Program Number: 6174 Poster Board Number: A0077

Presentation Time: 11:00 AM–12:45 PM

Assessing the morphology of high speed videookeratoscopy recordings for the evaluation of tear film surface quality in contact lens wear

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Purpose: To evaluate pre-lens tear film surface quality (TFSQ) using high speed videokeratoscopy (HSV) with two types of Si-Hy contact lenses (CL1: Somofilcon A, CL2: Sterifilcon A).

Methods: Ten subjects (6F & 4M, aged 20-40) participated in the study. Assessment on day one included fluorescein tear film break up time (BUT), measuring meniscus height, assessing lid margins redness and roughness, meibomian glands, tarsal, and palpebral conjunctiva. Participants also completed the Ocular Surface Disease Index (OSDI) questionnaire and subjective assessment of CL comfort. On the second day each patient was randomly fitted with different types of CLs in each eye (masked for subjects and operators) and non-invasive assessment of TFSQ was evaluated before insertion, 20min after, and after 7h. The acquired recordings were then subjectively evaluated by two operators, noting morphological changes in Placido disk patterns in three score categories: adequate, satisfactory or inadequate, separately for five zones of the cornea. Further, non-invasive BUT (NIBUT) was estimated for each of the recordings.

Results: The differences between the scores of both eyes were recorded for bare eye condition and the results showed slightly better score for left eye especially in the central zone. Both lenses similarly decreased the quality of the tear film, except L2 in the superior zone. Although the decrease for both CLs was comparable, in absolute terms more adequate counts were found in the central zone of L2. Four patients indicated differences in lens comfort at the end of wear with three ranking L1 as less comfortable than L2. No association was found between subjective assessment of comfort and fluorescein BUT or scores of OSDI. NIBUT for baseline, morning and afternoon measurements ranged from 3.1-37.6s, 0-37.9s and 0-37.9s, respectively. Similarly, no association was found between those values and any other clinical parameter evaluated in the study.

Conclusions: The dynamics of TFSQ on CL surface is different to that of a bare eye. The superior zone may be more related to comfort due to the lid while the central zone is related to quality of vision. In our study NIBUT estimates did not add information in terms of differences in quality of TF especially during CL wear. However, assessing TF using HSV could optimise CL wear in the future.

Commercial Relationships: Clara Llorens Quintana, None; Maryam Mousavi, None; Dorota H. Szczesna-Iskander, None; D Robert Iskander, None

Support: This study was supported by Marie Sklodowska-Curie Innovative Training Networks grant, EDEN (European Dry Eye Network), ID 642760.
(Systane Ultra, Alcon Laboratories, Inc, Fort Worth, TX, USA) at the other visit. LLT was measured again 15 minutes after instillation. Visits were randomized so the data analysis was masked.

**Results:** Analysis was done on the worst eye (having the lowest LLT at baseline) for each patient. The average baseline LLT before using a lipid containing eye drop was 49.46 ± 9.18 nm. Fifteen minutes after instillation of the eye drop with lipid, the average LLT was 77.5 ± 29.3 nm, showing an increase of 28.04 ± 27.36 nm (p=0.001). Of the 35 subjects, 22 had a LLT increase of 15mm or greater. Fifteen minutes after instillation of the eye drop without lipid, the average LLT was 50.0 ± 12.9 nm which was not statistically significant from baseline (p=0.60). Of the 35 subjects, one subject had a LLT increase of 15 nm or greater.

**Conclusions:**
The lipid layer is the most anterior layer of the preocular tear film and is important for tear film stability and is dependent on meibomian gland function. Here we demonstrate that in subjects with MGD, lipid containing artificial tears increase the thickness of the lipid layer of the tears, while aqueous or nonlipid containing artificial tears do not make a significant difference in the LLT.

**Commercial Relationships:** Jennifer Fogt, Valeant Pharmaceuticals (R); Matthew Kowalski, None; Peter E. King-Smith, None; Joseph T. Barr, Valeant Pharmaceuticals (R), Alcon Laboratories, Inc (C); Valeant Pharmaceuticals (C)

**Support:** Valeant Pharmaceuticals

**Program Number:** A0079

**Presentation Time:** 11:00 AM–12:45 PM

**Effect of preservatives in artificial tears on bacterial growth in a blepharitis setting**

**Armin Mohi1, Susanne Hauswaldt1, Salvatore Grisanti2, Martin Rudolf3**

1 Department of Microbiology, University of Luebeck, Luebeck, Germany; 2 Eye Hospital, University of Luebeck, Luebeck, Germany; 3 Department of Microbiology, University of Luebeck, Luebeck, Germany.

**Purpose:** Blepharitis is a chronic inflammatory disease of the eyelids. Although the etiology is complex and not fully understood, it is general consensus that bacteria and inflammation are a main part of this pathology. A continuous treatment with artificial tears is fundamental treatment strategy. In this study we investigated the potential antimicrobial effect of different preservatives in artificial tears on the bacterial flora of blepharitis patients.

**Methods:** We incubated agar-plates with different bacteria which are commonly found in blepharitis patients (S. aureus; S. epidermidis; C. amycolatum, A. baumannii, P. acnes) to perform an agar diffusion test (Kirby-Bauer testing). We soaked the testing discs with different eye drops. We used commercial artificial tears. One with benzalkonium chloride (BAK) and two with polyhexamethylene biguanide (PHMB) in different concentrations (0,001 vs. 0.0015 mg/ml) as eye drop preservative. We compared this eye drops with a preservative free compound and two different antibiotic eyedrops (Ofloxacin, Gentamicin). In addition we tested PHMB in different concentrations (0,001, 0.01, 0.1, 0.2, 0.3 mg/ml) in the same setting. After 24-48 h (incubation, 37°C) we evaluated the effect of the different compounds on the bacterial growth.

**Results:** As expected Ofloxacin and Gentamicin showed a good reduction of bacterial growth in all tested bacteria (18-40 mm). The tested preservatives showed a poor reduction of the bacterial growth. The PHMB showed no reduction at all in the used concentrations. The BAK showed a significant reduction on the P. acnes culture. The preservative free eye drop showed no reduction at all.

The PHMB alone showed a significant reduction beginning from a concentration of 0.01 mg/ml on Staph. aureus, Staph epidermidis and P. acnes. Beginning from 0.1 mg/ml on Corynebacterium acylactum and beginning from 0.2 mg/ml on A. baumannii.

**Conclusions:** The collected data showed no significant reduction of bacterial growth by using PHMB in this low concentration as an eye drop preservative. By using a higher concentration (>0.01 mg/ml) we could see a significant reduction on 3 of the 5 tested bacteria cultures. The BAK showed a good effect on the growth of P. acnes. There could be a positive effect on the bacterial load and the chronic inflammation of blepharitis patients by using a higher concentration of PHMB as a preservative in artificial tears.

**Commercial Relationships:** Armin Mohi; Susanne Hauswald, None; Salvatore Grisanti, None; Martin Rudolf, None

**Program Number:** 6177 Poster Board Number: A0080

**Presentation Time:** 11:00 AM–12:45 PM

**Does the concentration of neuropeptides in tears differ based on extent of meibomian gland drop out?**

Stephanie Cox, Jason J. Nichols. School of Optometry, University of Alabama at Birmingham, Birmingham, AL.

**Purpose:** To determine if there is a difference in the concentration of vasoactive intestinal polypeptide (VIP), calcitonin gene-related peptide (CGRP), neuropeptide Y (NPY), or substance P (SP) in tears of subjects with varying amounts of meibomian gland (MG) drop out.

**Methods:** Subjects were recruited and assigned to a group based on extent of MG drop out (worse of two eyes). Those with a MG drop out score of 0 or 1 on the Arita scale (less than 33% MG drop out) for the lower eyelid were assigned to group 1, and those with a score of 2 or 3 (33% or greater) were assigned to group 2. Tears were collected from the inferior tear meniscus of the eye with the most drop out using a microcapillary tube and stored in glass amber vials at -80 degrees Celsius until analysis. Each sample was optimized for peptide testing using C18 spin columns, and the retrieved peptides were split into four for use in the four neuropeptide ELISA kits. The concentration of each neuropeptide in each sample was found using standard and curve and multiplied by four to find concentration in the entire sample. The maximum likelihood method was used to account for concentrations beneath the limits of detection. The concentrations of the various neuropeptides were normalized by dividing each concentration by the amount of sample collected for each subject. Group neuropeptide concentrations were compared using a Mann-Whitney U test.

**Results:** Subjects had an average age of 34.9 ± 15.5 years and 63% were female. Group 1 included 29 samples and group 2 included 31 samples. For VIP, the median normalized concentrations (interquartile ranges) were 35.12 ng/mL/mL (46.92) and 29.61 ng/mL/mL (26.25) for groups 1 and 2, respectively (p = 0.70). The median normalized concentrations of CGRP were 159.16 ng/mL/mL (479.51) and 91.97 ng/mL/mL (252.92) for groups 1 and 2, respectively (p = 0.05). For NPY, the median normalized concentrations were 34.53 ng/mL/mL (62.84) and 30.87 ng/mL/mL (36.03) for groups 1 and 2, respectively (p = 0.42). For SP, the median normalized concentrations were 58.90 ng/mL/mL (229.16) and 27.48 ng/mL/mL (116.66) for groups 1 and 2, respectively (p = 0.21).

**Conclusions:** A statistically significant difference between groups based on extent of MG drop out was not found. This suggests that tear film associated neuropeptides related to sympathetic, parasympathetic, or sensory nervous systems may not be related to MG drop out.

**Commercial Relationships:** Stephanie Cox, None; Jason J. Nichols, None
The purpose of this study is to investigate the feasibility of using a customized imager to evaluate the tear film dynamics in vivo, by simultaneously estimating the thickness of the lipid and aqueous layers of the tear film through an optical approach.

**Methods:** The imager consists of an advanced optical coherence tomography and a robust maximum-likelihood estimator, which has been validated using tear film phantoms. To evaluate the tear film dynamics in vivo, a chinrest with translational adjustments was developed for a rough alignment of the subject with the imager. A finer alignment has been realized through a dual axis galvo-scanner, which performs rapid scans over the corneal surface and identifies the corneal apex location. The scanner then immediately steers the light beam to the apex area and scans over a 0.5 mm by 0.5 mm area with 30 by 30 sampling points. Due to the curvature of the cornea and the telecentric scanning scheme of the current system, only the point with the maximum spectrum intensity is selected and processed for an estimation, which is considered to have a normal incidence of light. In the data acquisition process, the subject is asked to have a complete blink and then keep the eye open for 10 seconds.

**Results:** A tear film dynamics curve acquired from an Asian male (normal subject, age 29) is shown in Figure 1, which illustrates the temporal change of both the lipid and aqueous layer thicknesses in a 10 seconds time frame after a blink, in vivo. Right after a complete blink, the lipid layer gets thicker rapidly with a thickening rate of 12 nm/s, and it stabilizes after about 2 seconds; the aqueous layer gets thinner gradually, from about 5 micron right after a blink down to about 2 micron in 10 seconds, with an average thinning rate of 0.3 micron/s.

**Conclusions:** The temporal change of the simultaneous acquisition of the lipid and aqueous thicknesses has been successfully measured in vivo. The customized tear film imager is shown to be feasible to investigate the tear film dynamics. Future work will integrate a scanning scheme that will allow the normal incidence of the scanning beam along the corneal surface, enabling measurements of thickness maps.

Thickness dynamics of (a) lipid layer and (b) aqueous layer after a blink

**Commercial Relationships:** Jannick P. Rolland-Thompson, Jinxin Huang, University of Rochester (P); Holly B. Hindman, None
Estimating Fluorescein Concentration in the Tear Film via Two Methods

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Purpose: Fluorescein (FL) concentration in the tear film is needed to better estimate relative changes in tear film thickness via changes in fluorescence during tear break-up (TBU). The purpose of this study was to develop methods to measure FL concentration via image analysis of the inferior meniscus and by direct measurement of very small samples.

Methods: An eye meniscus model was constructed to mimic the inferior meniscus with an upper sphere mimicking the cornea with radius of curvature of 7.8mm and a lower sphere forming an angle of 76.5 degrees (Wu et al., 2015). Two microliters of various FL concentrations were imaged on a slit lamp biomicroscope using the same light levels and conditions used on human subjects. Images were converted to grayscale and FL (pixel) intensity measured by a custom MATLAB program. Fluorescence intensity of 5 microliter samples of the known concentrations was then measured on an RT-PCR (Real time polymerase chain reaction). Calibration curves for FL concentration were developed for the slit lamp biomicroscope and PCR data.

Results: Fluorescence intensity measured by the slit lamp biomicroscope image analysis reached its maximum at the 0.04% concentration and then rapidly decreased due to concentration self-quenching. A similar pattern was reached with PCR data, with a maximum at 0.14%FL. FL concentrations of 0.6%, 0.4%, 0.3%, 0.14 and 0.04%, yielded average pixel intensity values of 25.5, 53.2, 76.6, 149.1 and 150.55 (image analysis) and Rpre (Initial fluorescence from pre-read) values of, 21561, 30531, 25574, 47002 and 16433.

Conclusions: These methods show promise for measuring FL concentrations in the tear film from the inferior meniscus. During TBU, as visualized via tear film fluorescence, both FL concentration and tear film thickness are unknown. Knowledge of FL concentration within the tear film before TBU begins allows better estimation of relative tear film thickness changes during TBU.

Commercial Relationships: Deborah Antwi, None; Ashley Ryckman, None; Lindsey Becker, None; Jun Zhang, None; Richard J. Braun, None; Peter E. King-Smith, None; Carolyn G. Begley, None

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Program Number: 6181 Poster Board Number: A0084
Presentation Time: 11:00 AM–12:45 PM

Does hyperosmolality induce an irreversible process leading to human corneal epithelial cell death?

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Purpose: Tear film hyperosmolality is a core mechanism of dry eye disease. Hyperosmolality leads to increased ocular surface stress, friction, inflammation and damage, as well as symptoms of discomfort and visual impairment. These detrimental sequelae are mediated in large part through morphological and cytotoxic effects on corneal epithelial cells. We hypothesized that (1) short-term exposure to a hyperosmolar environment may alter cell morphology but not initiate an irreversible process leading to cell death, and (2) extended exposure to the same conditions would be cytotoxic.

Methods: Human corneal epithelial cells (gift from Dr. James Jester) were cultured in normal (290 mOsm/L) or hyperosmolar (308, 338, 400, 600 mOsm/L) keratinocyte serum-free medium for 1, 3, 6 and/or 24 hours. Cells were evaluated for appearance, apoptosis and death with light and fluorescent microscopes. Experiments included positive controls for DNA fragmentation and dead cells.

Results: Our findings demonstrate that hyperosmolality induces morphological and cytotoxic effects in human corneal epithelial cells and that these responses are both dose- and time-dependent. Very few cells die after a 24-hour exposure to 290 or 308 mOsm solutions, cells exposed to 338 mOsm medium exhibit membrane blebs (i.e. a sign of impending cell death), the 400 mOsm condition kills a large percentage of cells, and the 600 mOsm medium causes complete cell death after 3, 6 or 24 hours. Cell death does not appear to be mediated primarily through apoptotic DNA fragmentation. Following a 1-hour exposure to the 600 mOsm medium, cells appear rounded, but still adherent and alive. Limiting this 600 mOsm exposure to 1 hour and replacing with 290 mOsm medium for 23 hours increases cell survival.

Conclusions: Our results indicate that chronic exposure to a hyperosmolar challenge will kill the majority of human corneal epithelial cells, whereas changes in cell morphology due to brief exposures can be largely ameliorated by normalization of the surrounding environment. Our findings also raise the possibility that hyperosmolar conditions may induce forms of death other than apoptosis (e.g. necrosis) in these cells.

Commercial Relationships: Wendy Kam, TearLab Corp. (R); David A. Sullivan, TearLab Corp. (S), TearLab Corp. (R); Benjamin D. Sullivan, TearLab Corp., TearLab Corp. (P), TearLab Corp. (I); Manoj Venkiteshwar, TearLab Corp.

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mice into two groups, SE and EE respectively, and compared tear secretion among all groups.

Results: Tear secretion in the EE group was significantly higher (2.5±1.0 mm, 3.0±1.6 mm, 4.2±1.3 mm) than in the SE group (1.7±0.7 mm, 2.2±0.9 mm, 2.8±1.2 mm, P<0.01, P<0.05, P<0.01, respectively) at week 1, week 2 and week 3 in C57BL/6Jcl mice. After the acute stress test, tear secretion decreased only in the SE group (pre-: 3.8±1.9 mm, post-: 2.3±1.7 mm, P=0.06). Tear secretion in the EE group didn’t change after the acute stress. Tear secretion significantly decreased in BDNF STOpetO hetero mice (1.9±1.0 mm, P<0.05) and homo mice (0.5±0.5 mm, P<0.01) compared to wild type mice (2.4±0.9 mm). Tear secretion increased only in the EE group (4.0±1.6 mm, P<0.01) compared to the SE group (2.4±1.1 mm) in wild type mice at week 2. Tear secretion in hetero mice and homo mice didn’t change under EE compared to under SE.

Conclusions: The results of this study suggest that environmental factors are related to tear secretion. Moreover, these results suggest an association between tear secretion and BDNF gene expression.

Commercial Relationships: Kokoro Sano, None; Motoko Kawashima, None; toshihiro imada, None; Ryuji Hisamura, None; Shigeru Nakamura, None; Fumiya Izumiseki, None; Kenji F. Tanaka, None; Mitsuhiro Watanabe, None; Masaru Mimura, None; Kazuo Tsubota, None

Program Number: 6183 Poster Board Number: A0086
Presentation Time: 11:00 AM–12:45 PM

Evaluation of the SIRT5 deficient mice as dry eye model mice

Purpose: Sirtuins (SIRTs) are nicotinamide adenine dinucleotide (NAD) - dependent protein deacetylases which play important roles in modulating the aging process, metabolism and longevity. SIRTs are important genes regulating the physiological function. Various diseases are caused by aging, SIRTs are an important regulatory factor, of protein modification. The change of various factors and the progression of dry eye syndrome are caused by aging, the involvement of SIRTs are considered as one of the causes. Here, we focused on SIRT5 gene which is related to aging, mitochondrial function and metabolism. We investigate the tear secretion, histopathological analysis, related protein expression and metabolome analysis and evaluate the possibility of whether SIRT5 deficient mice can be used as a dry eye model mice.

Methods: SIRT5 deficient mice were used in all experiments. As comparison, C57BL/6J mice were used. The tear volume was measured by the phenol red thread into nasal side of the eyelid margin for 30 seconds once every two weeks. The mice were sacrificed and the lacrimal glands were removed. Histopathological analysis was performed to analyze tissue sections using an optical microscopy after hematoxylin and eosin (H/E) and Oil Red O staining. Lacrimal gland tissue was examined by metabolome analysis, evaluated by Western blotting about nearby changed protein expression.

Results: Tear secretion was measured by the phenol red thread test, results decreased in SIRT5 deficient mice compared to that in control mice at 10-30 weeks old. Moreover, H/E staining of the lacrimal glands in SIRT5 deficient mice showed histological change with an accelerated aging phenotype, accumulated the lipid droplet in SIRT5 deficient mice. In addition, after comparing the relations of the tear secretion with the metabolism change in the lacrimal gland, the change of the signal about metabolism of the fatty acid including the HMG-CoA was cleared.

Conclusions: In SIRT5 deficient mice, the decrease of tear secretion and the accumulation of the lipid droplet were confirmed, the possibility that the metabolism change of the fatty acid including the HMG-CoA was suggested.

Commercial Relationships: YASUHISA TANAKA, None; Takaaki Inaba; Kazuo Tsubota, None

Program Number: 6184 Poster Board Number: A0087
Presentation Time: 11:00 AM–12:45 PM

Biophysical interactions of essential fatty acids with human meibomian lipids at an air-tear interface

Purpose: Dry-eye is a debilitating condition of the ocular surface. One of the therapies of dry-eye is the use of essential fatty acids (EFAs) which reduce the inflammation and symptoms in dry-eye patients. EFAs are mainly given by dietary supplementation. Recently, eye drops containing EFAs have been developed and are being investigated. EFAs applied topically to the ocular surface might interact with the lipid layer of the tear film and affect its integrity. Therefore, the aim of this study was to investigate the biophysical interactions of EFAs with meibomian lipids at an air-tear interface.

Methods: Human meibomian lipids were spread on an artificial tear solution in a Langmuir trough maintained at 35°C. The lipids film at the air-tear interface was compressed and expanded to record pressure-area isocycles. Human meibomian lipids were mixed with EFAs (linoleic acid and α-linolenic acid) in different mole fraction ratios and their interactions were studied by recording pressure-area isocycles of the mixed films.

Results: Linoleic acid added to meibomian lipids film increased its lift-off area and the maximum surface pressure at the highest compression. At 0.2 mole fraction, it increased the lift-off area from 30 to 34 Å², and maximum surface pressure from 14 to 23 mN/m. Increasing amounts of the fatty acid further increased lift-off area and maximum pressure till 0.6 mole fraction (40 Å², 34 mN/m) after which there was no further increase. α-Linolenic acid showed a different effect on meibomian lipids. At 0.2 mole fraction, it decreased the lift-off area from 30 to 24 Å², and maximum surface pressure from 14 to 12 mN/m. Increasing amounts of the fatty acid further decreased lift-off area (17 Å² at 0.8 mole fraction) but there was no further decrease in the maximum surface pressure.

Conclusions: EFAs added topically to the ocular surface are likely to interact with the lipid layer of the tear film and affect its biophysical function. Linoleic acid expands meibomian lipids film and makes it exert more surface pressure which can be beneficial for tear stability. α-Linolenic acid condenses meibomian lipids film with less surface pressure which may not be beneficial for tear stability. Further studies supplemented with clinical evaluations of ocular surface parameters will be needed to determine the benefits of topical application of EFAs for dry-eye.

Commercial Relationships: Poonam Mudgil, None

Program Number: 6185 Poster Board Number: A0088
Presentation Time: 11:00 AM–12:45 PM

Palmitolenic acid, a constituent of the sea buckthorn (Hippophae rhamnoides) oil fatty acid, restores tear secretion in a murine dry eye model

Purpose: Sea buckthorn (Hippophae rhamnoides) seed and pulp oils have traditionally been used for a food and medicinal ingredient in eastern countries. Consumption of daily oral sea buckthorn (SB)
oil was reported to attenuate the symptoms of dry eye patients. The purpose of this study was to investigate orally intake of SB pulp oil and a constituent palmitolenic acid on tear secretion using mouse stress induced dry eye model.

Methods: Seven-week-old female C57/B6J mouse were used for this study. Mice were physically restrained in a 50-ml plastic conical tube and subjected to a stream of air directed at the animal’s head at a rate of 0.5-1.0 m/s for 4 hours. They were individually placed in cages with water and food ad libitum for the remaining time. This series of treatments was repeated for up to 5 days. SB pulp oil and palmitolenic acid was orally administered at 2.5mL/kg and 0.22mg/kg daily prior to the stress exposure respectively. Change in tear secretion was measured by the cotton thread test. Tear secretion was measured before (initial value) and 5 days. Five mice were assigned to each treatment group.

Results: A significant decrease in tear secretion was observed in the vehicle value compared with the initial value (p < 0.01). In the SB oil and palmitolenic acid application, slight decreases in the tear secretion were observed, although the differences were not significant compared with the initial values. Change in tear secretion was significantly suppressed in the SB oil and palmitolenic acid compare to the vehicle (p < 0.05).

Conclusions: These results indicate that SB oil restored dry eye symptoms by acting tear secretion capacity by a constituent palmitolenic acid and may represent a very potent nutritional treatment for the prevention of dry eye.

Commercial Relationships: Shigeru Nakamura, None; Yuki Kimura, None; Daisuke Mori; Michiko Shibuya, None; Kazuo Tsubota, Tsutuba Lab Co., Ltd (F)

Support: Supported by Tsutuba Lab Co., Ltd

Program Number: 6186 Poster Board Number: A0089

Presentation Time: 11:00 AM–12:45 PM

Mucin (Qniumucin) extracted from jellyfish can be applied to ophthalmologic researches and/or diagnoses as a substituting material of human mucin: A study on contact lenses

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Purpose: Human mucins which show severe inhomogeneity in a complex chemical structure are not easy to be applied to basic medical studies especially in ophthalmology because of the extremely small amount of each individual tear sample. Instead, a mucin extracted from mesoglea of jellyfish (qniumucin: Q-mucin), which were discovered in 2005 by one of the present author and co-workers, can be used as a substituting material which can be obtained in a large scale. As a test example, we analyzed the surface interaction of contact lenses with Q-mucin solutions which reproduces the interaction between a real tear film and lens surfaces.

Methods: Q-mucin and hyaluronan (HA) was dissolved in saline for various concentrations. Solutions were dropped on various surfaces including soft and/or rigid gas permeable (RGP) contact lenses. The incidentally formed contact angle (CA) and its transient behavior were observed by a TV camera. Values and the transient lineshapes of contact angles were examined to clarify their dependence on the Q-mucin concentration.

Results: Q-mucin solutions (0.01-1.00 wt%) dropped on a hydrophobic glass surface formed very stable droplet of which CA=46°. CA decreases on increase of mucin concentration towards better wettability. For glass plates with additional hydrophilic coatings, CAs became slightly smaller (CA=43°) showing similar dependence on mucin concentrations. On RGP contact lenses, however, CA decreased dynamically within one second, e. g. from 46° down to 8°. Similar transient lineshapes were obtained for various Q-mucin concentrations where the initial peaking values decreased on increase of mucin concentrations. CA of HA solutions increased on increase of its concentrations because the increase in viscosity affects stronger.

Conclusions: Jellyfish mucin was used to mimic the interaction between tear films and contact lens surfaces. Reasonable concentration dependence of wettability was monitored by CA measurements. This method can be applied to estimate unknown mucin concentration of real single tear drop using Q-mucin as a standard.

Commercial Relationships: Kiminori Ushida, None; Ayaka Oohata; Gai Kawamura, None; Yuichi Hori, Santen Pharmaceutical Co., Ltd. (R), Otsuka Pharmaceutical Co., Ltd. (C), Otsuka Pharmaceutical Co., Ltd. (R), Santen Pharmaceutical Co., Ltd. (C), Santen Pharmaceutical Co., Ltd. (F)

Program Number: 6187 Poster Board Number: A0090

Presentation Time: 11:00 AM–12:45 PM

General importance of the wettability of tear films on ocular surfaces which is magnified by mucin molecules involved in tear liquids as a main constituent: Evidence of surface activation property of mucin solutions

Ayaka Oohata, Kiminori Ushida, Yuichi Hori. Department of Chemistry, School of Science, Kitasato University, Sagamihara, Japan.

Purpose: The typical chemical structure of mucins which are involved in tear liquids as a main constituent implies the surface activation property while its evidence has never been confirmed. If they act as surfactants, the tear liquids effectively spread over ocular surfaces forming a very thin and stable tear film without dry islands. In other words, the wettability of mucin solution seems magnified by the existence of mucin molecules. As a result, the lubrication between the ocular surface and the eyelid, and probably that between the surface and a contact lens, are controlled by the concentration of mucin. In order to justify this newly suggested molecular mechanism of dry eye, we measured the surface tension of mucin and hyaluronan (HA) solutions both of which are solutions of polyanions.

Methods: A mucin extracted from mesoglea of jellyfish (qniiumucin: Q-mucin) was used as a standard mucin. The surface tension of each solution was measured in a pendant drop method which can be applied to a single droplet. Droplet shapes were monitored by a TV camera and automatically analyzed by a laptop computer using Young-Laplace equation.

Results: The surface tension of Q-mucin solutions (0.1-10mg/mL) dissolved in saline and pure water was measured. On increase of Q-mucin concentrations in pure water, the surface tension values were gradually decrease from 72 mN/m (that of water) down to 60 mN/m for (10 mg/mL). The lineshapes were similar to that known as a typical behavior of surfactants. In saline solutions of Q-mucin, essentially the same lineshapes were obtained. Contrastingly, saline solutions of HA showed almost no change in surface tension on increase of HA concentration. These results indicate that the mucin solutions have surface activation property but HA solutions do not.

Conclusions: Wettability and lubricating property of tear films seem to be sustained by mucin molecules in tear liquids. Only mucin has this ability because it can effectively act as a surfactant. While it is used as an additive to eye drops, HA did not show any surface activation properties.

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Concentration (mg/mL) dependence of saline solutions of qunimucin (Q-mucin) and hyaluronan (HA)

**Commercial Relationships:** Ayaka Oohata, None; Georgi A. Georgiev, Santen SAS, Evry, France (F); Norihiko Yokoi; Slavyana Ivanova, None; Vesselin Tonchev, None; Philippe Daull, Santen SAS, Evry, France

**Support:** Collaborative study grant by Santen SAS, Evry, France.

**Program Number:** 6188 **Poster Board Number:** A0091

**Presentation Time:** 11:00 AM–12:45 PM

**Surface chemistry of the interactions of cationic nanoemulsions with human meibum films**

Georgi A. Georgiev1, Norihiko Yokoi2, Slavyana Ivanova1, Vesselin Tonchev3, Philippe Daull4, 1Optics and spectroscopy, University of Sofia “St. Kliment Ohridski”, Sofia, Bulgaria; 2Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan; 3Phase Formation, Crystalline and Amorphous Materials, R.Kaischew Institute of Physical Chemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria; 4Research and Development, Santen SAS, Evry, France.

**Purpose:** As cationic nanoemulsions (CNE) are showing promise, as eyedrops and ophthalmic drug (e.g. cyclosporine) delivery vehicles, it is important to know their interplay with the tear film lipid layer. Therefore the interactions of three CNE (Cationorm, Ikervis and Ikervis Vehicle) were studied with human meibum (MGS) films. Both Cationorm and Ikervis formulations contain cetalkonium chloride (CAC) as 0.2 wt% or 0.24-0.25 wt% of their oil phase respectively.

**Methods:** MGS were collected from 4 healthy volunteers (25-36 [30.75±5.12 SD] years old) and then dissolved in chloroform to a unified 1 mg MGS/mL stock solution. MGS and CNE were spread at the air/phosphate buffered saline interface of a Langmuir surface balance to ensure range of MGS/CNE oil phase 2D ratios: 100/1, 50/1, 20/1, 10/1, 5/1, 3/1, 2/1 and 1/1. The films capability to reorganize during dynamic area changes were evaluated through the surface pressure-area compression/expansion isocycles. The layers dilatational rheological properties were probed via the step/relaxation method through Fourier analysis (in the 1-10−4 Hz range) and by exponential decay modeling of the relaxation transients. Films structure was monitored with Brewster Angle microscopy. All the samples were evaluated at 25 and 35°C.

**Results:** At high (≥ 50/1) MGS/oil phase ratios the inclusion of CNE had no noticeable effects on the film properties. In the range of 20/1-2/1 MGS/Ikervis formulations and 20/1-3/1 MGS/Cationorm oil phase ratios the layers showed improved spreading, higher maximum surface pressures and increased film thickness compared to pure MGS. The contribution of the elastic modulus to the film dilatational viscoelasticity also increased. At 1/1 MGS/Ikervis and ≤2/1 MGS/ Cationorm oil phase the layers remained primarily elastic, but the contribution of long (≥ 500 s) viscous relaxation process started to increase and slight heterogeneities in the MGS/Cationorm layers were observed.

**Conclusions:** Under physiologically relevant high MGS/oil phase ratios CNEs interact favorably with MGS films and enhance their structure and surface properties. Only at low MGS/oil phase ratios slight perturbations are observed in the layers structure and viscoelasticity. The latter can be related with the increased amount of water soluble surfactant compounds in the film subphase at high CNE concentrations, which however might be rapidly diluted by the aqueous turnover in vivo.

**Commercial Relationships:** Georgi A. Georgiev, Santen SAS, Evry, France (F); Norihiko Yokoi; Slavyana Ivanova, None; Vesselin Tonchev, None; Philippe Daull, Santen SAS, Evry, France

**Support:** Collaborative study grant by Santen SAS, Evry, France.

**Program Number:** 6189 **Poster Board Number:** A0092

**Presentation Time:** 11:00 AM–12:45 PM

**Treating inadequate lid seal in patients with dry eye using an overnight ointment reduces discomfort upon awakening and overall dry eye symptoms**

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**Purpose:** To retrospectively evaluate whether treating inadequate lid seal in patients with dry eye with an overnight ointment would improve overall symptoms and the specific symptoms of discomfort upon awakening.

**Methods:** The de-identified data of consecutive patients (n=21, 9 males, 12 females), from a single clinic in Boston, MA, who met the inclusion criteria for the study and were fully consented, were included. Inclusion criteria: over the age of 18, no history of lid surgery, no lagophthalmos, no current infective ocular disease, and no ocular surgery within the last 6 months. The patients also all had: 1. A prior diagnosis of dry eye and a history of treatment; 2. A dry eye symptom score of 7 or higher (max score = 28) using the SPEED questionnaire; 3. An ‘eye discomfort upon awakening’ score of 1 or higher (0=no compromise, 1=mild, 2-=moderate, 3=severe). All patients were treated for the inadequate lid seal using a petrolatum ointment at the air/phosphate buffered saline interface of a Langmuir surface balance to ensure range of MGS/CNE oil phase 2D ratios: 100/1, 50/1, 20/1, 10/1, 5/1, 3/1, 2/1 and 1/1. The films capability to reorganize during dynamic area changes were evaluated through the surface pressure-area compression/expansion isocycles. The layers dilatational rheological properties were probed via the step/relaxation method through Fourier analysis (in the 1-10−4 Hz range) and by exponential decay modeling of the relaxation transients. Films structure was monitored with Brewster Angle microscopy. All the samples were evaluated at 25 and 35°C.

**Results:** The mean (± standard deviation) age and symptom scores of the patients were as follows. Mean age: 57.5 ±12.8 years. The SPEED score decreased from 12.2±2.3 pre-treatment to 7.0±3.0 post-treatment (p<0.0001). The discomfort upon awakening score decreased from 1.8±0.6 pre-treatment to 0.6±0.7 post-treatment (p<0.0001).

**Conclusions:** Treatment of compromised lid seal with ointment during sleep improves symptoms of discomfort upon awakening and overall ocular surface symptoms as measured by the SPEED questionnaire. This therapy should be considered as adjunctive therapy for those patients with dry eye and inadequate lid seal whenever discomfort upon awakening is present, including those managed with other therapies.

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Release of Lacritin’s C-Terminal Bacterialic Domain by Esp, a Serine Protease Highly Expressed in Coagulase Negative Clinical Isolates from Bacterial Keratitis, but not from Endophthalmitis or Blepharitis

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Purpose: Lacritin is a multifunctional tear protein with latent bactericidal activity. Its C-terminal bactericidal fragment is constitutively released into tears, likely by a serine protease. Released fragment (alone or in synergy with others) underlies the sterility of human tears (McKown et al, JBC ’14). Here we asked whether commensal S. epidermidis may contribute to lacritin C-terminal proteolysis via its serine protease Esp that was previously shown to disrupt S. aureus biofilm in nasal colonization (Iwase et al, Nature, ’10).

Methods: Recombinant lacritin alone, or mixtures of recombinant lacritin, recombinant Esp and thermolsynin, or recombinant lacritin and thermolsynin were incubated for 20 hours at 37°C, separated by SDS-PAGE and blotted for lacritin. Eight coagulase negative clinical isolates such as from bacterial keratitis, blepharitis and endophthalmitis were expanded, supernatants collected, pooled, separated by SDS-PAGE, and then blotted for Esp. All keratitis supernatants were also individually blotted for Esp. Recombinant Esp without incubation served as an internal blotting control.

Results: Esp, but not Esp activating enzyme thermolsynin, releases a <10 kDa band characteristic of the C-terminal bactericidal fragment. Lacritin alone incubated in parallel remained intact. Clinical isolate blotting detected Esp only in bacterial keratitis, and when examined separately in most keratitis isolates.

Conclusions: Release of lacritin’s C-terminal bactericidal fragment appears to be in part under the control of commensal S. epidermidis via the release of serine protease Esp that releases this latent activity into tears.

Purpose: A chalazion is chronic inflammatory granuloma primarily caused by the retention of a meibomian gland secretion, and it has generally been believed that it is a non-infectious form of inflammation. However, in meibomitis-related ocular surface inflammation, e.g., meibomitis-related keratoconjunctivitis, which is reportedly caused by Propionibacterium acnes (P. acnes), patients often have a past history of chalazion. The purpose of this present study was to evaluate the role of P. acnes in the pathogenesis of chalazion.

Methods: This study involved 8 patients (age range: 3-37 years) who underwent surgery to remove a chalazion cyst. In 4 of the 8 patients, solid meibum in the chalazion was obtained at the time of surgery, stored in chloroform/methanol, and analyzed using gas chromatography mass spectrometry for composition analysis. The chalazion tissue of each patient was soaked in 10% buffer formaldehyde, embedded in paraffin, sectioned, and stained by hematoxylin and eosin as well as by the monoclonal antibody of P. acnes.

Results: Our findings showed positive for P. acnes in 2 patients with typical granuloma formation with epithelialoid cells and polymuclear giant cells, which had not been treated with antimicrobial agents and/or steroids. On the other hand, they showed negative for P. acnes in the other 6 patients without typical granuloma formation treated with antimicrobial agents and/or steroids. Compared to that of the 12 normal control subjects (NC) which we previously reported (ARVO 2014), analysis of the fatty acid composition of the solid meibum in chalazion revealed a significant increase of straight-chain saturated fatty acid (17.7%, NC: 2.8%) (p<0.05) and a significant decrease of monounsaturated fatty acids (45.0%, NC: 51.7%) (p<0.05).

Conclusions: The findings of this study show that P. acnes proliferation in a meibomian gland could be a trigger for the pathogenesis of chalazion and that P. acnes lipase could change the lipid composition of meibum, thus resulting in the chronic inflammation.

Purpose: To establish the effect of lipid supplements on tear lipid biochemistry and their influence on lens wear comfort in habitual lens wearers.

Methods: Forty habitual soft contact lens wearers were recruited to a double-masked, randomized crossover trial. An emulsion drop containing phosphatidylglycerine (Systane Balance, Alcon) or a liposomal spray containing phosphatidylcholine (Tears again, BioRevive) both with saline drop/spray as placebos, were used three times a day for two weeks with 48 hours washout between each intervention. Visits and collections occurred at baseline, 1 day and 14 days during intervention. Ocular comfort was measured using the Ocular Comfort Index. Basal tears (15 µl from each eye) was collected with lenses in-situ and assayed for the concentration and activity of phospholipase (sPLA2) and the concentration of a malondialdehyde (MDA). Electrospray ionization mass spectrometry characterized the tear lipidome.
Results: Neither of the lipid supplements improved ocular comfort from baseline. The lipid drop and placebo increased the concentration of sPLA, (p=0.02 & p=0.04) cholesterol esters (p=0.04, p=0.05), free cholesterol (p<0.001, p<0.001), triglycerides (p=0.001, p=0.01), phospholipids (p=0.001, p=0.02), and (O-acyl)-ω-hydroxy fatty acids (OAHFA) (p=0.004, p=0.01) compared to baseline at day 1. However, by day 14, levels of sPLA, and all lipid classes except cholesterol esters, free cholesterol and OAHFA returned to baseline concentration. The spray treatment had no significant effect on the concentration and activity of sPLA, and the concentration of MDA and majority of lipid classes either at day 1 or at day 14 compared to baseline (p>0.05). Symptomatic wearers showed higher levels of lysophospholipids with lipid spray (p=0.02) and with lipid drop (p=0.02) at day 14. Ocular comfort improved with lower levels (r=−0.21, p=0.007) and activity of sPLA, (r=−0.20, p=0.01). Higher levels of sPLA resulted higher levels of lysophospholipids (r=0.41, p<0.001 & r=0.40, p=0.001) and, lower levels of OAHFA (r=−0.30, p=0.03).

Conclusions: Lipid supplements did not affect lens wear comfort. Drop supplements showed a transient effect on tear lipolipid. Lysophospholipids and OAHFA could be potential biomarkers in lens wear discomfort.

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Influence of omega 3 and 6 fatty acids on human meibomian gland epithelial cells

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Purpose: Oral supplementation with omega 3 (ω-3) and/or 6 (ω-6) fatty acids (FAs) has been reported to alleviate the signs and symptoms of dry eye disease (DED), and to improve the expressibility and quality of meibum, in patients with meibomian gland dysfunction (MGD). We hypothesized that these FA effects may reflect a direct FA action on human meibomian gland epithelial cells (HMGECS). Our purpose was to test this hypothesis.

Methods: Immortalized (i) HMGECs were cultured with ω-3 (10 μM), ω-6 (10 μM) or both FAs together (5μM each) for up to 7 days in the presence or absence of serum. Cells were evaluated for neutral lipid (LipidTox) staining, as well as the appearance of lysosomes (Lysotracker) and lysosomal markers, Lamp-1 and LC3 (Western blot). The lipid composition of cellular lysates was analyzed by high performance thin-layer chromatography.

Results: Our research shows that ω-3 and ω-6 stimulate the accumulation of small neutral lipid-containing vesicles, but not lysosomes, in HMGECS. This vesicular effect was associated with a 2.4- to 3.7-fold increase in the cellular content of triglycerides following ω-3 and ω-6 treatment, respectively. The combination of both FAs together also enhanced triglyceride levels. Of particular interest, culture of HMGECS with ω-3 and azithromycin (AZM; 10 μg/ml), a known inducer of HMGEC differentiation, led to a significantly greater amount of total neutral lipids, relative to that found with AZM alone. Cellular exposure to the FAs did not alter the expression of free or esterified cholesterol, or phospholipids. Further, these FAs, alone or together, reduced the proliferation of HMGECS in serum-free, but not serum-containing, media.

Conclusions: Our findings support our hypothesis and demonstrate that ω-3 and ω-6 can act directly on HMGECS to influence the quality and quantity of intracellular lipids. It is possible that these cellular effects may contribute to the beneficial actions of these FAs on the signs, symptoms and meibum quality of patients with MGD and DED.

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Effect of Androgens on Human Lacrimal Gland Cells In-vitro

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Purpose: Dry eye syndrome (DES) has been shown to have gender predilections with females, especially post-menopausal or ovarietomized, being more susceptible than their male counterparts. Sexual dimorphism and neuro-hormonal control of Lacrimal gland (LG) secretion has been studied in rats and mice and these studies have implicated androgen levels to be responsible for this gender susceptibility. However, similar studies from humans are lacking. The present study aimed to investigate the role of androgens on human LG secretions.

Methods: Human LG cultures (n=3) were established according to the published protocol using tissues from patients undergoing therapeutic exenteration after IRB approval. The cultured cells were stimulated with Testosterone (10μM) and Dihydrotestosterone (10μM) on day 3 and the supernatants collected on day 7 for analysis. Control (unstimulated) and test (stimulated) supernatants were used for total protein quantification by Bradford assay, protein profiling by SDS-PAGE and quantification of scIgA, Lysozyme and Lactoferrin by sandwich ELISA.

Results: Human LG cells were cultured as adherent monolayer on Matrigel™. Testosterone stimulation of cultures led to an increase in total protein (236.84±6.96mg/ml vs 165.17±30.04mg/ml control; p=0.08) whereas DHT stimulation did not have a significant increase in protein output (227.96±30.61mg/ml vs 165.17±30.04mg/ml control; p=0.21). SDS-PAGE profiling showed a statistically significant increase in the levels of 78kD protein (corresponding to scIgA) and 7kD protein (corresponding to transferrin) on Testosterone and DHT stimulation. The stimulated supernatant also showed a statistically significant increase in scIgA (28.57±5.9ng/ml vs 20.3±6.6 control; p=0.01, 0.02) levels whereas similar increase was not observed with Lysozyme (p=0.43,0.19) and Lactoferrin (p=0.13,0.61) secretion.

Conclusions: The present study provides preliminary evidence that androgens augment total protein output and scIgA production/secretion by the human LG cells. These results warrant further experimentation to evaluate the mechanism of androgens action towards the long-term goal of using them in pharmacological management of DES.

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