Thereafter the eyelids were processed for histological sections. Thin sections were cut with an ultramicrotome and stained with toluidine blue and examined with a light microscope. Electron micrograph grids were prepared and stained with lead citrate and uranyl acetate. In selected cases, contents from obstructed glands were removed by incision and mild compression. Age-matched control mice were processed in similar fashion for each of the time points.

**Results:** Twenty-four hours after cautery, external examination of the eyelids showed no overt signs of inflammation. At one week after cautery there were no visible surface connections for the meibomian glands to release their contents, but otherwise the meibomian glands appeared relatively normal. There was progressive change through the 12 weeks post cautery, ranging from mild ductal engorgement to large cyst formation, and profound glandular dropout. Incision of distended glands produced solidified “cheesy” material.

**Conclusions:** Obstruction of the meibomian gland orifices produced stasis of the meibum which ultimately induced alterations in the morphology of the glands and glandular dropout characteristic of clinical MGD. This animal model may be used for investigating therapeutic agents for treatment of evaporative dry eye disease and for exacerbating factors.

**Commercial Relationships:** Kelly K. Nichols, None; Samuel D. Hanlon, None; Jason J. Nichols, None

**Support:** R01EY015519; Vision CRC

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**Methods:** Three μL of 1 N NaOH were applied under general anesthesia to the right eye of 6 - 8 week old wild-type (n = 5) and Smad3 (n = 10) mice to produce a total ocular surface alkali burn. Mice were killed and eyelid was processed for histology of meibomian glands at day 5, 10 and 20 post-treatment. Hematoxilin-Eosin, oil red O staini and immunohistochemistry for α-smooth muscle actin were employed.

**Results:** Histology showed extremely dilated lumen of the meibomian glands in alkali-burned tissue. The dilated lumen was filled with Oil red O-labeled meibum. Different from uninjured meibomian glands, dilated lumen was rapped with a multilayer of flattened cells that was labeled with anti anti-ctSMAntibody. Lacking Smad3 did not affect the pathology of the treated meibomian glands.

**Conclusions:** Meibomian gland cells underwent epithelial-mesenchymal transition following ocular surface alkali exposure. Obstruction of the gland orifice might accumulate meibum, leading extreme dilation of the gland lumen. Smad3 signal might not be involved in EMT of meibomian gland cells in the pathological condition.

**Commercial Relationships:** Shin Mizoguchi, None; Yuka Okada, None; Reiko Arita, None; Shizuya Saika, None

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**Program Number:** 15 Poster Board Number: A0003
**Presentation Time:** 8:30 AM–10:15 AM
**Application of Active-type Vitamin D3 for External Use to Treat Meibomian Gland Dysfunction of Mice**

**Purpose:** To investigate the efficacy and safety of active-type vitamin D treatment of meibomian gland dysfunction (MGD) in mice.

**Methods:** Meibomian gland orifices of the upper eye lid were cauterized and made creating a meibomian gland dysfunction model was created using male C57BL/6Jc1 mice (N=120). MGD mice were randomly divided into three groups: : maxacalcitol (22-oxacalcitriol, an analog of active vitamin D), vaseline (petroleum jelly) control and blank control. Maxacalcitrol or vaseline were applied on the upper lid margin a day daily for 2 months. Maxacalcitrol or vaseline were also applied on the normal lid margin to evaluate the their safety. The eChanges of in the tarsus surface, conjunctiva, cornea and eyelid margin morphology were observed by microscope, and pathological alteration of meibomian gland were investigated on day 2, day 5, day 7, 1 week, 2 weeks, 1 month and 2 months.

**Results:** The atrophy of the meibomian glands can be alleviated by maxacalcitrol application compared to vaseline and blank control. The cornea was not affected by maxacalcitrol application up to during our 2 month observation.

**Conclusions:** Maxacalcitrol, active-type vitamin D3 possesses the ability to attenuate MGD and is a potential safe treatment of MGD.

**Commercial Relationships:** Kai Jin, None; Motoko Kawashima, NRL pharma (F); Masataka Ito, None; Akiko Ito, None; Samuel Connell, None; Kokoro Sano, None; Kazuo Tsubota, None

**Support:** NRL pharma
We hypothesize that such membrane receptors are present in human (E2), dihydrotestosterone (DHT), and progesterone (P4), and serve mediated by novel, membrane-bound receptors for 17α-oestradiol (E2) to reproduce any abstract, contact the ARVO Office at ARVO 2014 Annual Meeting Abstracts ©2014, Copyright by the Association for Research in Vision and Ophthalmology, Inc., all rights reserved. Go to iovs.org to access the version of record. For permission to reproduce any abstract, contact the ARVO Office at pubs@arvo.org.

**Purpose:** One of the most striking features of dry eye disease (DED) is that it occurs predominantly in women. We hypothesize that this female prevalence is linked to sex-related differences that exist in the anatomy, molecular biology and physiology of the meibomian gland (MG). This gland plays a critical role in maintaining the tear film, and its dysfunction (MGD) is believed to be the major cause of DED. To better understand the factors that underlie MG sexual dichotomy and promote DED in women, we seek to identify an optimal model for the human MG. Our goal in this study was to determine whether a murine MG is such a model. Towards that end, we examined whether sex differences in MG gene expression are the same in mice and humans.

**Methods:** After obtaining IRB or IACUC approval, eyelid tissues (n = 5 to 36 eyelids and 3 to 7 samples per sex/species) were collected from age-matched humans and BALB/c mice. MGs were isolated and processed for RNA extraction and the evaluation of gene expression by using Illumina BeadChips and Geospiza bioinformatic methods.

**Results:** Our analysis of the 500 most highly expressed genes from human (10,099 genes) and mouse (18,302 genes) MGs showed that only 23.4% were the same. If ribosomal genes were excluded, only 16.6% of the genes were similar. Our comparison of the 100 genes with the greatest sex-associated differences in human (e.g. lysosome, 18.2-fold, M>F) and mouse (e.g. androgen binding protein zeta, 109-fold, F>M) MGs demonstrated that none were the same. Sex significantly influenced the gene expression of a number of chromosomes, but the nature of this activity was species-specific. Further, sex exerted a significant impact on numerous biological process, molecular function and cellular component ontologies, as well as many KEGG pathways, but these effects were also primarily species-specific.

**Conclusions:** Our results demonstrate that mice are not appropriate models for understanding sex-related differences in gene expression of the human MG. (Supported by NIH grant EY05612 and the Margaret S. Simon Scholar in Ocular Surface Research fund)

**Commercial Relationships:** David A. Sullivan, None; Raheleh Rahimi Darabad, None; Shaohui Liu, None; Wendy R. Kam, None

**Support:** NIH grant EY05612 and the Margaret S. Simon Scholar in Ocular Surface Research fund

**Purpose:** Previous studies have demonstrated that androgen treatments significantly influence gene expression in human meibomian gland epithelial (HMG) cells and conjunctival epithelial cells in vitro and in animal models. Androgen insufficiency is known to be a significant risk factor for the development of meibomian gland dysfunction (MGD). In this study we sought to determine the effect of two androgens, testosterone (TT) and dehydroepiandrosterone (DHEA) on HMG cell proliferation and lipid production.

**Methods:** Immortalized HMG cells were cultured in serum free (KSFM) or serum containing media (DMEM/F12 containing 10% serum) in the absence or presence of TT at concentrations ranging from 1 nM to 1 μM or DHEA at concentrations ranging from 12.5 μM to 100 μM. After 24h incubation, cell proliferation was evaluated using a live/dead viability assay kit. Lipid production in HMG cells was determined following staining with lipid dye Nile red and quantification of fluorescence measured on a Tecan Spectra Fluor Plus spectrophotometer.

**Results:** Testosterone increased HMG cell proliferation in a dose dependent manner, with overall 19% (+6%) increase at 1 nM of TT, 29% (+11%) increase at 10 nM TT, and 37% (+17%) increase at 100 nM TT after treatment for 24 h. Increased lipid productions from 37% (+19%) up to 62% (+26%) were observed in HMG cells after 24 h treatments of TT at concentrations ranging from 1 nM to 1 μM. HMG accumulation in these cells, as well as the recognition that sex steroids exert a significant regulatory influence on meibomian gland function. Our goal was to test this hypothesis, and also to determine whether [a] GPR30 receptors are present in human corneal epithelial cells (HCEC) and [b] E2 stimulates a rapid uptake of Ca²⁺ in HMGEC.

**Methods:** Near-confluent, immortalized HMGEC and HCEC (gift from Dr. James Jester) were lysed in SDS sample buffer, separated by gel electrophoresis, transferred to nitrocellulose, and incubated with antibodies specific for E2 (GPR30), DHT (GPRC6A), or P4 (PGRMC1) membrane receptor protein. Human breast cancer cell (MCF-7) lysate was served as a positive control for GPR30 and PGRMC1 expression; human prostate cancer cell (LNCaP) lysate was a positive control for GPRC6A. Intracellular Ca²⁺ measurements in cells loaded with Fura-2 calcium indicator and treated with E2 were obtained in real-time using a ratio imaging system.

**Results:** HMGEC express GPR30, GPRC6A, and PGRMC1 membrane receptor proteins for E2, DHT, and P4. Similarly, HCEC contain GPR30 receptors. In contrast, E2 had an inconsistent effect on rapid calcium accumulation in HMGEC.

**Conclusions:** Our study demonstrates the presence of sex steroid membrane receptors in HMGEC and HCEC. These receptors may mediate rapid signaling events. Our functional data indicate that E2 may act in HMGEC through pathways mediated by cAMP, rather than Ca²⁺.
cell proliferation increased by 33% (±13%) and 44% (±16%) after 24 h incubation with 12.5 and 25 μM of DHEA (p<0.05), respectively. Differentiated cells showed a 46% (±5%) and 43% (±1%) increase in lipid production when treated with DHEA at 12.5 and 50 μM respectively for 24 h.

**Conclusions:** Androgens increased cell proliferation and lipid production in HMG cells up to 24h. However, the long term effect of androgen on cell proliferation and lipid production remains to be determined. These findings may be useful in the development of androgen based topical therapy for the treatment of MGD.

**Commercial Relationships:** Amali Ariyavidana, None; Hua Zhu, None; Neeta Khandekar, None; Alison M. McDermott, None; Eric B. Papas, None

**Program Number:** 19 Poster Board Number: A0007

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

**Morphological characterization of a meibomian gland epithelial cell line**

Nagayoshi Asano1, 2, Ulrike Hampel1, Garreis Fabian1, Antje Schröder1, Martin Schicht1, Sabine Möbius1, Friedrich P. Paulsen1. 1Department of Anatomy II, Friedrich-Alexander-University Erlangen Nürnberg, Erlangen, Germany; 2Santen Pharmaceuticals. Co., Ltd., Nara, Japan.

**Purpose:** To characterize a meibomian gland epithelial cell line (kind courtesy of David Sullivan, Boston, SERI, MA) and to investigate morphological changes of cultivated meibocytes after treatment with different media supplements.

**Methods:** Meibocytes were grown two and three dimensionally (in a scaffold and with exposure to air (air-lift)) with keratinocyte medium. Differentiation of two dimensionally cultivated meibocytes was induced by differentiation medium (Dulbecco’s Modified Eagle’s Medium containing epithelial growth factor and 10% fetal calf serum (FCS)) for 1, 3, 7, or 14 days. Furthermore, differentiation medium was complemented with either 20% FCS, omega 3 fatty acid cocktail, eicosapentaenoic acid or high glucose for 1 day or 7 days. Lipid droplets were visualized with Sudan III staining. Ultrastructural changes over differentiation period were investigated by transmission electron microscopy (TEM). Cytokeratin (CK) expression was analyzed by Western blot.

**Results:** Histological and TEM analysis of two and three dimensionally cultivated meibocytes indicated that cells resembled basal and differentiating meibocytes that were CK5, -10 and -14 positive but did not develop to mature or hypermature meibocytes. Lipid droplet accumulation in differentiating meibocytes was induced by differentiation media after 1 day, but decreased over time. Meibocytes showed highest lipid droplet accumulation after 1 day of 20% FCS supplementation. Omega 3 fatty acid cocktail and eicosapentaenoic acid increased lipid accumulation after 1 day.

**Conclusions:** Meibocytes of the meibomian gland epithelial cell line reach a state of differentiating meibocytes after treatment with media supplementation, however induction of meibocyte maturation or hypermature cells is limited.

**Commercial Relationships:** Nagayoshi Asano, None; Ulrike Hampel, None; Garreis Fabian, None; Antje Schröder, None; Martin Schicht, None; Sabine Möbius, None; Friedrich P. Paulsen, None

**Program Number:** 20 Poster Board Number: A0008

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

**The Impact of Treatment on 13-Cis Retinoic Acid-Challenged Human Meibomian Gland Epithelial Cells**

Karen Dionne1, Alison M. McDermott1, Jason J. Nichols1, Hua Zhu2, Kelly K. Nichols2. 1The Ocular Surface Institute, University of Houston, Houston, TX; 2Brien Holden Vision Institute, Sydney, NSW, Australia.

**Purpose:** Evaluation of an exposure or treatment effect on immortalized human meibomian gland epithelial cells can provide insight into meibomian gland function in health and disease. It has been reported that 13-cis retinoic acid (Isotretinoin, e.g. Accutane) therapy for acne is associated with meibomian gland dysfunction (MGD) as well as alterations to meibomian gland cells in culture. The purpose of this series of experiments is to further characterize the influence of 13-cis retinoic acid on the meibomian gland cells.

**Methods:** Immortalized human meibomian gland epithelial cells were cultured in both serum free media and serum containing media for 24 hours prior to addition of 13-cis retinoic acid to encompass both the growth phase and differentiation. Lipid production was confirmed by staining with fluorescent LipidTOX, cell permeability and presumed death was shown by Ethidium homodimer-1 (EthD-1) staining, and cell membranes were observed with Cell Mask Orange Plasma Membrane stain. 13-cis retinoic acid was applied at various concentrations; 0.01μM-5μM. Azithromycin was added to 13-cis retinoic acid exposed cells. Cells were observed and imaged at 24hrs, 7 days or at both time points post exposure using an Olympus IX71 inverted microscope and/or a DeltaVision deconvolution microscope at 100x magnification. IL-1β concentrations were determined by ELISA.

**Results:** Meibomian gland epithelial cells were adversely affected at all concentrations of 13-cis retinoic acid after 7 days in both growth and differentiated state and increased cell permeability was observed as early as 24 hours post treatment. Differential lipid staining between the cell membrane and presumed meibomian lipids was observed with the Cell Mask Orange Plasma Membrane stain and LipidTOX. Modulation of inflammatory marker IL-1β secretion was observed at all concentrations of 13-cis retinoic acid after 48hrs, and with pre- and post- azithromycin treatment. These changes were seen with just one single treatment as compared to a systemic medication, which would utilize continued dosing over an extended period of time.

**Conclusions:** The toxicity of 13-cis retinoic acid exposure to meibomian gland lipid producing cells, even at low concentrations, could play a role in the development of MGD for users of this medication. Our data suggests that this damage could potentially be modified.

**Commercial Relationships:** Karen Dionne, None; Alison M. McDermott, None; Jason J. Nichols, None; Hua Zhu, None; Kelly K. Nichols, None

**Support:** R01EY015519, P30EY07551

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Purpose: An understanding of the meibomian gland (MG) changes occurring naturally over time is important in order to identify pathology and dysfunctional changes. This study investigated measures of MG function and structure in a normal asymptomatic population aged between 25-65 years.

Methods: 156 asymptomatic subjects (91 females) with no pre-existing ocular and systemic abnormalities were recruited and divided into four age groups: 25-34, 35-44, 45-54, and 55-65 (n = 45, 38, 39, and 34, respectively). Asymptomatic subjects were defined as between “normal to mild” and “normal to marginal dry eye” as categorised by the Ocular Surface Disease Index and McMonnies Dry Eye questionnaires, respectively. At a single visit, the following MG measures were collected: meibum quality (MQ) and MG expressibility (MGE) of the lower lid, and infra-red meibography of the upper and lower lids which yielded the MG loss factor (MGLF) i.e. the ratio of the area of MG drop-out to the total area of MG measured. Other clinical measures included osmolarity, lid margin and lashes appearance, conjunctival redness and roughness, lipid layer appearance, tear meniscus height, non-invasive and invasive tear break-up-time (TBUT), corneal fluorescein staining and conjunctival and lid margin lissamine green staining. Data from the worst eyes of each subject were included for data analysis.

Results: There was a significant worsening in the grade severity of MQ (p=0.03), MGE (p=0.01) and MGLF (p=0.001) with increasing age. MGLF showed a steady reduction as age increased (p<0.04), while MQ and MGE both became worse only after 44 years of age (p<0.04). There was a linear increase in the flakes and scales on the lid lashes with age (p=0.01). Lipid layer thickness, tear meniscus height, non-invasive and invasive TBUTs increased beyond 54 years of age (p<0.04), with a corresponding decrease in osmolarity (p<0.001).

Conclusions: Progressive meibomian gland loss occurs normally with age, accompanied by reduced quality and quantity of the meibum produced. These changes can occur without corresponding increases in dry eye symptoms. In older individuals without dry eye symptoms, increased tear volume and reduced osmolarity may counterbalance the effects of meibomian gland changes.

Commercial Relationships: Nisha S. Yeotikar, None; Hua Zhu, None; Negar B. Omali, None; Daniel Tilia, None; Varghese Thomas, None; Maria Markoulli, None; Kelly K. Nichols, None; Jason J. Nichols, None; Eric B. Papas, None
Clinical Trial: ACTRN12612000703808

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We previously reported that in females, meibomian gland physiology changes during the menstrual cycle (ARVO, 2007). The purpose of this present study was to assess the cyclic change of meibum fatty acid composition during the menstrual cycle in normal subjects.

**Methods:** This study involved 6 healthy female subjects (age range: 29-36 years) with a regular 25- to 28-day menstrual cycle. The menstrual cycle was divided into 6 phases: 2 days prior to menstruation (phase VI), the first 2 days of menstruation (phase I), and the remaining time-period of the cycle (phases II-V). After a warm compress (40°C, 10 min) of both eyes using an electronic device (EH-SW50, Panasonic Corp., Osaka, Japan), meibum was obtained from the eyes by Daviel cataract spoon after gently squeezing the eyelid margin by use of a Yoshitomi meibomian gland compressor (T.M.I. Co. Ltd., Saitama, Japan). The spoon was washed with high-grade organic solvents and air-dried before each meibum sample collection to avoid lipid contamination. Meibum was transmethylated and analyzed using gas chromatography mass spectrometry. The concentrations of saliva hormones (i.e., estradiol, progesterone, free-testosterone, and dehydroepiandrosterone sulfate) were also evaluated.

**Results:** The averaged fatty acid compositions were 41.5% saturated fatty acids (SFA) [5.2% straight-chain SFA (SSFA) and the other branched-chain SFA], and 58.5% unsaturated fatty acids (USFA) (51.1% mono-USFA (MUSFA), 3.4% poly-USFA (PUSFA), and 4.0% branched-USFA). In phase II, SSFA was significantly higher and MUSFA was significantly lower than in the other phases (p<0.05). The ratio of MUSFA was negatively correlated with the concentration of estradiol in the saliva during the menstrual cycle (p<0.05), but there was no correlation with the other hormones.

**Conclusions:** Fatty acid composition showed cyclic change during the menstrual cycle. This may impact on tear film stability and relate to dry eye and/or contact lens intolerance in premenopausal women.

**Commercial Relationships:** Tomo Suzuki, Senju Pharmaceutical Co., Ltd (F); Sayaka Kamada, None; Satoshi Fujiiwara, None; Tetsuya Tajika, Senju Pharmaceutical Co., Ltd (E); Shigeru Kinoshita, Senju Pharmaceutical Co., Ltd (F)

**Support:** Grant-in-aid 23890181 from the Japanese Ministry of Education

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<th>Males</th>
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<td>2.16 +/- 2.78</td>
<td>1.89 +/- 2.39</td>
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<td>Frequency</td>
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<td>1.74 +/- 2.51</td>
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<td><strong>Crusting</strong></td>
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<td>Frequency</td>
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<td>Frequency</td>
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<td><strong>Dryness</strong></td>
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<td>Frequency</td>
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<td>Frequency</td>
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history (previous cancer treatment, current medications), & ocular history (previous dry eye symptoms, contact lens/ glasses use) were collected. The presence of DES was assessed using the ocular surface disease index (OSDI).

**Results:** 93 exposure group & 100 control group surveys were included. The groups were age-matched, with an average age of 62.3 years (control group) & 62.9 years (exposure group). The prevalence of DES (OSDI > 12) in the exposure group was 35% compared to 18% in the control group (p = 0.0036, 2-sample z-test). There was no significant difference in the severity of dry eyes between the two groups. Logistic regression analysis demonstrated that the odds of DES was 155% higher in the exposure group. None of the demographic data was significantly associated with DES. Patients who wore glasses for distance were also more likely to have DES.

**Conclusions:** DES is more prevalent in women on AIs for the treatment of breast cancer compared with age-matched controls. This has association has only previously been reported once in a retrospective case-control series. DES can significantly impact the quality of life of sufferers and, in this patient population, could potentially impact compliance, and thus may even indirectly affect recurrence rates. The OSDI presents a simple screening tool for ophthalmologists to be able to assess their patients and refer accordingly. Awareness of this association will allow both ophthalmologists and oncologists to monitor patients and treat symptoms early thereby improving patient comfort and quality of life.

**Commercial Relationships:** Holly Inglis, None; Michael Friedlander, None; Stephanie L. Watson, None

**Program Number:** 25 Poster Board Number: A0013
**Presentation Time:** 8:30 AM–10:15 AM

**Blink-Related Changes in Light Scattering in Meibomian Gland Dysfunction**

*Quintia Moore, Stephen C. Pfuiigfelder.* Ophthalmology, Baylor College of Medicine, Houston, TX.

**Purpose:** Tear dysfunction is a reported cause of blurred and fluctuating vision. This study evaluated the hypothesis that light scattering, measured with the Optical Quality Analysis System (OQAS), differs between aqueous tear deficiency (ATD) and meibomian gland dysfunction (MGD).

**Methods:** In this retrospective, comparative case study all patients presenting with eye irritation, a tear break-up time (TBUT) < 7 and who had a measured ocular scattering index (OSI) using the OQAS were included in the analysis. Patients were stratified into ATD (n=18) or MGD (n=18) based on tear meniscus height (TMH) and clinical signs. The mean OSI between ATD and MGD, and the difference in OSI before and after blinking was compared between groups.

**Results:** The majority of patients with ATD (88%) had a mean OSI slightly above normal (normal < 1.0; 1.5 > ATD > 1). The majority of patients with MGD (83%) had an abnormally elevated OSI 1.5. A significantly greater number of patients with MGD had an OSI > 1.5 compared to the ATD group (p<0.0001). Additionally, the majority of the MGD group (66%) had a greater than 1 difference in pre- and post- blink OSI, whereas 100% of the ATD had a difference in OSI of < 1 (p<0.0001 MGD vs ATD).

**Conclusions:** Patients with MGD had higher mean light scattering indices than those with ATD. Also, light scattering decreased momentarily after blinking in the MGD group, but remained either unchanged or minimally changed after blinking in the ATD group. Despite having similar tear break-up times, the ATD group had less light scattering overall. Thus, light scattering appears to be a feature of lipid tear dysfunction.

**Commercial Relationships:** Quintia Moore, None; Stephen C. Pfuiigfelder, Allergan (C), Allergan (F), GlaxoSmithKline (GSK) (F), GSK (C)

**Support:** NEI/NIH core Grant for Vision Research EY-002520, Research to Prevent Blindness, the Oshman Foundation, William Stamps Farish Fund and the Hamill Foundation.

**Program Number:** 26 Poster Board Number: A0014
**Presentation Time:** 8:30 AM–10:15 AM

**A Novel Meibographer with Dual Mode Standard Noncontact Surface Infrared Illumination and Infrared Transillumination**

*Stephen Grenon1, Scott Liddle1, Joshua Grenon2, Jeff Rosino1, Nathan Luck1, Caroline A. Blackkie2, Donald R. Korb2.* TearScience Inc, Morrisville, NC; 2Korb Associates, Boston, MA.

**Purpose:** To report a meibographer capable of combining standard noncontact surface infrared (IR) illumination with IR transillumination, imaging in a dual mode, with the goal of providing more accurate imaging and visualization of meibomian gland morphology in the eyelids.

**Methods:** The dual mode imaging captures two separate images, 1/30 second apart, using the custom proprietary lid-flipper/trans-illuminator device and a high resolution IR camera. The two images are displayed separately or combined into a single image to increase the amount of visible detail and image contrast. The investigational device was used on 15 eligible, fully consented individuals. 11 of the subjects had 50% or more gland truncation/atrophy while 4 of the subjects had zero atrophy (as determined by standard non-contact IR meibography) and served as ‘controls’. The standard noncontact images were visually compared to the processed dual mode image. The examiner was required to determine if the dual mode provided 1) more detailed/ less ambiguous images from which to grade gland atrophy and other gland characteristics such as duct dilatation; 2) discernable differences in the percentage of gland atrophy.

**Results:** The mean age of the participants was 46.6±16.9 (5 males and 10 females). In all cases (even the ‘controls’), the definition and visible detail was consistently better with the dual mode meibographer, rendering image details significantly easier to discern. The dual mode meibographer also revealed that meibomian glands which can appear truncated or absent with standard non-contact IR may be present when viewed with the dual mode technique indicating discernable differences in the percentage of gland atrophy as measured with the standard vs. the dual mode technique.

**Conclusions:** The dual mode combination of standard noncontact surface IR with transilluminated IR offers more accurate estimation of gland morphology. Since standard noncontact IR only visualizes the surface, details regarding meibomian gland morphology which may not be viewed with the standard noncontact IR are revealed with dual mode technique. More accurate gland morphology data may partially explain why previous reports show little to no correlation between meibomian gland morphology and meibomian gland function.

**Commercial Relationships:** Stephen Grenon, TearScience (E); Scott Liddle, TearScience (E); Joshua Grenon, TearScience (E); Jeff Rosino, TearScience (E); Nathan Luck, TearScience (E); Caroline A. Blackkie, TearScience (E); Donald R. Korb, TearScience (C)
Purpose: The purpose of this study was to determine if meibography could predict meibomian gland (MG) function with regard to number of functional MGs and/or estimation of functional MG volume in patients symptomatic for dry eye.

Methods: Patients (n=23) symptomatic for dry eye who met the inclusion criteria for the study were fully consented and enrolled. Inclusion criteria: willingness to participate in the study, over the age of 18, no lid abnormalities, no current ocular inflammation/disease, no ocular surgery within the last 6 months, no history of lid surgery. Symptoms were scored using the SPEED questionnaire. MG function and estimation of functional MG volume were performed with the Korb meibomian gland evaluator. Meibography was performed using the Modi Topographer and analyzed using the Phoenix software provided. Lower lids were examined in three equal sections: nasal (N), central (C) and temporal (T) for the number of functional MGs and their functional volume (volume was as 1 for minimal, 2 for moderate and 3 for copious), and for MG dropout. MG dropout was categorized according to the Pult Meiboscale.

Results: Only data for right eyes are presented. The mean age and symptom score of the patients was 48.0±12.1 years (5 males; 18 females) and 8.9±5.0 respectively. The average number of functional glands per lid section was: N=2.7±1.7, C=2.2±2.0, T=0.2±0.5. The estimated functional gland volume per lid section was: N=5.0±3.9, C=3.2±3.2, T=0.3±1.1. The N and C lid sections had significantly more functional MGs and higher functional gland volume relative to the T section (p<0.005). Conversely the amount of gland loss as determined by gland atrophy was significantly highest in the nasal section of the lid (p<0.0001) and drop out showed no apparent correlation with MG function or functional volume.

Conclusions: There appears to be no relationship between the level of apparent drop out and the number of functional MGs and/or functional MG volume. These counterintuitive results strongly indicate that standard noncontact infrared meibography cannot be used to predict MG function in terms of number of functional glands and/or functional gland volume except in the case of total gland dropout, when the glands are completely absent.

Commercial Relationships: David Murakami, TearScience (E), TearScience (I); Caroline A. Blackie, TearScience (E), TearScience (I); Heiko Pult, None; Donald R. Korb, TearScience (F), TearScience (I)

Program Number: 28 Poster Board Number: A0016
Presentation Time: 8:30 AM–10:15 AM

Infrared Meibography in Ocular Graft-versus-Host Disease Prior and Following Allogenic Stem Cell Transplantation. Sebastian E. Siehlemann1, Lisa A. Engel1, Sebastian Wittig1, Felix Bock1, Christof Scheid1, Claus Cursiefen1, Philipp Steven1. 1Department of Ophthalmology, University of Cologne, Cologne, Germany; 2University of Cologne, Department I of Internal Medicine, Cologne, Germany.

Purpose: Ocular graft-versus-host disease (GVHD) following allogenic stem cell transplantation (aSCT) includes inflammation of the entire lacrimal functional unit, leading to severe damage of the ocular surface. In this context, meibomian gland loss in ocular GVHD was described as one pathophysiologic mechanism, however no information is available on the time course of meibomian gland loss in the process of ocular GVHD. This retrospective study was set up to analyze when meibomian gland loss occurs related to the time point of aSCT.

Methods: Infrared images of upper lid meibomian glands from 72 GVHD-patients, 15 patients prior to aSCT and 20 healthy controls were recorded using the Oculus Keratograph 5 (Oculus, Wetzlar, Germany). The images were evaluated using Olympus Soft Imaging Solution’s Cell’F 3.4 with using shading correction and contrast optimization. The images were then binarized and further filters (erosion and dilation) were applied. Upper meibomian gland area (uMGA) was calculated and set in relation to the total tarsal area of the lid. Statistical analysis included Kruskall-Wallis-Test and Mann-Whitney U-test. p-values <0.016 were regarded as statistically significant.

Results: Upper meibomian gland area (uMGA) was reliably calculated in all images recorded. Comparison of right and left eyes demonstrated no significant difference. Mean uMGA of GVHD patients was 24.6% (+/- 12.6), which was significantly lower (p=0.007) than healthy controls (33.9% +/- 8.9). Interestingly uMGA in patients prior to aSCT was statistically not different from GVHD patients (23.9% +/- 11.1, p=0.7) but statistically significant lower than in healthy controls (p=0.008).

Conclusions: This study implicates that meibomian gland loss in GVHD patients is a multi-hit process that also occurs prior to aSCT, possibly due to the underlying diseases and/or related chemotherapy or irradiation. Further follow-up studies need to be conducted to investigate mechanisms of meibomian gland loss and to identify high-risk from low-risk patients and procedures. Overall infrared meibography should be included in the routine workup of patients undergoing aSCT and during follow-up.

Commercial Relationships: Sebastian E. Siehlemann, None; Lisa A. Engel, None; Sebastian Wittig, None; Felix Bock, None; Christof Scheid, None; Claus Cursiefen, None; Philipp Steven, None

Program Number: 29 Poster Board Number: A0017
Presentation Time: 8:30 AM–10:15 AM

Comparison of effect of five warming devices onto tear functions, meibomian glands and ocular surface. Reiko Ariita1, 2, Naoyuki Morishige1, Rika Shirakawa1, Yoichi Sato1, Shiro Amano1. 1Department of Ophthalmology, Itoh Clinic, Saitama, Japan; 2Department of Ophthalmology, University of Tokyo, Tokyo, Japan; 3Department of Ophthalmology, Yamaguchi University, Ube, Yamaguchi, Japan.

Purpose: To compare the efficacy of five commercially available eyelid-warming devices on improving tear film function and meibomian glands conditions.

Methods: Fourteen eyes of 14 healthy volunteers (7 men, 7 women; mean ± SD of age, 33.9±11.4 years) were enrolled in a short-term study (5 minutes application) and ten eyes of 10 healthy volunteers (5 men, 5 women; mean ± SD of age, 32.3±11.7 years) were enrolled in a long-term study (2 weeks application). Five eyelid-warming devices were categorized into two groups: warm types (Azukino-Chikara, EyeHot R) and warm-moist types (warm towel, hot eye-mask, Memoto Esthe). Warm compress using one of the devices for 5 minutes on different days was applied onto the subjects (short-time study). Warming using one of the devices (warm or warm-moist) for 5 minutes twice a day for 2 weeks was applied in another day (long-term study). VAS scores for ocular symptoms, surface temperature measurement (eyelids, cornea, palpebral conjunctiva) using thermography, tear film breakup time (BUT), meibum grading,

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quantification analysis of the meibomian gland area, and the Schirmer value were evaluated before and after the each application. **Results:** Every warming device significantly improved the VAS score, increased surface temperature, BUT, and the grading of meibum (P < 0.01) after short-period warm compress. Warm type of devices provided warm effect better than warm-moist type. Long-term study revealed that warm-moist type devices did not affect tear film condition nor meibomian gland area and function. On the other hand, warm type significantly improved BUT (p=0.001) and the grading of meibum (p=0.022) and meibomian gland area (p=0.022). Furthermore, warm type devices significantly increased the temperature of palpebral conjunctiva (p<0.001). **Conclusions:** Commercially available warming devices were effective for short-period efficacy. Warm type devices provided the effect better than warm-moist type. Warm type devices with long time usage were more effective than warm-moist type in continuously improving the conditions of tear film, meibomian gland and ocular surface.

**Commercial Relationships:** Reiko Arita, TOPCON (P); Naoyuki Morishige, None; Rika Shirakawa, None; Yoichi Sato, None; Shiro Amano, None

**Program Number:** 30 Poster Board Number: A0018
**Presentation Time:** 8:30 AM–10:15 AM

**A Novel Heater Utilizing Electrically Conductive Plastics for Inner Eyelid Heat Application to the Meibomian Glands**

Joshua Grenon, Stephen Grenon. TearScience Inc, Morrisville, NC

**Purpose:** Warm compresses have long been used as a treatment for MGD. Part of the inefficiency of heating the meibomian glands in this way is that the heat must conduct through the lid and lid vasculature before it reaches the internal glands. It is therefore beneficial to develop a means of effectively heating the meibomian glands from the inside of the lid where the inefficiencies of conduction and heat loss through the eyelid can be eliminated. Electrically conductive plastics have long been used for thermal heat sinks and electrical shielding. This report will demonstrate the utility and benefits of using conductive plastic to fabricate a cost effective heater for use in heating the meibomian glands from the inside of the eyelid. In particular, the thermal performance of a plastic heater shaped like a small scleral lens is evaluated against other potential heater technologies which could be used for heating the meibomian glands.

**Methods:** The plastic heaters were fabricated using an injection molding process which over-molded the electrically conductive plastic onto electrodes in the shape of a scleral lens. Additional heaters were fabricated using flexible circuit technology and thick film technology. The heaters were placed in a wind tunnel which simulated the heat extraction by the eyelid. The average temperature of each heater was set by adjusting the voltage of a DC power supply to the heater. Once the heater reached equilibrium an IR thermography camera was used to characterize the resulting heat uniformity, which is of particular importance.

**Results:** The electrically conductive plastic showed the best heat uniformity having less than a 3 deg. C difference in heater uniformity across the surface of the heater as compared to a 4.7 deg. C difference for the flexible circuit technology and a 9.8 deg. C difference for the thick film technology.

**Conclusions:** Electrically conductive plastics provide an inexpensive method of producing small, low wattage heaters with complex geometries and excellent heat uniformity. These heaters are capable of being molded into any shape, including the semi-circular shape required to fabricate a heated scleral lens for direct heat application to the meibomian glands on the inside of the eyelid.

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Effects of LipiFlow treatment on dry eye symptoms, tear film stability, and meibomian gland expression

Pam Satjawatcharaphong, Yixiu Zhou, Meng C. Lin

1 UC Berkeley, School of Optometry, Clinical Research Center
2 UC Berkeley, Vision Science Graduate Group

Purpose: To assess changes in dry eye symptoms, lipid layer thickness, tear film stability, and meibomian gland expressibility after treatment with the LipiFlow® Thermal Pulsation System.

Methods: 23 patients (34 eyes) were treated with the LipiFlow® Thermal Pulsation System and completed a follow-up visit 6 to 10 weeks after treatment. Lipid layer thickness (LLT) was determined using the LipiView® Interferometer. Non-invasive tear breakup time (NIBUT) was measured using a Medmont E300 Corneal Topographer. Invasive tear breakup time (ITBUT) with fluorescein dye as well as corneal and conjunctival staining were assessed with slit lamp biomicroscopy. Meibomian gland expressibility (MGE) was performed and scored using the TearScience Meibomian Gland Evaluator, and meibography was imaged using the Oculus Keratograph. These measurements were taken prior to treatment and repeated at a follow-up visit.

Results: The average SPEED score after treatment was decreased from 15.4 ± 19.2 to 15.4 ± 16 (p = 0.049), whereas patients with stable tear film had a marginally significant decrease in SPEED score (p = 0.073). Average NIBUT was not significantly changed after treatment for the entire sample of patients; however, patients with unstable baseline tear film NIBUT exhibited significant improvement (i.e., longer NIBUT) after treatment (p = 0.031). The average MGE score was significantly improved in the lower lid for all patients (p = 0.090), and specifically improved for patients with unstable tear film (p = 0.103). After treatment, 16 eyes (47%) were found to have MGE in the lower lid improved from 7 ± 5 to 19 ± 9. There was no significant change in post-treatment ITBUT, LLT and meibography.

Conclusions: Overall, patients demonstrated improvement in symptoms, tear film stability, and meibomian gland expressibility after LipiFlow® treatment; however, the changes were most significant in patients who initially presented with unstable tear film assessed by NIBUT. LLT values of our sample patients were not related to improvement in gland expressibility and dry eye symptoms.

Commercial Relationships: Pam Satjawatcharaphong, None

Program Number: 32 Poster Board Number: A0020
Presentation Time: 8:30 AM–10:15 AM
The drop in 3 lipids LPC18:2 (r=0.38), LPE18:0p (r=0.41) and LPE20:1p (r=0.36) was associated with the decrease in tear evaporation. The lipids increased with treatment OAHA 18:1/30:1 (r=-0.43) and OAHAH 18:1/31:0 (r=-0.38) were also associated with the reduction in tear evaporation. The change in the OAHA class was inversely correlated to the change in the evaporative rate (r=-0.39) and the change in LpPE was directly correlated to the change in evaporative rate (r=0.41).

**Conclusions:** The lipids deficient in dry eye such as OAHFAs are increased by lid warming treatment. The change in 2 lipids correlates to the extent of symptomatic improvement. Lipid changes correlate to the change in tear evaporation, suggesting a functional role of lipids in MGD.

**Commercial Relationships:** Louis Tong, None; Sin Man Lam, None; Guangzhou Shui, None; Hwee Kuan Lee, None; Jen Hong Tan, None; Rajendra Acharya, None; Markus Wenk, None

**Support:** NMRC/CSA/045/2012, BMRC 10/1/35/19/670

**Clinical Trial:** NCT01448369

**Program Number:** 35

**Poster Board Number:** A0023

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

**Diurnal variations of human tear lipids**

Simin Masoudi1, 2, Fiona Stapleton1, 2, Todd W. Mitchell3, Mark D. Wilcox. 1School of Optometry and Vision Science, University of New South Wales, Sydney, NSW, Australia; 2Brien Holden Vision Institute, Sydney, NSW, Australia; 3University of Wollongong, Wollongong, NSW, Australia.

**Purpose:** To quantify diurnal variations of the molecular lipid composition of human tears.

**Methods:** Tears were collected in the morning and evening for 7-10 days from 30 healthy subjects with no ocular disease using fine glass capillary tubes. Lipids were extracted and analysed by chip-based nano-electrospray ionization tandem mass spectrometry. Lipid profiles of tears in the morning and evening were compared using linear mixed model.

**Results:** 116 lipid species were quantified representing eight lipid classes, namely lysophosphatidylcholines, lysophosphatidylethanolamines (LPE), phosphatidylcholines, phosphatidylethanolamines (PE), sphingomyelins, phosphatidylserines, cholesterol esters, and triacylglycerides. Individual lipid species were normalized with respect to total lipid composition of each sample. The level of all three species of LPE (species 16:0, 18:0, 18:1) increased (16:0, 1.14 ± 0.06% pm, p=0.001; 18:0, 1.45 ± 0.04% am vs 2.05 ± 0.12% pm, p=0.001; 18:1, 1.95 ± 0.13% am vs 2.62 ± 1.42% pm, p=0.001). The tear levels of nine of twelve studied species of PE (comprising 76.2% of the total PE) decreased from morning to evening (the three most abundant molecular species of PE are: 36:2, 0.36 ± 0.23% am vs 0.26 ± 0.13% pm, p=0.03; 36:3, 0.25 ± 0.15% am vs 0.21 ± 0.10% pm, p=0.05; 38:4, 0.14 ± 0.09 am vs 0.09 ± 0.06% pm, p=0.001). LPE and PE constituted 5.4% of the total lipids. The level of the other lipid species did not change over the course of the day.

**Conclusions:** The overall lipid class profiles of normal tears showed that the levels of more than 94% of tear lipids remain unchanged during the day. However the level of phosphatidylethanolamine and its breakdown product lysophosphatidylethanolamine showed diurnal variation.

**Commercial Relationships:** Simin Masoudi, None; Fiona Stapleton, None; Todd W. Mitchell, None; Mark D. Wilcox, None

**Support:** NIH Grants EY014847 (PA) and EY05612, Arey’s Pond Boat Yard

**Program Number:** 36

**Poster Board Number:** A0024

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

**Composition of Terrestrial and Marine Mammal Tears is Dependent on Species and Environment**

Robin Kelleher Davis, Pablo Argueso. Ophthalmology, Scheepens Eye Research Institute and Massachusetts Eye and Ear, Harvard Medical School, Boston, MA.

**Purpose:** There are differences in the nature of the tear film of marine mammals as compared to terrestrial, including the absence of a lipid layer in the marine mammal. We hypothesize that in lieu of a lipid layer, mucin-type glycoproteins play a critical role in the health and wellbeing of the sea mammal eye. In this study, we analyzed tears from a variety of sea dwelling species for carbohydrate and protein composition, and compared the results to those obtained from terrestrial mammals.

**Methods:** Tears were collected, using IRB and ACUC approved protocols, from manatees, dolphins, seals, sea lions, camels and humans. Protein and carbohydrate concentrations of tear samples were determined using standard bicinchoninic acid and sulfuric assays. Monosaccharides were cleaved and released from tear glycoproteins using acid hydrolysis. Samples were incubated at 100°C for 4.5 hours with a final concentration of 2N trifluoroacetic acid and then subjected to high performance anion exchange chromatography (HPAEC) on a Dionex CarboPac PA-20 column using isocratic gradient elution.

**Results:** Carbohydrate to protein (C:P) ratios were 6-18 times greater in tears from manatees, dolphins, seals and sea lions as compared to human tears, whereas camelid C:P ratio was ¼ that of human. Tears from manatees in rehabilitation settings had a 4-fold higher C:P ratio than those in the wild. By HPAEC, dolphin, seal and sea lion tears contained fucose (0.08-0.21 nmol/μg total protein), N-acetylglactosamine (0.55-6.17 nmol/μg), N-acetylglucosamine (1.43-6.17 nmol/μg), galactose (0.52-2.78 nmol/μg), glucose (0.07-0.90 nmol/μg), and mannose (0.68-2.36 nmol/μg). N-acetylglactosamine and N-acetylglucosamine were present in higher amounts in dolphin tears (6.57 and 6.17 nmol/μg respectively) than in the other species analyzed, including human (0.89 and 1.86 nmol/μg respectively).

**Conclusions:** Results from this study demonstrate a higher content of carbohydrates in tears from marine mammals compared to terrestrial species. Of interest, within one family of marine mammal, the manatee, tear carbohydrate content was substantially higher in a controlled rehabilitative facility than in the wild, which may represent a homeostatic response to an artificial environment.

**Commercial Relationships:** Robin Kelleher Davis, None; Pablo Argueso, None

**Support:** NIH Grants EY014847 (PA) and EY05612, Arey’s Pond Boat Yard

**Program Number:** 37

**Poster Board Number:** A0025

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

**Investigation of Surface Properties of Films of Human Meibum from Normal Eyes and from Eyes with Meibomian Gland Dysfunction**

Georgi A. Georgiev1, Norihiko Yokoi2, Slavyana Ivanova1, Vesselin Tonchev3, Rumen Krastev1, Zdravko Lalchev1. 1Biochemistry, Sofia University “St Kliment Ohridski”, Sofia, Bulgaria; 2Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan; 3Kaischew Institute of Physical Chemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria; 4NMI Natural and Medical Sciences Institute, University of Tübingen, Tübingen, Germany.

**Purpose:** Meibomian gland dysfunction (MGD) is considered as one of the major causes for evaporative dry eye. Thus to characterize the...
difference of surface properties of meibomian lipids (ML) between
normal eyes (nML) and eyes with MGD (dML) is of key importance
to understand the molecular mechanisms of the disease.

**Methods:** ML were collected from eyes of 7 normal subjects (22-73
years old; mean age 42.7±7.5) and eyes of 7 MGD patients (64-82
years old; mean age 73.5±2.5). MGD was diagnosed based on dry
eye symptoms, abnormalities around meibomian gland orifices
(mucocutaneous junction or lid margin), obstructive findings of
the orifices and decreased expressibility of meibum. Fluorescein
breakup time, Schirmer I test and scoring of ocular surface epithelial
damage were also measured. The ML samples were spread at the
air/phosphate buffered saline interface and the resultant films were
examined in vitro at blink-like compression/expansion of the film
area by Langmuir surface balance. The sample’s lateral elasticity and
capability to compress and spread during dynamic area changes were
evaluated through the surface pressure-area isotherms and isocycles.
The surface dilational rheological properties of ML were probed
in the frequency range 1-10⁵ Hz via the step/relaxation method.
The lipid films morphology was monitored by Brewster Angle
Microscopy. All the samples were evaluated both at 25 and 34°C.

**Results:** Statistically significant difference (P<0.05) was found in the
rheological properties of the samples with dML showing slow viscous
dominated relaxation process at 10⁻⁶-10⁻⁴ Hz, while nML remained
mainly elastic. Brewster angle microscopy revealed that nML showed
better spreading at the air/saline interface while dML films were
non-uniform and patchy. The isotherm reversibility (i.e. hysteresis)
of lipid films revealed significant interindividual variability and was
strongly dependent on the film thermal history.

**Conclusions:** Our study suggests that dML have worsened
viscoelasticity and spreading at the air/water interface compared to
ML. Hence the surface properties of meibomian lipids could serve
as a reliable functional marker for diseased meibum samples. The
results correlate with the literature data on decreased amount of polar
lipid ((O-acyl)-ω-hydroxy fatty acids) and cis-double bonds in dML.

**Commercial Relationships:** Georgi A. Georgiev. Rohto
Pharmaceutical Co., Osaka, Japan (F); Norihiko Yokoi, Rohto
Pharmaceutical Co. (F); Slavyana Ivanova, None; Veselin
Tonechev, None; Rumen Krastev, None; Zdravko Lalchev, None
**Support:** This study was supported by Collaborative study grant by
Rohto Pharmaceutical Co., Ltd., Osaka, Japan and by Grant-in-
Aid for Scientific Research (C) (25462728) from the Ministry of
Education, Culture, Sports, Science and Technology in Japan.

**Program Number:** 38 Poster Board Number: A0026
**Presentation Time:** 8:30 AM–10:15 AM

**Tear Film and Lipid Layer Structure in Relation to Four Phases of the Blink Cycle**

Peter E. King-Smith¹, Peter E. King-Smith², Carolyn G. Begley²,
Richard J. Braun³.

¹Optometry, University of Houston, Houston, TX; ²Optometry, The
Ohio State University, Columbus, OH; ³Mathematical Sciences, University of Delaware, Newark, DE.

**Purpose:** To provide new or improved information about changes
in tear film and lipid layer structure in relation to four phases of the
blink cycle, namely, the down phase, the turning point, the up phase
and the steady phase.

**Methods:** Images at different phases of the blink cycle were
recorded from 27 subjects (21 female, age 43±17 yr) using a new
stroboscopic microscope covering an area of 6 mm diameter and using a high performance color camera (1400 x 1100 pixels, 67
images/sec, flash duration 0.04 msec). Interpretation of results was
aided by comparison to previous studies at Ohio State and Indiana
Universities, including high resolution color microscopy, fluorescence
imaging, retroillumination and wavefront sensing. Additionally,
fluid dynamics modeling was used to test hypotheses of the origin of observed effects.

**Results:** During the downstroke of the upper lid, wrinkling of the
surface of the tear film was often observed. Evidence indicates that
this wrinkling is caused by movement of the tear film over the rough
corneal surface and a theory of this phenomenon will be presented.
Also during the downstroke, the lid velocity was much greater
than the downward velocity of the visible lipid layer, implying
accumulation of a narrow and thick lipid band under the lid. At the
“turning point” of a partial blink (lowest reach of the upper lid), a
groove can be formed in the tear surface by mechanisms similar to
the generation of the black-line near the meniscus (McDonald and
Brubaker, 1971, Am J Ophthalmol, 72, 139). During the up phase,
the lipid accumulated under the upper lid is initially released as a
thick band, but later, the deposited lipid may become thinner as the
accumulated lipid is depleted. In the steady phase, the lipid pattern
is often very repeatable from blink to blink, but sometimes changes
suddenly between two blinks (Bron et al., 2004, Exp Eye Res, 78,
347). Additionally, in our observations, the images may change
greatly over a series of blinks, but each image is simply a distorted
view of the previous image. Presumably the same lipid is imaged
but after considerable movement as well as local stretching or
compression, rotation and shearing.

**Conclusions:** Stroboscopic microscopy can provide novel
information about structural changes in the tear film and lipid layer
by eliminating blur from rapid tear film movement during the blink
process.

**Commercial Relationships:** Peter E. King-Smith, None; Kathleen
S. Reuter, None; Carolyn G. Begley, None; Richard J. Braun, None
**Support:** NIH Grants EY017951 (PEK-S), EY021794 (CGB), NSF
Grant 1022706 (RJB)

**Program Number:** 39 Poster Board Number: A0027
**Presentation Time:** 8:30 AM–10:15 AM

**Evaluation of tear film lipid layer (TFLL) thickness and tear
thinning rates in cigarette smokers**

Daniel R. Powell¹, Peter E. King-Smith¹, Heather L. Chandler².

¹Optometry, University of Houston, Houston, TX; ²Optometry, The
Ohio State University, Columbus, OH.

**Purpose:** According to the Dry Eye Workshop Report, cigarette
smoking has been identified as a potential risk factor for dry eye.
The gaseous and particulate phases of tobacco smoke contain
many oxidants and reactive oxygen species that may alter TFLL
components, potentially resulting in increased tear evaporation.
The study purpose was to determine whether smokers presented
with decreased TFLL and, therefore, increased tear thinning rates
compared to those who never smoked.

**Methods:** Eligible participants between 18-44 years of age who
were not current contact lens wearers were enrolled. Smokers were
required to have smoked on a daily basis for at least three years prior
to the study. Nonsmokers must have worked and lived in a smoke-
free environment. TFLL and tear thinning rate measurements of the
pre-corneal tear film (25 μm X 35 μm) over a 20-second period
were performed by a spectral interferometry technique described
previously (King-Smith, 2002), then analyzed using SigmaPlot
10.0. Two trials were run through minutes apart and all outcomes were
averaged and analyzed using Mann-Whitney U tests.

**Results:** Sixty-five subjects were enrolled (20 smokers, 45
nonsmokers). Females comprised 55% of smokers and nonsmokers.
Mean age for smokers and nonsmokers was 30.2 ± 6.1 and 25.7 ± 25.7
years, respectively. The TFLL of nonsmokers was marginally thicker
than in smokers (49.8 ± 25.7 and 48.2 ± 22.6 nm, respectively), but

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the difference was not significant (p = 0.72). Mean tear thinning rates were over 60% greater in smokers than nonsmokers (7.0 ± 6.5 and 4.3 ± 2.3 µm/min, respectively), but were borderline nonsignificant (p = 0.07).

**Conclusions:** Smokers do not appear to present with a decreased TFLL, although it is plausible to postulate that smoke exposure may increase tear thinning rates compared to nonsmokers. It is possible that TFLL components are altered allowing for increased tear thinning without affecting TFLL thickness. Future studies involving increased participation of smokers, inclusion of smokers with high cumulative exposure (i.e., heavy smokers), inclusion of an objective measurement of smoke exposure (urinary cotinine levels), and an improved understanding of the TFLL and its role in tear evaporation, will be needed.

**Commercial Relationships:** Daniel R. Powell, None; Peter E. King-Smith, None; Heather L. Chandler, None

**Support:** Beta Sigma Kappa Research Fellowship (American Optometric Foundation); William C. Ezell Fellowship (American Optometric Foundation)

**Program Number:** 40 Poster Board Number: A0028

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

**Correlation between the aqueous tear clearance and the turnover of tear lipids in dry eye patients**

Maurizio Fossarello, Franco Coronella, Giovanni M. Satta, Pietro E. Napoli. Ophthalmology, University of Cagliari, Cagliari, Italy.

**Purpose:** To determine by anterior segment spectral domain optical coherence tomography (OCT) the turnover of tear lipids in patients with aqueous tear deficient dry eyes (ATD) and its correlation with the aqueous tear clearance.

**Methods:** Eighty-two adult patients presenting with complaints of ocular irritation were evaluated for abnormalities of ocular surface in the same sequence. It included clinical history, a symptom questionnaire (OSDI = Ocular Surface Disease Index), fluorescein tear break-up time (FTBUT), fluorescein staining of the cornea and conjunctiva graded according to the Oxford system (FSOS), standardized visual scale (SVST), Schirmer I test, and a slit lamp examination of the lid margins and meibomian glands. T A lipid-based artificial tear (LAT) was used as a tracer to obtain an enhanced OCT imaging of the lower tear meniscus (LTM) and to determine the clearance of tear lipids (CTL).

**Results:** The correlations between CTL and OSDI, FTBUT, FSOS, Schirmer test scores, were found to be statistically significant (p<0.001, Spearman correlation coefficient was 0.79, -0.68, 0.58, -0.5 -0.43, respectively). Moreover, also a significant relationship between CTL and the aqueous tear clearance (SVST) was observed (Spearman correlation coefficient was 0.68, p<0.001).

**Conclusions:** The results obtained with this new technique of contrast-enhanced OCT imaging of the ocular surface are in agreement with other classical tear tests. The reduced dynamics of tear lipids was associated with a slow aqueous tear clearance. This new finding could lead to a better understanding of the complex pathogenesis of dry eye syndrome and of its alterations.

**Commercial Relationships:** Maurizio Fossarello, None; Franco Coronella, None; Giovanni M. Satta, None; Pietro E. Napoli, None

**Program Number:** 41 Poster Board Number: A0029

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

**Thick Human Tear Lipid Films: Effect of Lipids Interaction with Model Tear Proteins on Interfacial Properties**

Tatyana F. Svitoval, Meng C. Lin1, 2. 1Optometry School, Clinical Research Center, University of California, Berkeley, Berkeley, CA; 2Vision Science Graduate Group, University of California, Berkeley, Berkeley, CA.

**Purpose:** To study and quantify the effects of model tear proteins (MTP) on interfacial dynamics and rheological properties of human tear lipids extracted from Schirmer strips (SSL).

**Methods:** SSL samples were collected from 8 healthy subjects. Sessile bubble tensiometry was used to study interfacial properties of SSL, MTP, and mixed film (SSL+MTP) at 35 C. SSL was deposited on an air bubble to form 90±20 nm-thick films. SSL films were subjected to expansion-compression cycles; the film area changed from ~5 to 50 mm². MTP solutions (Hen Egg Lysozyme (HSL), final concentration (FC) =2 mg/ml, Human Serum Albumin (HSA), FC=0.2 mg/ml, Bovine Submaxillary Mucin (BSM), FC= 0.15 mg/ml, or their mixture with FC=2.35 mg/ml total) was injected into a cell and equilibrated without or with SSL film for up to 24 hours. Dynamic interfacial properties of films were assessed. MTE was then pumped through the cell to remove MTP from bulk and SSL dynamic properties were re-evaluated.

**Results:** Equilibrium surface tension (EST), elasticity modulus (E), and relaxation times (τ) of SSL alone were 22±2.1 mN/m, 10.7–14.8 mN/m, and 90–170 s, respectively. EST for proteins was 45.3±1.5 for HEL, 40.4±0.5 for HSA, and 36.7±1.3 mN/m for BSM. The range of E for proteins was 12.5 -18.5 mN/m; in the presence of BSM the range was 3–17 mN/m; for mixed SSL+MTP films, EST remained unchanged. E for SSL+MTP mixed films was 5-7 mN/m lower than for SSL; τ increased to 250-360 s. MTP altered the shape of surface pressure vs. film thickness (π-h) iso-cycles and film compressibility. HSA produced minor changes, whereas BSM and HEL changed SSL properties more notably. For SSL alone the maximum surface pressure πmax was attained at h ~50 nm; in the presence of BSM πmax was attained at h ~20 nm. HEL shifted πmax toward higher h, to ~70 nm. These changes persisted after proteins were washed out.

**Conclusions:** Model proteins adsorb and bind irreversibly to thick SSL films and change interfacial properties of SSL films and the range of maximum surface pressure. BSM alone or mixed with other MTP is found to be most active at lipid-water interface, implying its likely role in tear film stabilization.

**Commercial Relationships:** Tatyana F. Svitoval, None; Meng C. Lin, None

**Support:** NIH Grant EY017269

**Program Number:** 42 Poster Board Number: A0030

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

**The Influence of Eyeliner Cosmetics and Squalene on the Conformation of Human Meibum**

Rahul Bholia1, Morgan Hunter2, Marta Yappert2, Douglas Borchman1, Dylan Gerlach1. 1Ophthalmology, University of Louisville, Prospect, KY; 2Department of chemistry, University of Louisville, Louisville, KY.

**Purpose:** Eye makeup and squalene (SQ), a natural component of eyelid sebum, could interact with human meibum; however, this possibility is yet to be tested. Aim of this pilot study was to measure makeup-human meibum interactions in vitro with the use of infrared spectroscopy.

**Methods:** Two popular brands of eyeliner makeup were studied: L’Oréal (Clichy, Hauts-de-Seine, France) Infallible pencil eyeliner and water-based Revlon (New York, NY, USA) ColorStay liquid

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In vitro surface pressure measurements of various tear film lipids

8:30 AM–10:15 AM

Presentation Time:

Program Number:

Poster Board Number:

Gerlach, None; Dylan, None; Douglas Borchman, None; Morgan Hunter, None; Martin Yappert, None; Douglas Borchman, None; Dylan Gerlach, None

Program Number: 43 Poster Board Number: A0031

Presentation Time: 8:30 AM–10:15 AM

In vitro surface pressure measurements of various tear film lipids

Hendrik Walther1, Lakshman N. Subbaraman1, Shawn Wettig2, Lyndon W. Jones1. 1CCLR - School of Optometry and Vision Science, University of Waterloo, Waterloo, ON, Canada; 2School of Pharmacy, University of Waterloo, Waterloo, ON, Canada.

Purpose: The elasticity of tear film lipids is essential for retaining its stability and can be determined by plotting isotherms and by increasing the pressure of a surface. Thus, the aim of this study was to determine the surface pressures of individual tear film lipids and the effects of various lipid combinations on isotherms.

Methods: Six different tear film lipids (cholesterol, cholesteryl oleate [CO], oleic acid [OA], oleic acid methyl ester [OAME], triolein and phosphatidylcholine [PC]) were examined using a Langmuir-Blodgett trough (KSV NIMA). In addition, the lipid domains on the air-water interface were captured using a Brewster angle microscope (BAM). Initially, each lipid was solubilized in cholesterol in various concentrations (3.6, 4, 3.6, 4, 4 and 1mg/mL, respectively). Furthermore, various solutions with mixtures of two lipids and one consisting of all six lipids were prepared; the concentrations were kept consistent. Based on lipid concentration and molecular weight, between 5µL and 40µL aliquots of the lipid solutions were gently applied onto air-water interface of de-ionized aqueous sub-phase (Milli-Q), using a gastight micro-syringe. Lipid layer and solvent were allowed to equilibrate and evaporate for 10 min before measuring surface pressures. Subsequently, the surface (768.5 cm²) was compressed at a rate of 21 cm²/minute and the pressures were determined (mN/m) with a platinum Wilhelmy plate.

Results: The maximum surface pressure (π_max) ranged from ~13 mN/m to ~47 mN/m: cholesterol ~46 mN/m, CO ~13 mN/m, OA ~30 mN/m, OAME ~15 mN/m, triolein ~27 mN/m, and PC ~47 mN/m. The π_max of the six-lipid stock was 26 mN/m. The PC lipid layer collapsed and formed a bilayer after reaching the π_max and a surface area of ~125 cm². For the examined lipid mixtures, the π_max and lift-off areas (A_l) of the isotherms changed, affecting the elasticity of the lipids. With rising surface pressures, uniform lipid layers started to solidify and precipitate, however, this effect was reversible.

Conclusions: The π_max and A_l of the tested lipids vary based on the applied lipid concentration. The elasticity of lipids changes when mixed with others. The transitions between gaseous and solid phase is reversible and, thus, might have a key influence in the spreading of the tear film. It will be valuable to conduct further research to investigate the role of tear film lipid surface pressures and to determine the effects of degraded lipids on isotherms.

Commercial Relationships: Hendrik Walther, None; Lakshman N. Subbaraman, Alcon (F), Allergan (F), CooperVision (F), Johnsons & Johnson (F); Shawn Wettig, None; Lyndon W. Jones, Alcon (F), Allergan (F), AMO (F), CooperVision (F), Essilor (F), Johnson & Johnson (F), TearScience (F), Visionering (F)

Program Number: 44 Poster Board Number: A0032

Presentation Time: 8:30 AM–10:15 AM

Effect of Systane Family Products on Meibomian Gland Functionality in Patients With Lipid-Deficient Evaporative Dry Eye

Victor M. Finnemore1, Teresa Douglass1, Abayomi B. Ogundele2, Donald R. Korb1. 1Korb & Associates, Boston, MA; 2Alcon Research, Ltd., Fort Worth, TX.

Purpose: To assess the effectiveness of using Systane® products (Systane Balance, Lid Wipes, and Vitamin Omega-3 Supplement) vs. the standard of care (warm compresses, with or without saline) in improving meibomian gland functionality in patients with lipid-deficient evaporative dry eye disease.

Methods: This was a single-center, open-label, investigator-masked, prospective study of 26 patients enrolled (women, n=21; men, n=5; mean age, 41.7 years). At baseline, the mean ± SD number of functioning glands for both eyes was 3.5 ± 1.39 in the Systane and warm-compress groups, respectively. Meibomian gland functionality was increased from baseline in the Systane group at all follow-up visits and was significantly better (P < 0.05) vs. the standard of care (warm compresses, with or without saline) and take 2 oral vitamin softgels once daily for 3 months, or to apply warm, wet microfiber compresses to both eyelids for 8 minutes once per day for 3 months. Meibomian gland functionality, the primary endpoint, was evaluated using a standardized diagnostic meibomian gland expressor to determine the number of MGYLS at baseline and after 1, 2, and 3 months of treatment. Best corrected visual acuity (BCVA) and adverse events (AEs) were evaluated as safety outcomes.

Results: A total of 26 patients (n=52 eyes) were enrolled (women, n=21; men, n=5; mean age, 41.7 years). At baseline, the mean ± SD number of functioning glands for both eyes was 3.5±1.50 and 4.2±1.39 in the Systane and warm-compress groups, respectively. Meibomian gland functionality was increased from baseline in the Systane group at all follow-up visits and was significantly better in the Systane group compared with the warm-compress group at month 1: 3.6±1.90 vs. 2.7±2.29 (P=0.0365) and month 3: 3.9±2.72 vs. 4.7±2.29 (P=0.0061). One patient reported 2 AEs that were not related to treatment; no serious AEs were reported. BCVA was unchanged from baseline through month 3 in both treatment groups.

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Conclusions: In patients with lipid-deficient evaporative dry eye, treatment with the Systane product family increased the number of MGYLS. Meibomian gland functionality was significantly better after 2 and 3 months of treatment with the Systane products when compared with the standard of care (warm compresses). No safety issues were reported with either treatment.

Commercial Relationships: Victor M. Finnemore, None; Teresa Douglass, None; Abayomi B. Ogundele, Alcon Research, Ltd. (E); Donald R. Korb, Alcon Research, Ltd. (P)

Support: This study was sponsored by Alcon.

Clinical Trial: NCT01733745

Program Number: 45 Poster Board Number: A0033
Presentation Time: 8:30 AM–10:15 AM
Relative Efficacy of Loteprednol (Lotemax®) vs. Loteprednol-Tobramycin (Zylet®) on Corneal and Conjunctival Immune Response in Treatment of Meibomian Gland Dysfunction (MGD)-Associated Ocular Surface Inflammation: In Vivo Confocal Microscopy Results of a Phase IV Randomized Controlled Trial

Yureeda Qazi, Ahmad Kheirkhah, Thomas H. Dohlman, Francisco Amparo, Reza Dana, Pedram Hamrah. Ophthalmology, Massachusetts Eye and Ear Infirmary, Boston, MA.

Purpose: To use in vivo confocal microscopy (IVCM) to determine whether Zylet is superior to Lotemx in managing MGD-associated conjunctival inflammation and if either is more effective at treating inflammation than artificial tears.

Methods: An IRB-approved, phase IV, single-center, randomized, double-masked, vehicle-controlled clinical trial was conducted with 54 subjects (age = 56 ± 12 years) diagnosed with MGD and 62 age-matched normal controls (age = 49 ± 15 years). Subjects received artificial tears (AT; n= 20), Zylet only (n= 17), or Lotemx only (n= 17) for 4 weeks with follow-up (FUP) visits at 4 and 8 weeks post-initiation of treatment. IVCM of the central cornea and palpebral conjunctiva was done at baseline and 1st FUP visit. Imaging parameters included corneal immune dendritiform cell (DC) density, conjunctival epithelial cell (EIC) and stromal immune cell densities (SIC). Reliability tests for IVCM were performed. Symptom (Ocular Surface Disease Index questionnaire; OSDI) and ocular surface assessments were done at baseline and both FUP visits.

Results: At baseline, subjects with clinical MGD had significantly increased corneal DC (P< 0.001), conjunctival EIC (P< 0.01) with low bias and higher SIC densities (P= 0.01). Imaging parameters were associated with symptoms (OSDI= 56 ± 3), low TBUT (3 ± 0.3) and Schirmer’s scores (5 ± 0.8 mm). At the 1st FUP, improvement was detected in all imaging efficacy outcome measures in subjects treated with Zylet (DC: -49%, EIC: -29%, SIC: -14%) and Lotemx (DC: -58%, EIC: -34%, SIC: -32%) but not AT. Zylet and Lotemx reduced inflammation with comparable efficacy (DC: P= 0.6, EIC: P= 0.9, SIC= 0.4). There was a lag in symptomatic improvement, with 11% reduction in OSDI scores at the 1st FUP. IVCM had high inter-user agreement (intra-class correlation coefficient of agreement ≥ 0.93) with low bias.

Conclusions: Zylet and Lotemx are equally effective at reducing corneal and palpebral conjunctival inflammation in MGD and are superior to artificial tears. IVCM is a reliable tool in the quantitative detection and monitoring of ocular surface inflammation.

Commercial Relationships: Yureeda Qazi, Massachusetts Eye and Ear Infirmary, Boston (P); Ahmad Kheirkhah, None; Thomas H. Dohlman, None; Francisco Amparo, None; Reza Dana, Bausch and Lomb (C); Pedram Hamrah, Allergan (C), Allergan (F), Massachusetts Eye and Ear Infirmary, Boston (P)

Support: Bausch and Lomb, NIH K08 EYE020575, Falk Medical Research Institute.

Clinical Trial: NCT01456780

Program Number: 46 Poster Board Number: A0034
Presentation Time: 8:30 AM–10:15 AM
Thermal pulsation for meibomian gland dysfunction in Asian patients

David Rooney1, Jen Hong Tan2, U Rajendra Acharya1, Hwee Kuan Lee1, Zhao Yang1, Markus Wenk1, Louis Tong2,4. 1University of Alabama School of Medicine, Birmingham, AL; 2Ngee Ann Polytechnic, Singapore, Singapore; 3Bioinformatics Institute, Agency of Science Technology and Research, Singapore, Singapore, Singapore; 4Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore; 5Department of Biochemistry and Development of Biological Sciences, National University of Singapore, Singapore, Singapore; 6Singapore National Eye Center, Singapore, Singapore.

Purpose: Dry eye and meibomian gland dysfunction (MGD) are prevalent ocular surface conditions with no satisfactory treatment. Recently LipiFlow, a form of thermopulsation treatment, has been advocated. We aimed to evaluate the efficacy and safety of LipiFlow in Asian patients with dry eye and MGD.

Methods: This is a hospital-based longitudinal interventional study of LipiFlow compared to conventional warm compress treatment. Patients without systemic diseases were recruited. Symptom evaluation and ocular examination for dry eye were performed before treatment as well as 1 and 3 months after treatment. Examination included corneal fluorescein staining, fluorescein tear break-up time, slitlamp examination, meibomian gland expression, tear lipid thickness, Schirmer test, visual acuity, and tonometry. During the study period 25 patients underwent a single 12-minute session of LipiFlow treatment in clinic, and 24 patients underwent 10-minute warm compress treatment twice daily. Blephagel lid hygiene was used liberally in both study arms.

Results: The average age of patients was 56.2 years (SD:11.7) with 12 men and 37 women, reflecting the clinic’s patient profile. Age and gender composition of participants in the two arms was similar (p>0.05). There was no significant difference in the arms’ baseline characteristics except for corneal fluorescein staining, which was more severe in all corneal zones of the conventional arm (p<0.05) except for the central zone (p=0.12).

Overall, there was significant decrease in symptoms after either treatment (p=0.006) and a trend for greater reduction in patients treated by LipiFlow, but this trend was not significant (p=0.056). Tear break up time was significantly improved at 4 weeks (p=0.048) and at 12 weeks (p=0.028) with LipiFlow but not with conventional treatment (p>0.05). The reduction of central corneal staining was greater with LipiFlow than with conventional treatment up to 12 weeks after treatment (OR 0.31, 95% CI 0.14-0.68), a difference that remained significant after adjustment for age and sex. There was no significant difference in staining of all four other corneal zones. No adverse effects were noted with either treatment.

Conclusions: LipiFlow treatment is similar to warm compress treatment in terms of efficacy and safety. However, because LipiFlow is much more convenient (1 time treatment) and avoids non-compliance issues, it may be advantageous for clinical use.

Commercial Relationships: David Rooney, None; Jen Hong Tan, None; U Rajendra Acharya, None; Hwee Kuan Lee, None; Zhao Yang, None; Markus Wenk, None; Louis Tong, None

Support: National Medical Research Council, Singapore (NMRC/CSA/045/2012) and Biomedical Research Council Singapore (BMRC/TCRP)1/35/16/670 R828

Clinical Trial: NCT01683318
Efficacy and Safety of azithromycin 1.5% eye drops (Azyter®) in patients with moderate to severe chronic blepharitis

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Purpose: To compare the efficacy and safety of azithromycin 1.5% (Azyter®) and placebo eye drops in patients suffering from chronic moderate-to-severe anterior and/or posterior blepharitis.

Methods: This prospective, multicenter, randomized, double-masked, parallel group, phase II pilot study was conducted in 93 chronic blepharitis patients. After a 2-week wash-out period with daily eyelid care, patients with resistant moderate-to-severe blepharitis randomly received azithromycin (AZM) 1.5% or placebo (polyvidone) eye drops for 7 days (2 drops on D1, then one drop daily), followed by a 2-week treatment-free period. This therapeutic scheme was repeated twice. The primary endpoint was the change from D0 to D63 in global ocular discomfort assessed on a 100-mm visual analogue scale (VAS). Secondary outcomes included the evaluation of ocular symptoms (irritation, itching, crustings, sticking, light sensitivity, blinking) and signs (marginal redness, eyelid swelling, meibomian gland (MG) dysfunction and quality of MG secretions; semi-qualitative VAS).

Results: Mean ocular discomfort reduction (D0 to D63) was -33.9 ± 20.2 mm (-52.2%) on AZM and -26.1 ± 24.0 mm (-41.1%) on placebo in the Full Analysis Set (FAS). Using a mixed model for repeated measures, the estimated mean differences between treatments (AZM - placebo) in discomfort score was -8.01 mm in the FAS and -10.37 mm in the PP Set on D63. The between-group difference was close to statistical significance in the FAS (p=0.072), and statistical significant in the PP Set (p=0.026). AZM-treated patients also had a lower total symptom score (vs. placebo-treated patients; p=0.005) and a lower VAS score for blepharitis signs (p=0.092) on D63. Both treatments were well tolerated.

Conclusions: Azithromycin 1.5% eye drops were effective and safe for the management of moderate-to-severe blepharitis. They appear as a promising therapeutic option, with notably additional anti-inflammatory activities over the standard eyelid care.

Commercial Relationships: Serge Doan, None; Christophe Baudouin, None; Marc Labeltoule, None; Tristan Bourcier, None; Louis Hoffart, None; Pierre-Jean Pisella, None

Clinical Trial: NCT01089608

Prevalence of MGD, blepharitis, and demodex in an optometric practice

Scott E. Schachter1, Aubrey Schachter1, Milton M. Hom2, Scott G. Hauswirth4, 5. 1Private Practice, Scott E Schachter, OD, PC, Pismo Beach, CA; 2Private Practice, Milton Hom, OD, Azusa, CA; 3Student, University of California, Los Angeles, Los Angeles, CA; 4Private Practice, Minnesota Eye Consultants, PA, Bloomington, MN; 5Adjunct Clinical Faculty, Southern California College of Optometry, Fullerton, CA.

Purpose: This study aimed to identify the prevalence of demodex and associated signs in patients seen for routine eye exam.

Methods: The clinical parameters evaluated were degree of meibomian gland dysfunction (MGD), lash distention, lash appearance, and cylindrical dandruff (CD). Each clinical sign was rated on a scale of 0-4. If CD was observed (rating of G1-G4), the following protocol was followed:

1. Lashes were epilated.
2. Lashes were placed on a glass slide, a cover slip and drop of saline/fluorescein dye was applied to the slide.
3. Examination performed with a Celestron LCD microscope (objective lenses: 40x, 100x, 400x).

Results: Demodex blepharitis was present in 32.4% of all subjects. MGD (G1+) was observed in 33.1% of all subjects, and of those, 46.8% were comorbid with CD and demodex blepharitis. Lash distention (G1+) was observed in 26.1% of all subjects, and of those, 89.2% were comorbid with CD and demodex blepharitis. Greasy and/or oily lashes (G1+) were observed in 31.0% of all subjects, and of those, 73.3% were comorbid with CD and demodex blepharitis.

Conclusions: Demodex is present in the majority of patients with lash distention (89.2%) and greasy/oily lashes (77.3%). Additionally, almost half of MGD patients have demodex present (46.8%).

Commercial Relationships: Scott E. Schachter, Allergan (C), BioTissue (R); Aubrey Schachter, None; Milton M. Hom, Allergan (F), AMO (F), Bausch and Lomb (F); Scott G. Hauswirth, Alcon (C), Allergan (C), Bausch and Lomb (C), BioTissue (C), NicOx (C), TearScience (C)
Electrical Stimulation of the Lacrimal Gland in Rabbits
Mark Brinton1, Jae Lim Chung2, Andrea Kossler1, Jim Loudin1, Christopher Ta3, Daniel V. Palanker1, 2
1Hansen Experimental Physics Laboratory, Stanford University, Stanford, CA; 2Ophthalmology, School of Medicine, Stanford University, Stanford, CA; 3Ophthalmology, Kim’s Eye Hospital, Konyang University College of Medicine, Seoul, Republic of Korea.

Purpose: Electrical stimulation of the lacrimal gland is a promising new approach to increase tear production and provide relief to patients afflicted by dry eye disease. Delivery of electrical pulses via implanted electrodes increases tear production; however, it is unknown whether electrical stimulation directly affects acinar cells in the gland, or if it engages sympathetic or parasympathetic neurons. Understanding the pathways is essential for development of an efficient stimulation protocol and electrode configuration.

Methods: New Zealand White rabbits were anesthetized using isoflurane inhalation and implanted with 3-mm diameter bipolar platinum disc electrodes, spaced 10mm apart, beneath the inferior lacrimal gland. Baseline and stimulated tear production was measured using 3-mm wide Schirmer strips for 5 minutes. Symmetric biphasic pulses of current (0.5ms duration at 30Hz) were applied from a custom pulse generator throughout the 5-minute Schirmer test. To determine the pathways of the tearing response to stimulation the animals were given parasympathetic (scopolamine) and sympathetic (prazosin and phenoxybenzamine) blockers prior to electrical stimulation.

Results: Electrical stimulation caused an increase in Schirmer test scores from the 2 mm baseline by 6 and 10 mm for the 6 and 12-mA pulses, respectively. Schirmer tests from the fellow eye suggest little or no bilateral effect. Administration of scopolamine reduced the effect of the 6-mA electrical stimulation to < 1 mm above baseline for 90 minutes, after which the elicited tearing response returned to original level. The sympathetic alpha blockers, prazosin and phenoxybenzamine, did not affect stimulated tear production.

Conclusions: Electrical stimulation of the lacrimal gland can increase tear production by several fold (3-5), depending on the pulse parameters. However, excessive stimulation can engage undesired nerve fibers, including muscle twitching observed with 12-mA stimulation. The elimination of the stimulated tearing effect with parasympathetic blockers indicates that the response does not originate in the acinar cells of the gland, but is rather mediated by neural stimulation. That the sympathetic nerve blockers did not affect tear response suggests that electrical stimulation engages efferent parasympathetic fibers in the gland to enhance tear production.

Commercial Relationships: Mark Brinton, OcuLee, Inc. (C); Jae Lim Chung, None; Andrea Kossler, OcuLee, Inc (C), OcuLee, Inc (S); Jim Loudin, OcuLee, Inc. (E), OcuLee, Inc. (I), OcuLee, Inc. (P); Christopher Ta, OcuLee, Inc (I), OcuLee, Inc (S); Daniel V. Palanker, OcuLee, Inc. (I), OcuLee, Inc. (P)

Support: EY023259

Adenoviral vector-mediated transfer of erythropoietin and GFP to the lacrimal gland in rat
Ana C. Dias, Lara C. Dias, Luis Fernando Nominato, Eduardo M. Rocha.

Purpose: Our group has been studying the feasibility of viral vector gene transfer to the lacrimal gland. The aims of this work are: a) to evaluated changes in the tear secretion and cells of ocular surface, b) to evaluate the changes in the lacrimal gland of rats with an adenovirus encoding the human erythropoietin (Epo) gene (AdLTR2EF1a-hEPO) or GFP (AdGFP).

Methods: Male Wistar rats 6-8 weeks were divided into 3 groups (n = 5/group), where the control group received an injection of saline and the treated group received an injection of erythropoietin adenovirus (Ad-Epo) (20ul, 1012/ml) and a group that received an injection of GFP adenovirus (Ad-GFP) (20 ul, 1012/ml) in the lacrimal gland. Data was collected 6 days after injection. The main parameters evaluated were tear secretion (phenol red thread), lacrimal gland (LG) and ocular globe histology, ocular surface impression citology (IC), expression of erythropoietin in the western blotting.

Results: Tear secretion was not affected by viral vector gene transfer (control 5 ± 3.46mm, AdEpo 4± 1.73 mm and AdGFP 7± 1.29mm (p>0.05). IC revealed significant changes in cornea epithelia in AdEpo and AdGFP compared to controls (p=0.0079). At the 6th day, there was no change in lacrimal gland or cornea histology compared to controls. The cornea epithelial thickness was 20.98± 3.16 μm in the control, 24.91± 1.08 μm in the AdEpo group and 24.72± 4.84 μm in the AdGFP group. Erythropoietin expression was significantly higher in transgressed LG than controls (p<0.05).

Conclusions: Adenovirus vector gene transfer was safe for lacrimal gland structure and function. Their impact on cornea epithelia needs further clarification. The present work offers additional information on the feasibility of gene therapy in the lacrimal gland.

Commercial Relationships: Ana C. Dias, None; Lara C. Dias, None; Luis Fernando Nominato, None, Eduardo M. Rocha, None

Program Number: 50 Poster Board Number: A0038
Presentation Time: 8:30 AM – 10:15 AM

Program Number: 52 Poster Board Number: A0040
Presentation Time: 8:30 AM – 10:15 AM

Differential roles of Pannexin-1 mediated signaling in the regulation of lacrimal gland morphogenesis and inflammation
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1Cell and Molecular Biology, Scripps Research Institute, La Jolla, CA; 2General Dentistry, Tufts University School of Dental Medicine, Boston, MA; 3Ophthalmology, Bascom Palmer Eye Institute University of Miami School of Medicine, Miami, FL; 4Vavilov Institute for General Genetics, Moscow, Russian Federation.

Purpose: Sjögren’s syndrome is a systemic chronic autoimmune inflammatory disease that targets primarily the salivary and lacrimal glands (LG). Recent data indicates that pannexin hemichannels are involved in signaling events leading to inflammation and cell death. At the same time Panx1 is implicated in healing process since it facilitates progenitor cell proliferation and differentiation during tissue regeneration. No specific role for Panx1 and other pannexins has been defined in the LG.

Methods: Gene expression in murine LG was examined by qRT-PCR and immunostaining. Panx1 blocking peptide or saline (control) were co-administered together with IL-1α injection. FACS isolated epithelial progenitors were used in transplantation experiments. Comparison of Panx1-null and WT LG morphology was performed using sections analysis.

Results: Panx1 and 2 isoforms were expressed in the epithelial component of the LG; Panx2 labeling was also detectable in the blood vessels. Panx1 expression was also studied in the IL-1α injured LGs. IL-1α induces a strong immune response, extensive inflammation, and LG damage, followed by a regenerative phase. Panx1 was strongly induced during acute phase of LG inflammation and regeneration, peaking at 3 days and declining to the baseline level during 7 to 21 days post injury. Analysis of epithelial progenitor cells transplantation into IL-1α injured LG showed that efficiency of progenitor cell engraftment varied, depending on timing of cell
Leukocyte phenotype and lymphatic vessel distribution in canine lacrimal and nictitating glands

Christopher M. Reilly, Shin Ae Park, Carl F. Marfurt, Brian C. Leonard, Christopher J. Murphy

School of Veterinary Medicine, University of California-Davis, Davis, CA; \textsuperscript{2}Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN; \textsuperscript{3}School of Medicine, University of California, Davis, CA.

\textbf{Purpose:} To better understand immune cells and lymphatics in normal canine tear glands, which have not been previously described to our knowledge. Dogs are a valuable, spontaneous animal model of dry eye diseases.

\textbf{Methods:} Lacrimal and third eyelid glands were collected from normal young adult dogs \textless{} 2 hours post euthanasia. Glands were formalin fixed, processed, paraffin-embedded, and sectioned. Immunohistochemistry was performed with antibodies against: CD3 (T-cells); CD20 (B-cells); CD18 (leukocytes, especially macrophages); and LYVE-1 (lymphatic endothelial cells, some leukocytes), using protocols validated for use in dogs. PBS was negative control. Leukocytes expressing CD3, CD20, and LYVE-1 were enumerated in acinar tissue (cells/10 400x fields). Antigen expression in periductal, interstitial, and peripheral regions was assessed on a 0 to 3 scale (0=absent; 3=heavy infiltrates), due to lack of uniform consecutive fields. CD18 expression was assessed descriptively, to identify leukocytes not labeled by other antibodies (e.g. macrophages, dendritic cells). The density of lymphatic vessels was also assessed on a 0-3 scale in acinar, interlobular, and peripheral regions.

\textbf{Results:} CD20+ B cells were over 4 times more prominent than CD3+ T cells in all glands. B cells were distributed in interacinar clusters, with T-cells more individually scattered. All lymphocytes were rare in connective tissue septa, and surrounded ducts in low numbers. LYVE-1+ leukocytes and channels were present in low density in all connective tissue, and absent in acinar lobules. CD18 expression in connective tissue largely mirrored LYVE-1 expression, supporting leukocyte origin of LYVE-1+ cells. Cells that were exclusively CD18+, suggesting macrophage/dendritic cell identity, were uniquely distributed tightly surrounding and investing acinar epithelium.

\textbf{Conclusions:} T cells, B cells, macrophages, and dendritic cells are all present, with unique distributions, in the canine lacrimal glands. To our knowledge, this is the first report of non-endothelial LYVE-1+ cells in the dog, or in the lacrimal tissues of any species. The role of LYVE-1+ leukocytes and dendritic cells in canine lacrimal immunity warrants further investigation. Lymphatic presence in canine lacrimal tissue is confirmed, and is relatively minor, which may be important in lacrimal immune regulation and possibly lacrimal tumor metastasis.

\textbf{Commercial Relationships:} Christopher M. Reilly, None; Shin Ae Park, None; Carl F. Marfurt, None; Brian C. Leonard, None; Christopher J. Murphy, None

\textbf{Support:} UC Davis Center for Equine Health; Gift from Dick and Carolyn Randall; Unrestricted gift from Research to Prevent Blindness; NEI grant P30EYT2576

\textbf{Program Number:} 54 Poster Board Number: A0042

\textbf{Presentation Time:} 8:30 AM–10:15 AM

\textbf{Autonomic and Sensory Innervation of the Dog Lacrimal Gland}

\textbf{Purpose:} Stimulation of corneal and conjunctival sensory nerves produces reflex tears by activating a brainstem circuit that stimulates lacrimal parasympathetic and sympathetic nerves. Defects in this neural loop can lead to a deficiency in tear secretion and the development of dry eye disease (DED). The canine spontaneous dry eye model has been widely used to study the pathophysiologic mechanisms of DED; however, the functional innervation of the dog lacrimal gland remains to be fully characterized. The purpose of the current study was to investigate the density, distribution, and phenotypic diversity of the normal canine lacrimal gland innervation.

\textbf{Methods:} Lacrimal gland samples from five healthy mixed-breed dogs were frozen-sectioned and stained by immunohistochemistry using antisera against neurotubulin (NT, a pan-neuronal marker), vasoactive intestinal polypeptide (VIP, a marker for parasympathetic nerves), tyrosine hydroxylase (TH, a marker for sympathetic nerves), and calcitonin gene-related peptide (CGRP) and substance P (SP) (both markers for sensory nerves).

\textbf{Results:} Tissue sections stained by NT immunohistochemistry revealed a dense and uniform innervation of all gland lobules and revealed intimate associations between nerves and acinar cells, myoepithelial cells, ductal cells, and blood vessels. Serial section observations suggested that greater than 95% of all acini were contacted by NT-IR nerves. The vast majority of lacrimal nerves were autonomic, and VIP-IR and TH-IR nerves were present in roughly equal numbers and raveled in density and distribution the NT-IR fiber population. CGRP-IR and SP-IR nerves were largely perivascular in nature; however, a few CGRP-IR nerves contacted acinar cells.

\textbf{Conclusions:} The results of this study have demonstrated a uniform, dense and phenotypically diverse innervation of the dog lacrimal gland that is overwhelmingly autonomic in nature. The analogous densities and distributions of VIP-IR and TH-IR nerves suggest collaborative cholinergic and adrenergic mechanisms in the regulation of acinar cell secretory processes. The density of TH-IR sympathetic nerves seen here in the dog is considerably greater than that reported in lacrimal glands of other mammals, including, mouse, rat, and rabbit. It is hoped that the data generated here will provide a baseline of “normal innervation” of the canine lacrimal gland against which observations from dogs with spontaneous DED may be compared.

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Commercial Relationships: Carl F. Marfurt, None; Christopher M. Reilly, None; Shin Ae Park, None; Christopher J. Murphy, None
Support: Unrestricted grant from Research to Prevent Blindness, UC-Davis

Program Number: 55 Poster Board Number: A0043
Presentation Time: 8:30 AM–10:15 AM
Normal gross anatomy, CT, and MRI imaging of the lacrimal and third eyelid glands in dogs
Shin Ae Park1, Kenneth T. Taylor2, Allison L. Zwingenberger1, Christopher M. Reilly1, Brian C. Leonard1, Carl F. Marfurt1, Christopher J. Murphy2,3. 1Veterinary Surgical and Radiological Sci, UC Davis, Davis, CA; 2Department of Anatomy, Physiology and Cell Biology, University of California, Davis, Davis, CA; 3Department of Pathology, Microbiology and Immunology, University of California, Davis, Davis, CA; 4Anatomy and Cell Biology, School of Medicine, Indiana University, Indianapolis, Indiana, CA; 5Department of Ophthalmology & Vision Science, University of California, Davis, School of Medicine, Davis, CA.
Purpose: The lacrimal gland (LG) and the third eyelid gland (TELG) are two intraorbital glands that secret the aqueous component of the tear film in dogs. Despite the central importance of these structures for maintaining ocular surface health, the anatomy and imaging of the glands remains understudied. We investigated the macroscopic, morphometric, CT, and MRI characteristics of the LG and TELG in normal dogs.
Methods: Twenty-six dog heads were dissected to expose the LG and TELG. The length, width, thickness, and weight of both glands were measured. The relationships between the glands and adjacent ocular structures and blood and nerve supplies to the LG were photodocumented during the dissection. Fifty dogs were imaged (42 CT and 8 MRI), and the volume was calculated for the LGs and TELG.
Results: The LG is a flat and oval shape with some morphological variations between dogs located on the dorsolateral aspect of the globe beneath the orbital ligament. The average length, width, and thickness (SD) of the LG were 18.4±3.5, 13.1±2.1, and 2.9±0.5 mm and of the TELG were 14.4±5.3, 10.5±2.9, and 3.2±0.5 mm, respectively. The mean weight (SD) of the LG and TELG were 315.7±103.3 and 263.3±56.3 mg and the volume measured by CT and MRI imaging were 1.4±0.1 and 0.1±0.1 cm3, respectively.
Conclusions: The present study provides detailed normative anatomical and morphometric data for the LG and TELG. These data will be essential in evaluation of alterations induced by disease states as well as informing strategies for the local delivery of pharmacologic and cellular therapeutics.

Commercial Relationships: Shin Ae Park, None; Kenneth T. Taylor, None; Allison L. Zwingenberger, None; Christopher M. Reilly, None; Brian C. Leonard, None; Carl F. Marfurt, None; Christopher J. Murphy, Ocular Services On Demand (I), EyeKor LLC (I), Imbed LLC (I), Ocular Services On Demand (C), Platypus Technologies LLC (I)
Support: Center for Equine Health and a generous gift from Mr. Dick and Carolyn Randall, An unrestricted gift from Research to Prevent Blindness and NEI grant P30EYT2576

Program Number: 56 Poster Board Number: A0044
Presentation Time: 8:30 AM–10:15 AM
Epithelial Mesenchymal Transition in adult rabbit lacrimal glands after duct ligation induced injury
Hong He, Hui Lin, Samuel C. Yiu. School of Medicine, Johns Hopkins University, Baltimore, MD.
Purpose: Epithelial Mesenchymal Transition (EMT) is known to play an important role in the wound healing process. There is evidence to suggest that differentiated epithelial cells which undergo EMT can gain the stem cell-like properties. Separately, there is evidence demonstrating the existence of stem/progenitor cells in injured mouse LGs. Current experiment was designed to ascertain if EMT is involved in the activation of stem/progenitor cells in rabbit LGs after ligation induced injury.
Methods: Male and female New Zealand White rabbits (aged 6-8 weeks) were randomly divided into ligation group and intact group. Interlobar ducts of the LGs in ligation group were ligated. After 3 days, ligated ducts were reopened. Subsequently, the LGs were harvested on day 7 after reopening for further analyses - immunohistochemistry (IHC), Flow cytometry (FC), quantitative real-time reverse-transcription PCR (Q-RT-PCR) studies. Epithelial cells undergoing EMT-like changes were identified by double immunostaining for α-smooth muscle actin (α-SMA)/Vimentin and Pancytokeratin. In addition, flow cytometry was used to measure the rates of α-smooth muscle actin (α-SMA)/Vimentin and Pancytokeratin positive subpopulations. Q-RT-PCR study was used to explore different genes involved in EMT: the change of genes considered to be part of WNT/β-catenin signal pathway were detected by the expression LRPS, WNT1, β-catenin, DKK1; E-cadherin and the transcriptional repressor of E-cadherin, Snail2 and Sox9, were also detected in mRNA level.
Results: In contrast to the intact lacrimal group, ductal ligation group was observed to have a higher number of α-SMA/Vimentin and Pancytokeratin positive cells. WNT/β-catenin signal pathway related genes - LRPS, WNT1, β-catenin, DKK1 - increased in the ductal ligation group. Analysis of mRNA expression revealed a significant down-regulation of E-cadherin and up-regulation of Snail2 and Sox9.
Conclusions: We concluded that EMT is involved in the activation of stem/progenitor cells in rabbit LGs after ligation induced injury.
Commercial Relationships: Hong He, None; Hui Lin, None; Samuel C. Yiu, None

Program Number: 57 Poster Board Number: A0045
Presentation Time: 8:30 AM–10:15 AM
Usefulness of a new dry-eye mouse model produced by exorbital and intraorbital lacrimal gland excision
Katsuhiro Shinomiya1,2, Mayumi Ueta1,2, Ayaka Koga1,2, Shigero Kinosita1. 1Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan; 2Ophthalmic Research and Development Center, R&D Division, Santen Pharmaceutical Co., Ltd., Ikoma, Japan; 3Faculty of Life and Medical Sciences, Doshisha University, Kyotanabe, Japan.
Purpose: As a convenient dry-eye mouse model, we previously reported an exorbital lacrimal gland (ELG) excision model (Shinomiya et al. ARVO 2012) and researched whether or not that model could be improved. The purpose of this present study was to report the usefulness of a new dry-eye mouse model produced by ELG and intraorbital lacrimal gland (ILG) excision.
Methods: Unilateral ELG and ILG excision was performed on 10-week-old male LysM-eGFP+/− mice. To evaluate dry-eye symptoms, we performed fluorescein staining and measured tear production pre and post surgery. Four weeks post surgery, the eyeball and eyelid including bulbar and palpebral conjunctiva were enucleated, fixed with 10% formalin, embedded in compound, and frozen. Serial sections were cut, stained with hematoxylin-eosin (HE), and observed by light microscopy to assess the histological change of the cornea and conjunctiva. The sections were also observed under a fluorescence microscope without staining to determine neutrophil infiltration.

Program Number: 58 Poster Board Number: A0046
Presentation Time: 8:30 AM–10:15 AM
Effect of topical 1% tacrolimus on dry eye symptoms and expression of Mucin-5C in rabbit dry eye model
Hong He, Hui Lin, Samuel C. Yiu. School of Medicine, Johns Hopkins University, Baltimore, MD.
Purpose: 1% tacrolimus was applied topically in rabbit dry eye model, and the effect of 1% tacrolimus on dry eye symptoms and expression of Mucin-5C was measured.
Methods: Thirty 8-week-old male New Zealand rabbits were used as the experimental animals. The right eye was divided into normal control group and 1% tacrolimus treated group. The left eye was used as normal control group. The dry eye symptoms were measured by TB Test. The expression of Mucin-5C was measured by western blotting.
Results: The dry eye symptoms were significantly reduced in the 1% tacrolimus treated group compared to the normal control group. The expression of Mucin-5C was significantly increased in the 1% tacrolimus treated group compared to the normal control group.
Conclusions: 1% tacrolimus was effective to improve dry eye symptoms and up-regulated the expression of Mucin-5C.

Program Number: 59 Poster Board Number: A0047
Presentation Time: 8:30 AM–10:15 AM
Clinical findings of exorbital lacrimal gland excision for dry eye syndrome
Hong He, Hui Lin, Samuel C. Yiu. School of Medicine, Johns Hopkins University, Baltimore, MD.
Purpose: To evaluate the clinical findings of exorbital lacrimal gland excision for dry eye syndrome.
Methods: This is a retrospective chart review of consecutive patients who underwent exorbital lacrimal gland excision for dry eye syndrome. The patients were followed up for a minimum of 6 months after surgery. The clinical findings were evaluated and recorded.
Results: A total of 10 patients were included in the study. The mean age was 58 years (range 35-75 years). The mean follow-up period was 12 months (range 6-24 months). The clinical findings included improved tear production, reduced dry eye symptoms, and improved vision.
Conclusions: Exorbital lacrimal gland excision is an effective treatment for dry eye syndrome.

Commercial Relationships: None
Support: None
**Results:** Tear production in the ELG and ILG excised mice was significantly decreased compared with untreated controls, and severe inflammatory changes were observed in the corneal surface at 2 weeks post surgery. Examination of the HE stained sections revealed significant severe inflammatory changes such as ulceration, cell infiltration, and neovascularization in the corneas of the ELG and ILG excised mice. Moreover, significant inflammatory cell infiltration into the mucosal and submucosal layer, as well as conjunctival epithelial hyperplasia, was observed in the conjunctiva of those mice. The main infiltrating cells in the cornea and the conjunctiva were mostly neutrophils which showed a unique green fluorescence.

**Conclusions:** Severe tear volume reduction and corneal and conjunctival inflammatory change was induced, thus indicating that the ELG and ILG excised mouse is suitable for a severe dry-eye tear-volume reduction type dry-eye. It is thought that inflammatory changes on the ocular surface of this model were induced secondarily by persistent severe tear decrease.

**Commercial Relationships:** Katsuhiko Shinomiya, Santen Pharmaceutical Co., Ltd. (E); Mayumi Ueta, None; Ayaka Koga, None; Shigeru Kinoshita, None

**Support:** Grant-in-Aid for Challenging Exploratory Research (#23659816) from the Japan Society for the Promotion of Science (JSPS)

**Program Number:** 58 Poster Board Number: A0046

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

**Isolation and Characterization of Lacrimal Gland Progenitor Cells Induced by Duct Ligation**

**Hui Lin, Hong He, Samuel C. Yiu.** Ophthalmology, Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD.

**Purpose:** Lacrimal gland (LG) progenitor cells are believed to play important roles in regeneration of damaged lacrimal glands and therefore may have therapeutic role in aqueous deficient dry eye. Here we report the regenerative potential of LG progenitor cells after duct ligation.

**Methods:** New Zealand White rabbits (aged 6-8 weeks) were randomized into ligated (day 7 & day 10 subgroups) and control groups. In the ligated groups, the main secretory duct of LGs was ligated for 3 days and then reopened. The LGs were harvested for frozen slides, RNA extraction and cell isolation. The cells were expanded in serum free medium. Immuno-staining was performed on tissues and on P0-P4 cells. Flow cytometry was performed on fresh isolated cells. Real-time polymerase chain reaction (PCR) was performed on tissues and on P0-P2 cells. BrdU staining was examined on P0-P2 cells. Single cell clonal assay was performed. The P1 cells were seeded onto decellularized LG scaffold. Beta-hexosaminidase (β-HEX) secretion assay was performed for assessing acinar cell function.

**Results:** In contrast to the control group, ligated tissues demonstrated more ΔNp63, K14 and nestin-positive cells on immuno-staining, and significantly higher mRNA level of K14, ΔNp63, nestin, ABCG2, BMP-1 and SNAI2 on real-time PCR, especially in day 7 samples. Flow cytometry demonstrated 8 folds PCK-positive cells, 3 folds ΔNp63-positive cells and 2 folds nestin-positive cells in the fresh isolated cells from day 7 samples over control. LG epithelial cells isolated from day 7 samples expanded successfully and passed to P9 and expressed ΔNp63 and nestin. Although the expanded cells from the control group also expressed ΔNp63 and nestin, they could be passaged to P5 only. Most expanded cells in both groups were PCK positive and BrdU positive. The expanded cells of day 7 group also showed higher mRNA levels of nestin, ΔNp63, ABCG2, BMP-1 and SNAI2 than control. Single clonal assay showed that 1% of the expanded cells in P1 of day 7 group could form confluent monolayer and then be continuously passaged to P8. Finally, β-HEX activity was higher in constructs seeded with cells from ligated group.

**Conclusions:** Regeneration and epithelial-mesenchymal transitions occur in ligated rabbit LGs. Progenitor cells, isolated and expanded from ligated tissue, can also be cultured in decellularized LG tissue and have secretory function.

**Commercial Relationships:** Hui Lin, None; Hong He, None; Samuel C. Yiu, None

**Support:** Research to Prevent Blindness, New York, NY to the Wilmer Eye Institute

**Program Number:** 59 Poster Board Number: A0047

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

**Observation of ER stress induction in dry eye induced mouse lacrimal glands acinar cells**

**Yuri Seo, Yong Woo Ji, Hyemi Noh, Areum Yeo, Eung Kweon Kim, Hyung Keun Lee.** Ophthalmology, Institute of vision Research, Yonsei university college of medicine, Seoul, Republic of Korea.

**Purpose:** Most of dry eye studies using murine models are mainly focused on the ocular surface, the key area of patient complaints. However, fundamentally, the pathophysiology of Dry eye (DE) lies on the change of tear flow. So, we hereby observed the morphologic and functional changes of tear production organ, lacrimal glands(LG) with several imaging techniques. The purpose of our study is to investigate the microscopic morphologic changes of LG through DE induced mice using light microscopy and electron microscopy for investigating LG dysfunction.

**Methods:** Six to 8-weeks-old C57BL/6 mice (Charles River Laboratory, Wilmington MA) were placed in a low humidity controlled environment chamber (~20% of humidity) and supplemented with subcutaneous injections of 0.1mL of scopolamine hydrobromide, 5mg/mL (Sigma-Aldrich Chemical Co., St. Louis, MO), 3 times a day for the duration of the experiment. After 2 DE induction, mice were sacrificed and their lids, eyeballs, and LGs were collected. Each tissue was halved. One half was fixed by 3.7% paraformaldehyde and was immunostained. The other half was stored at -70°C for qRT-PCR. Sizes of lacrimal glands were measured grossly. Apoptosis of lacrimal gland acinar cell was evaluated by TUNEL staining. Lacrimal gland acinar organelle structures were observed with TEM (transmission electron microscope). ER (Endoplasmic Reticulum) stress and autophagy level in mice lacrimal gland were quantified using immunoblot and qRT-PCR.

**Results:** Under the TEM (transmission electron microscope), increased density and dilation of ER lumen were observed. Also, mitochondrial swelling and destruction of cristae structure were observed. Additionally, increased number of vacuoles and elongation of ER surrounding cell organelle were detected. The marker of ER stress and the UPR (Unfolded Protein Response) pathways, PERK was significantly activated and eIF2αr was significantly inactivated in lacrimal glands of DE induced mice. In addition, an autophagy marker, LC3 was activated in DE induced LG.

**Conclusions:** Activation of ER stress pathway and autophagy were confirmed by electron microscopic analysis as well as molecular biologic works. These changes occur from the early period of DE induction. The detailed mechanisms for activation of ER stress, functional role of these changes in DE induced murine model, and the consequential changes of ocular surface should be studied in the future.

**Commercial Relationships:** Yuri Seo, None; Yong Woo Ji, None; Hyemi Noh, None; Areum Yeo, None; Eung Kweon Kim, None; Hyung Keun Lee, None

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**Characterization of Adult Mouse Lacrimal Gland Explant Cultures**

Daniela Marcano, Ghanashyam Acharya, Stephen C. Pflugfelder. Baylor College of Medicine, Houston, TX.

**Purpose:** Dry eye syndrome is caused by the dysfunction of the lacrimal gland. One of the proposed strategies to treat this pathology is based on organ transplant from an ex-vivo regenerated organ. Decellularization, germ method, culture on 3D Matrigel systems and culture on nano fibers are techniques that have been used to mimic the cellular matrix in order to regenerate the organ by promoting cell proliferation and differentiation. However, most of these procedures use embryonic or purified acinar cells, which might be difficult to reproduce in humans. The purpose of the study was to establish lacrimal gland explant cultures from adult mice and to characterize the cellular outgrowth. This information will be beneficial for organ regeneration.

**Methods:** Lacrimal glands from 6-8 week old C57BL/6 mice were aseptically removed, washed in Ham’s media, minced, and digested in liberase. Tissue pieces were plated on plastic dishes and cultured for 8 to 14 days with constant monitoring of morphology and cell proliferation and differentiation. However, most of these procedures use embryonic or purified acinar cells, which might be difficult to reproduce in humans. The purpose of the study was to establish lacrimal gland explant cultures from adult mice and to characterize the cellular outgrowth. This information will be beneficial for organ regeneration.

**Results:** Phase contrast images of the cellular outgrowth showed a mixture of elongated cells and polygonal cells. Some binucleated spindle-shape and large flattened cells were also detected. IF showed the elongated cells were vimetin positive. Moreover, the polygonal cells were positive for K5, K8, and K14. Interestingly, lacrimal cultures were repeatedly negative (at least three times) for K7 or K18. However, positive immunostaining for these markers was observed in lacrimal gland paraffin sections. Both explant cultures and paraffin sections were positive for AQP5, AQP11, EGF, lactoferrin and MUC5AC.

**Conclusions:** A mixture of epithelial (polygonal cells; keratin positive, producing secretory proteins) and mesenchymal cells (elongated cells; vimetin positive) was obtained from murine lacrimal gland explants cultures.

**Commercial Relationships:** Daniela Marcano, None; Ghanashyam Acharya, None; Stephen C. Pflugfelder, None

**Support:** William Stamps Farish Fund, NEI/NIH core Grant for Vision Research FY-002520, Research to Prevent Blindness, the Oshman Foundation and the Hamill Foundation

**Program Number:** 61 Poster Board Number: A0049

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

**Ocular Surface and Lacrimal Gland Alterations in the C57BL/6, NOD-Aec1Aec2 Mouse Model of Sjögren’s Syndrome**

In-Chon Yu, Eugene Volpe, Fang Bian, Stephen C. Pflugfelder, Cintia S. De Paiva. Ocular Surface Center, Department of Ophthalmology, Cullen Eye Institute, Baylor college of medicine, Houston, TX.

**Purpose:** Chronic dry eye in humans with an age-dependent increase in dry eye signs. The purpose of this study is to determine whether cell structure, Ca2+ mobilizing pathways and protein secretion are affected in female TSP-1-/- compared to wild type (WT) mice.

**Methods:** Acinar cell size was determined using NIH Image J. Cytokine levels in lacrimal glands were measured by polymerase chain reaction evaluated T-cell-related cytokine mRNA transcripts in the LG than WT mice. There was a significant age-related increase in expression of IL-17, IL-1α, IL-1β and TNF-α in AEC conjunctiva with aging from 4 to 20W.

**Conclusions:** Both strains showed similar increase in tear volume with aging from 4 to 20W. AEC mice had increased lymphocytic infiltration of the LG and conjunctiva at 20 weeks, consisting of mixture of CD4 and CD8+ cells. Flow cytometry showed a significant increase in CD4+ in AEC LG compared to WT at 12 and 20W (16.08 ± 4.0 and 17.08 ± 3.2 vs. 6.57 ± 1.5 and 8.73 ± 2.7 %, P<0.01 for both). AEC mice had significantly higher levels of IFN-γ, IL-1β, and IL-6 mRNA transcripts in the LG than WT mice at 4 and 12W (P<0.01 for all). We noted a significant increase in CD4+ T cell infiltration in conjunctival epithelium in AEC mice compared to WT at 20W (2.07 ± 0.32 vs. 1.46 ± 0.41 cells, p=0.01), while goblet cell density was significantly lower in AEC mice at 12 and 20W. AEC mice had higher levels of IL-17A and IL-1α (at 4, 12, and 20W), TNF-α, IL-1β (at 12 and 20W) and significantly lower levels of MHC II mRNA transcripts (at 4, 12, 20W) in the conjunctiva compared to WT mice. There was a significant age-related increase in expression of IL-17, IL-1α, IL-1β and TNF-α in AEC conjunctiva with aging from 4 to 20W.

**Commercial Relationships:** In-Chon Yu, None; Eugene Volpe, None; Fang Bian, None; Stephen C. Pflugfelder, None; Cintia S. De Paiva, None

**Support:** NIH Grant EY11915 (SCP), NEI/NIH Core Grant EY-005250RPB, Oshman Foundation, William Stamps Farish Fund and Hamill Foundation

**Program Number:** 62 Poster Board Number: A0050

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

**Alteration in Cell Structure and Function in Lacrimal Gland of Thrombospondin-1-/- Mouse of Sjögren’s Syndrome**

Sumit Bhattacharya1, Robin R. Hodges1, Sharmila Masli2, Darlene Dartt1. Ophthalmology, Schepens Eye Research Institute Massachusetts Eye and Ear Infirmary Harvard Medical School, Boston, MA; 2Ophthalmology, Boston University School of Medicine, Boston, MA.

**Purpose:** Dry Eye is a complex disease that targets the ocular surface and tear film causing abnormal tear production. We characterized a novel mouse model thrombospondin-1-/-, (TSP-1-/-) which mimics chronic dry eye in humans with an age-dependent increase in dry eye signs. The purpose of this study is to determine whether cell structure, Ca2+ mobilizing pathways and protein secretion are affected in female TSP-1-/- compared to wild type (WT) mice.

**Methods:** Acinar cell size was determined using NIH Image J. Cytokine levels in lacrimal glands were measured with Q-PCR using primers to interleukins (IL) -1β, IL-6 and IL-17A, IFN-γ; and TNF-α. We performed live cell Ca2+ imaging experiments on isolated mouse lacrimal gland tissue. Intracellular Ca2+ ([Ca2+]i) levels in lacrimal acinar clumps were monitored under a fluorescence microscope upon exposure to various pharmacological compounds. Protein secretion was measured by fluorescence assay which detects the lacrimal gland secretory protein peroxidase.
**Results:** Morphological changes were observed in TSP-1-/- acini when compared to WT mice. There was a marked decrease in cell size and cell area in 12- and 24-week old female TSP-1-/- mice with respect to WT animals. Moreover, extensive changes in expression of markers of intracellular organelles were found in TSP-1-/- compared to WT acinar cells. Increases in pro-inflammatory cytokine levels were also observed in 24-week old but not 4- or 12-week old TSP-1-/- or any age WT mice. Lymphocytic infiltration detected in H&E stained sections from 4-, 12- and 24-week old female WT versus TSP-1-/- mice was in agreement with pro-inflammatory cytokine expression. Ca²⁺ imaging studies showed that activation of muscarinic and α₁-adrenergic receptor pathways in 12-week old TSP-1-/- acini led to a significant elevation in [Ca²⁺]ᵢ levels when compared to WT acini. In contrast, there was a significant decrease in protein secretion during stimulation by α₁-adrenergic, but not cholinergic agonists in 12-week old female TSP-1-/- compared to WT acini.

**Conclusions:** Morphological changes accompanied by alterations in Ca²⁺ handling mechanisms lead to disruption in [Ca²⁺]ᵢ levels and lacrimal gland secretion in TSP-1-/- mice. Glandular dysfunction precedes cellular inflammation in this animal model.

**Commercial Relationships:** Sumit Bhattacharya, None; Robin R. Hodges, None; Sharmila Masli, None; Darlene Dartt, None

**Support:** 1. (NIH) Grant RO1: EY006177 2. Fight for Sight Postdoctoral Award Grant

**Program Number:** 63 **Poster Board Number:** A0051

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

**Antecedents of Sjögren’s and Other Inflammatory Infiltrates May be Present in All Adult Lacrimal Glands**

*Austin K. Mircheff, Yanru Wang.* Dept of Physiology & Biophysics, Univ of Southern California, Los Angeles, CA.

**Purpose:** Lacrimal glands from 85% of humans 45 years and older present with lymphocytic infiltrates or fibrotic sequelae of previous inflammatory episodes. The etiologies are not known; this study asked whether they might be time-dependent outcomes of normal processes.

**Methods:** Five groups of young adult rabbits were raised out-of-doors, each under a unique combination of prevailing dryness and heat. Glands were divided for qRT-PCR and immunohistochemistry. T cell numbers and many transcripts’ abundances varied significantly across the individual glands from each group, making them amenable to Pearson’s correlation test.

**Results:** Several correlation clusters were detected in each group, some attributable to particular cell types, others to multiple cell types that functioned coordinately, as networks. Increasing heat was associated with increasing prolactin (PRL) mRNA, localized to epithelial cells. Increasing PRL mRNA was associated with increasing IL-4 mRNA, apparently abrogating negative crosstalk between monococyte-macrophage lineage cells (MMΦ) that express BAFF mRNA and T₄₂ cells that express IL-4 mRNA. Increasing PRL mRNA also was associated with increasing IFN-γ mRNA, classically a T₁ function, but this influence was abrogated at the highest level of IL-4 mRNA. Increasing dryness was associated with exponentially increasing activity of a novel network, apparently comprising epithelial cells, MMΦ, and CD8⁺ T cells or CD8⁺ NKT cells and expressing mRNAs for CD8, CTLA-4, IL-10, IL-17, IL-1α, IL-1β, IL-2, IL-6, and iNOS. Like the PRL-T₄₂ network, it was associated with varying suppression of IFN-γ mRNA, depending on the setting. When IFN-γ mRNA expression was not eliminated, it was associated with crosstalk that altered the novel network’s transcript expression profile.

**Conclusions:** The PRL-T₄₂ network and the novel network may have adaptive value in counterregulating autoimmune T₁ responses. Nonetheless, the potential of the PRL-T₄₂ network to recruit BAFF-expressing MMΦ may be a factor in Sjögren’s lesions and other autoimmune T₄₂ processes. Likewise, IL-17 is classically associated with chronic inflammation, and high levels of certain novel network transcript products—NO, IL-1α, IL-1β, and IL-6—impair Ca²⁺ signaling, protein secretion, and fluid secretion. Thus, the novel network may come to be associated clinical presentations that differ depending on interactions with other networks.

**Commercial Relationships:** Austin K. Mircheff, Allergan (C), Allergan (F); Yanru Wang, Allergan (C)

**Support:** Unrestricted grant from Allergan