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1. Demographic Factors Associated with Differential Access to Treatment in Keratoconus Patients at a U.S. Urban Safety Net Hospital

Oluchi Ihionu

Purpose: There is limited information regarding the potential influence of demographic factors on keratoconus outcomes. The purpose of this study is to examine the effects of demographic factors of race/ethnicity, preferred patient language, health insurance, and area deprivation index (ADI) on the rates at which patients receive two different keratoconus interventions: corneal collagen crosslinking (CXL) and medical contact lenses (CL). Our primary hypothesis was that patients who live in more economically disadvantaged areas, as determined by ADI, have lower rates of access to CXL and CL, and that other demographic factors could influence this access to treatment as well.

Methods: Electronic medical records of keratoconus patients at Boston Medical Center from 2012-2020 were retrospectively examined to obtain CXL and CL treatment records, race/ethnicity, primary language, insurance (private, public, or none), and home address. National ADI quintile was determined by address, and patients were categorized as 1st, 2nd, or 3rd quintiles and above (most disadvantaged). Logistic regression models were used to calculate odds ratios for keratoconus treatment adjusted for age, ADI, insurance, race/ethnicity, and language. Chi-square likelihood ratios were used to test for associations between demographic factors and odds of treatment, and post-hoc analyses were performed using Tukey’s method at a 5% family-wise error rate.

Results: 572 charts were reviewed, and 32 patients were excluded due to incomplete records. Of 540 remaining KCN patients, 42 received CXL and 213 received and/or had a history of CL. There was no significant association between ADI and CXL treatment (p=0.688) or CL access (p=0.912). Multivariate analyses showed that patients whose primary language was not English were less likely to have CL access relative to patients whose primary language was English (ORadj=0.65, 95% CI 0.45-0.93, p=0.021). Insurance status (p=0.002) and race/ethnicity (p=0.003) had significant associations with CXL treatment in multivariate models. When adjusted for all other factors, including insurance status, White patients were more likely to receive CXL relative to Black patients (ORadj=8.54, 95% CI 2.82-27.09) and patients with private insurance were more likely to receive CXL than those without insurance (ORadj=10.00, 95% CI 2.59-66.22).

Conclusion: Our data demonstrate disparities in keratoconus treatment access for commonly disadvantaged groups.
2. Dysregulation of DNA repair genes in Fuchs Endothelial Corneal Dystrophy

Shazia Ashraf, Neha Deshpande, Shivakumar Vasanth, Geetha Melangath, Raymond Jeff Wong, Marianne Price, Francis Price Jr., Ula V. Jurkunas

**Purpose:** Fuchs Endothelial Corneal Dystrophy (FECD), a late-onset oxidative stress disorder, is the most common cause of corneal endothelial (CE) degeneration and is genetically associated with CTG repeat expansion in TCF4. We previously reported accumulation of nuclear DNA and mitochondrial DNA (mtDNA) damage causing CE cell death in FECD. Specifically, mtDNA damage was a prominent finding in the development of FECD phenotype in the ultraviolet-A (UVA) light-induced FECD mouse model. We hypothesize that increased oxidative stress leads to an aberrant DNA damage response comprised of the DNA repair pathway that may result in repeat expansions in TCF4.

**Methods:** To investigate whether a deficiency in DNA repair pathways leads to the accumulation of toxic DNA lesions in FECD, we analyzed the differential expression profiles of DNA repair genes by real-time PCR arrays and tested 84 genes and five housekeeping genes. Total RNA was extracted from Descemet’s membrane-CE stripped from age-matched normal donors (n=3) and FECD specimens (n=8) stratified as either with TCF4 repeat expansion (> 50 CTG repeats; TCF4+) or without expansion (< 50 CTG repeats; TCF4-). cDNA was subjected to real-time PCR analysis on Human DNA Repair RT Profiler plates. Change in mRNA expression of <0.5 or >2.0-fold in FECD relative to normal was set as the cutoff value for down- and upregulation. The downregulated mitochondrial genes were further validated using the UVA-based mouse model of FECD.

**Results:** FECD specimens showed significant downregulation (<0.5-fold; p< 0.05) of 11 genes and upregulation (> 2-fold, p <0.05) of 8 genes belonging to base excision repair (BER), nucleotide excision repair (NER) or mismatch repair pathways (MMR), compared to normal donors. We further validated that MSH2 (MMR) and POLB (BER) genes were preferentially upregulated in TCF4+ FECD. Key mtDNA repair genes LIG3, NEIL2, SMUG1, TOP3A, TREX1, XPC, and XRCC1 were downregulated in both TCF4+ and TCF4- specimens. Downregulation of mtDNA repair genes Lig3, Neil2, and Top3a in FECD specimens (with and without repeats) and UVA-based in vivo model suggest DNA repair deficiency is an important contributor to the final common pathway of FECD pathogenesis.

**Conclusion:** Our findings indicate impaired DNA repair pathways that are important for the repair of ROS-induced mtDNA damage noted in FECD. We provide new insights toward bridging the gap between the human genetics of FECD and DNA damage due to oxidative stress, establishing the role of CTG repeat expansions in TCF4.
3. Detection of cell-cell signaling in corneal epithelium

Nicholas Azzari

**Purpose:** When the corneal epithelium is injured, calcium signaling events occur in cells near the injury in response to ATP released into the extracellular environment. The calcium signaling events propagate between cells and have been found to be necessary for cellular motility and wound healing though the elimination of those events in cell culture models. We have developed and used computational analysis programs to characterize these signaling events in both culture and live tissue models. Alongside the computational programs, we have developed a method to perform live cell imaging of globes utilizing 3D printed holders that immobilize the globes to produce high-quality images.

**Methods:** Live cell imaging was performed on both ex vivo globes from male C57Bl6 mice from two age cohorts young (9 to 12 weeks old) and older (27 week old), and cultured Human Corneal Limbal Epithelial (HCLE) cells using the Zeiss LSM 880 confocal microscope. Globes were pre-incubated with CellMask DeepRed and Fluo-4AM. Globes were stabilized using a 3D printed holder designed with CAD and printed using the EnderPro3 printer, to keep eyes stable during imaging. Images of the central corneal epithelium were collected after injury to the corneal epithelium. Calcium signaling analysis was performed using Zen, MATLAB, and ImageJ.

**Results:** Differences in calcium signaling events were observed during the wound healing process between young and old eyes. We observed calcium events within the basal cell layers of corneal epithelium from 9 week mice that occur at a defined periodicity for the entire recording time. However, by 27 weeks, the events were minimal. Cell signaling events within apical cell layers of both ages were negligible. Studies in cell culture models a similar periodicity was observed with easier cell identification. Additional differences include the mode and timeline of cell migration into the wound bed and changes in stiffness with age.

**Conclusion:** The results indicate that age diminishes the cellular calcium communication of the wound response within corneal epithelium as well as the nature and timing of cellular migration. With cellular activity periodicity observed in both ex vivo and cell culture models, we are developing a computational program that will model the activity in corneas. Future studies will focus on the changes in wound healing response within disease states, more advanced aging, and conditions where healing in the cornea becomes inefficient.
4. Ocular surface mast cells promote nerve damage and trigeminal ganglion inflammation following corneal injury

Wonkyung Cho, Elsayed Elbasiony, Aastha Singh, Sharad Mittal, Sunil Chauhan

Purpose: Nerve damage following injury has been associated with impaired wound healing. We have previously shown that corneal injury leads to increased activation of ocular surface mast cells. Here, we investigated whether mast cells interact with corneal nerve and contribute to nerve degeneration and neuroinflammation.

Methods: Corneal injury was induced by mechanical removal of the epithelium (3 mm) and one-third of the anterior stroma in C57BL/6 mice using an Algebrush II. To evaluate the proximity of mast cells and damaged nerves, corneas were harvested 6 hours post-injury and stained with β-tubulin III (corneal nerves), and avidin (mast cells), for immunohistochemistry (IHC) analysis. Trigeminal ganglions (TGs) were harvested post-injury and lysates were prepared to measure levels of tryptase (mast cell activation marker), CD11b and Substance P (SubP), using colorimetric assay and PCR analysis. To assess direct interaction between mast cells and inflamed corneal nerves, primary TGs were co-cultured with bone marrow-derived mast cells for 24h. Brightfield images were captured, and neurite length was quantified using ImageJ software. TGs harvested from co-cultures were assessed for the expression of nerve activation marker SubP and CGRP. To assess the in vivo effect of mast cell activation on nerve damage, injured corneas were treated with mast cell inhibitor cromolyn (2% in PBS) and hyperactivation of nerves were measured using eye wipe test.

Results: IHC analysis demonstrated that ocular surface mast cells infiltrated into the injured cornea in close proximity to the damaged corneal nerves. Corneal injury resulted in a significant activation of TG mast cells and inflammation, as indicated by increased levels of tryptase (p=0.003), CD11b (p<0.001) and SubP (p=0.009). Co-culturing of TGs with mast cells resulted in an approximate 13-fold upregulation in CGRP (p<0.001), and ~1.5-fold increase in SubP (p=0.008), compared to TGs cultured alone. Moreover, mast cells resulted in significant neuronal degeneration, as indicated by 50% decrease in neurite length compared to control TG cultures (p<0.001). Pharmacological inhibition of ocular surface mast cell activation resulted in a significant decline in TG inflammation and hyperalgesia, as shown by decreased eye wipes (p=0.005).

Conclusion: Ocular surface mast cells exacerbate nerve degeneration and promote inflammation in the trigeminal ganglion.
5. Ocular Surface Disease Patients with Microneuromas also have Reduced Nerves

Stephanie M. Cox, OD, Betul N. Bayraktutar, MD, Anya de Leeuw, MD, Gabriela Dieckmann, MD, Pedram Hamrah, MD

**Purpose:** The presence of microneuromas within the subbasal nerve plexus as identified via corneal in vivo confocal microscopy (IVCM) has been associated with corneal neuropathy, including with neuropathic corneal pain. However, the association of microneuromas with other nerve and immune cell parameters has not been established.

**Methods:** This is a secondary analysis of a retrospective study that involved the collection of IVCM images from ocular surface disease patients. All IVCM images were viewed by the grader, and presence or absence of microneuromas was determined for each patient based on all images. In addition, three representative images from the subbasal nerve plexus were selected for each patient. ImageJ with NeuronJ plug in was used to quantify each selected image for the total, main, and branch nerve densities and count. The results from these three images were averaged. In addition the number of immune cells were counted for each image and averaged. Patients with microneuromas were compared to those without using t-test or Mann-Whitney U test as appropriate.

**Results:** The patients showed an average of age of 58.3 ± 1.6 years and 76.2% were female. Compared to the non-microneuroma group (NMG), the microneuroma group (MG) had a lower average total density [11,995.28 µm/mm² (range: 3,580.00-14,173.55) vs 13,806.47 (range: 0.00-26,158.00); p=0.012], average total nerve number [6.7/frame (range: 1.7-10.3) vs 8.7 (range: 0.0-23.3); p=0.014], average branch nerve density [3,082.49 µm/mm² (range: 0.00-7,205.11) vs 4,553.88 (range: 0.00-14,601.73); p = 0.044] and average branch number [3.3/frame (range: 0.0-8.0) vs 5.0 (range: 0.0-19.3), p=0.024]. There was no significant difference in main nerve density (7,054.33 ± 541.44 µm/mm² for MG; 8,373.31 ± 271.68 for NG; p=0.070), main nerve number (2.9 ± 0.3/frame for MG; 3.5 ± 0.1 for NG; p=0.094), or immune cell density [14.6/mm² (range:0.0-175.0) for MG; 35.4 (range: 0.0-285.4) for NG; p=0.144].

**Conclusion:** Patients with microneuromas likely also have reduced nerve density, which is more apparent in branch nerves compared to main nerves, suggesting that patients with increased nerve loss have a higher likelihood of presenting with microneuromas.
6. Angiogenesis Modulation By locally Administered Regulatory T cells In High-risk Corneal Transplantation

Shima Dehghani, Katayoon Foruzanfar, Seokjoo Lee, Akitomo Narimatsu, Aytan Musayeva, Francesca Kahale, Hamid Alemi, Amir Reza Naderi, Tomas Blanco, Reza Dana

Purpose: Corneal allograft survival significantly decreases in hosts with vascularized recipient beds. It has been previously shown that subconjunctival injection of regulatory T cells (Treg) suppresses neovascularization (NV) in suture-induced corneal neovascularization model. Moreover, it has been stated that the antiangiogenic protein PD-L1 (Programmed death-ligand 1) mRNA is highly expressed on naïve Tregs. In this study we investigated the suppressive function of subconjunctivally injected Tregs on neovascularization in high-risk corneal transplantation. Additionally, we explored whether PD-L1 plays a role in the antiangiogenic effect of Treg.

Methods: In vitro: Mouse endothelial cells (MS-1) were co-cultured with CD4+CD25+ Tregs in the absence or presence of PD-L1 blocker (10 µg/ml) on a reduced growth factor basement membrane extract. Image acquisition was done at 4h (bright field microscopy) and indicators of tube formation, i.e., number of junctions and tube length, were measured using ImageJ software. In vivo: Allogeneic corneal transplantations on a vascularized corneal bed (high-risk model) were performed on BALB/c mice as hosts and C57BL/6 were used as donors (8 mice/group). Subsequent to transplantation, hosts were randomized into four groups, i.e. a control group and three groups each receiving a subconjunctival injection of saline, Treg (10^5 cells), or blocked Treg (for PD-L1)(10^5 cells), exclusively. Test subjects were examined on day 0, 3, 7 and 10 post-transplantation using a slit-lamp microscope. A standardized neovascularization grading system was used to score clinical findings.

Results: In vitro: The mean number of junctions and mean length were significantly less in MS-1+Treg group (10.0±7.7 and 3594±906) compared to control group (86.6±10.86 and 8995.5±630) at 4 hours after coculture. Blocking PD-L1 inhibited the antiangiogenic effect of Treg and rendered the latter difference insignificant (p>0.05). In vivo: Subjects undergoing corneal transplantation with subconjunctival injection of Treg had a significantly lower neovascularization score (6.8±0.9) compared to the saline injected (8±0) and control group (8±0) at day 7 post-transplant (p<0.05). Blocking PDL-1 on Treg inhibited the antiangiogenic effect of Tregs, raising the NV score (7.4±0.7), and made the aforementioned differences insignificant(p>0.05).

Conclusion: Locally administered Treg suppresses corneal neovascularization in high-risk corneal transplantation and PD-L1 protein appears to be impactful in the way Treg exert their antiangiogenic effect.
7. Mast cells augment neutrophil activation and their secretion of tissue-damaging factor

Elsayed Elbasiony, WonKyung Cho, Yilin Guan, Aastha Singh, Sharad Mittal, Sunil Chauhan

**Purpose:** Excessive neutrophil activation and secretion of their effector molecules result in tissue damage during ocular inflammation. Previously, our study demonstrated that mast cells initiate neutrophil recruitment to the ocular surface following injury. Here, we investigated whether mast cells promote activation of neutrophils to secrete tissue-damaging effector molecule.

**Methods:** Bone marrow cells were harvested from femurs and tibias of Balb/c mice and cultured for 3-4 weeks in the presence of IL-3 (10 ng/ml) and SCF (50 ng/ml) to generate mast cells. Neutrophils were magnetically sorted from the bone marrow cells of Balb/c mice using a neutrophil isolation kit (>95% purity). Mast cells were co-cultured with neutrophils at 1:1 ratio for 3 hours. Thereafter, neutrophils were harvested to evaluate the expression of their activation markers, CD11b and Ly6G, using flow cytometry. Supernatants of the co-cultures were collected to assess the activity of myeloperoxidase (MPO), an enzyme involved in neutrophil-mediated collateral tissue damage, using a colorimetric assay kit.

**Results:** Neutrophils co-cultured with mast cells expressed a significantly higher level of maturation marker CD11b (5-fold increase; p=0.0002), compared to neutrophils cultured in medium alone. Similarly, a significant 48% increase in the expression of Ly6G by neutrophils was observed in the presence of mast cells compared to neutrophils cultured alone (p=0.008). In addition, a significant 50% increase in MPO activity was observed in neutrophils when cultured in the presence of mast cells, compared to control neutrophil cultures (p=0.03).

**Conclusion:** Our data demonstrate that mast cells amplify neutrophil activation and promote the secretion of tissue damaging myeloperoxidase enzyme.
8. Contribution of Regulatory T Cells to the Regulation of Corneal Neovascularization in the Diabetic Cornea

Katayoon Forouzanfar, Shima Dehghani, Francesca Kahale, Akitomo Narimatsu, Seokjoo Lee, Aytan Musayeva, Hamid Alemi, Amirreza Naderi, Reza Dana, Tomas Blanco

Purpose: Diabetic corneal alterations are frequent but is an underdiagnosed complication in patients with diabetes mellitus (DM). The effect of DM on corneal neovascularization (CNV) associated inflammation has been not deeply investigated. We have previously shown that naïve regulatory T cell (Treg)-based cell therapy exerts a potent anti-angiogenic effect in non-diabetic vascularized corneas. Herein, we extend these findings in studying this effect on vascularized diabetic corneas.

Methods: Diabetes was induced by daily injection of streptozotocin (STZ) for 5 days in 8-week-old Balb/c mice. NV was induced in diabetic and non-diabetic mice by placing one suture in the nasal part of the cornea. Non-diabetic mice were subconjunctivally treated with saline (group 1), diabetic Treg (5x10^5) (group 2), or naïve Treg (group 3); and diabetic mice were treated with saline (group 4) or naïve Tregs (group 5). Seven days after, corneas were imaged, excised, and immuno-stained with anti-CD31 antibody (a blood vessel marker), and imaged with a confocal microscope. Pictures were analyzed with ImageJ software. CD4+CD25+ Treg from naïve or diabetic mice were cocultured with a mouse vascular endothelial cell line (MS1 VEC) in triplicates for 4 hours, and VEC tube formation was evaluated by brightfield microscopy and images were analyzed with Image J.

Results: Both in vivo and ex vivo assessments showed that diabetic mice showed higher NV than non-diabetic mice (p<0.05). While diabetic Tregs significantly increased NV in non-diabetic corneas (p<0.05), non-diabetic Tregs drastically reduced NV in diabetic corneas (p<0.01). Co-culture of naïve Treg with VECs yielded a decrease in tube length and the number of junctions compared to VECs alone (p<0.001), but this effect was not observed when co-cultured with diabetic Treg (p>0.05).

Conclusion: The diabetic state contributes to CNV-associated with inflammation. The anti-angiogenic functions of locally delivered Treg represents a new method for suppressing CNV in vascularized corneas and explore the underlying mechanisms for this effect.
9. The synthesis and biocompatibility of the mucin-mimicking glycopolymers

Thomas Fuchsluger, Jo Sing Julia Tang, Ruben R. Rosencrantz, Lars Dähne, Susanne Stählke.1, Peter Trosan

**Purpose:** Mucins together with the superficial lipid layer form the tear film. They are highly O-glycosylated proteins responsible for lubrication and protection of the ocular surface. We synthetized neutral, positively and negatively charged glycopolymers, which mimicking natural mucins and exhibiting high hydrophilicity and stability. Their biocompatible properties were tested in the cultures with the human corneal epithelial cells (HCE).

**Methods:** The various neutral, positively and negatively charged glycopolymers were synthetized by aqueous-based synthesis and cultured with HCE (in concentrations of 1%; 0,1% and 0,01% w/v). Several cytotoxicity/viability assays together with the light microscopy were used for the biocompatibility analysis (LIVE/DEAD assay, WST-8 assay, IHC of Ki-67, ABCG2 and Pax6).

**Results:** The neutral glycopolymers had no difference in cytotoxicity/viability assay compared to a control untreated cells. Most of the positively and negatively charged glycopolymers had significant cytostatic effects on HCE with lower viability in the highest concentration (1% w/v). The WST-8 results from the HCE cultures with selected glycopolymers were consistent with the viability assay. The expression of proliferative marker Ki-67 was same among all cultures with selected glycopolymers (0,01% w/v) and control cells. No decrease of viable cells was also detected by LIVE/DEAD assay (0,01% w/v). The expression of HCE markers (Pax6 and ABCG2) were detected in cells without any change after the 48h culture of HCE cells with selected glycopolymers (0,01% w/v).

**Conclusion:** Selected glycopolymers had no cytotoxic effect on HCE cells in the 0,01% w/v concentration. Moreover, they had no negative effect on the HCE viability and displayed both morphology and characteristic markers as untreated control cells. These selected glycopolymers are biocompatible and can be used with recently tested “layer-by-layer” coating system in prepared contact lens application.
10. IFNγ reduces viability in human conjunctival goblet cells in vitro

Pernille M. Hansen

**Purpose:** The homeostasis of the ocular surface is influenced by the well-being of conjunctival goblet cells which can be affected by cytokines. To explore the pathogenesis of ocular surface disease in patients with type 2 inflammatory diseases, the putative impact of interleukin (IL)-4, IL-13 and interferon (IFN)y on conjunctival goblet cell survival and function is of interest. The purpose of this study was to examine the effect of IL-4, IL-13 and IFNγ on human conjunctival goblet cell survival assessed with MTT assays.

**Methods:** Goblet cells were grown from conjunctival tissue from human donors. The human conjunctival goblet cells were seeded into wells and exposed for 48 hours with IL-4, IL-13 and IFNγ with 100 ng/mL, respectively. The cell viability was evaluated by MTT assays.

**Results:** Exposure with IFNγ showed significant decrease in goblet cell viability with MTT assay compared to no exposure (p<0.01), exposure to IL-4 (p<0.05) and IL-13 (p<0.01), respectively.

**Conclusion:** Our study indicates that IFNγ reduces human conjunctival goblet cell survival in contrast to the Th2-associated cytokines IL-4 and IL-13.
11. Cyto-protective Effect of Alpha-Melanocyte Stimulating Hormone Against Development of Fuchs Corneal Endothelial Dystrophy

Francesca Kahale, Neha Deshpande, Hamid Alemi, Amirreza Naderi, Shudan Wang, Tomas Blanco, Thomas Dohlman, Jia Yin, Ula Jurkunas, Reza Dana

Purpose: Fuchs Endothelial Corneal Dystrophy (FECD) is characterized by a progressive loss of corneal endothelial cells (CEnC). FECD is a leading cause of corneal transplantation worldwide, and no definitive pharmacologic treatment is available. We have previously shown the protective effect of neuropeptide alpha-Melanocyte Stimulating Hormone (α-MSH) on corneal donor buttons exposed to acute chemical oxidative stress. This study aims to investigate the protective effect of α-MSH in a mouse model of FECD induced by UV-A irradiation.

Methods: 8 week-old female C57BL/6 mice (n=8/group) were irradiated with UV-A (500J/cm²). Treatment with 1.67mg/kg of α-MSH was initiated immediately following irradiation and thrice weekly for 12 weeks. The control group received vehicle treatment. Corneal endothelium was visualized by in vivo confocal microscopy at week 2, 4, 8 and 12 post-irradiation. CEnC density, hexagonality, and coefficient of variation (CV) were calculated using Konan’s CELL CHEK software. Corneal edema was evaluated by measuring central corneal thickness (CCT) on Optical Coherence Tomography. In vitro, the mechanism of cyto-protection was evaluated by exposing human derived immortalized CEnC to oxidative stress prior to staining with γ-H2AX.

Results: At 12 weeks post-irradiation, mean cell density was significantly higher in the treated group compared to untreated (1137.5±398 cells/mm²). Polymegathism represented by CV was significantly lower in the treated group (37.2 ± 5.5 compared to untreated at 64.4±20.3. Pleomorphism represented by percentage of cells retaining hexagonality was significantly higher in the treatment group (54.3% ±3.4) compared to untreated (45.1% ±6.8). Corneal decompensation depicted by edema was noted in untreated corneas starting at week 4, and progressively worsened. By 12 weeks, CCT was significantly lower in the treated group at 87.3 μm ±5.9, compared to 138.6μm ±31.0 in the untreated. In vitro, DNA damage was significantly lower in the group treated with α-MSH.

Conclusion: Early treatment with α-MSH protects CEnC from UV-A induced cell damage. This leads to preservation of cellular morphology and lower CEnC loss. The conferred endothelial cyto-protection prevents corneal edema and decompensation.
12. pDC Secretome Promotes Corneal Nerve Survival and pDCs Modulate Cold Receptor Function

Brendan M. Kenyon, Olivia Esteirleiro, Deshea L. Harris, and Pedram Hamrah

**Purpose:** We have previously reported that depletion of corneal plasmacytoid dendritic cells (pDCs) leads to a robust loss of corneal innervation. Herein, we demonstrate neurotrophic properties of the pDC secretome by concurrent pDC depletion and topical treatment with pDC-derived supernatant. Furthermore, we characterize functional alterations of corneal nerves by ex vivo electrophysiology.

**Methods:** For rescue studies, pDCs were sorted from adult C57BL/6J mice and cultured in serum-free media for 3 days, at which point supernatant was collected. Adult BDCA2-DTR mice were used for depletion of pDCs by subconjunctival injections of diphtheria toxin (DT; 30 ng/eye) at day 0 and repeated every 2 days and were compared to non-depleted controls. In rescue studies, all BDCA2-DTR animals received DT injections every 2 days and were topically treated with pDC supernatant or serum-free media (control) 3 times/day for one week. Corneas were stained with βIII-tubulin, imaged by confocal microscopy, and nerve density quantified using NeuronJ. For electrophysiology studies, recordings were performed ex vivo on enucleated eyes using a glass recording micropipette at the ocular surface in a recording chamber perfused with 34°C physiologic solution. The activity of nerve terminal impulses (NTIs) was recorded at baseline and in response to cooling and warming ramps. Analyses were performed in Spike2 software.

**Results:** pDC supernatant treatment resulted in a significant increase in central cornea innervation compared to controls (131.49 + 10.49 vs 67.77 + 6.80 mm/mm2; p<0.01). This effect was due to a rescue of the subbasal plexus (100.10 + 8.92 vs 30.34 + 7.40 mm/mm2; p<0.001), as the stromal nerve density did not differ between groups. NTI activity of high-threshold cold receptors (HT-CRs) was altered by pDC depletion. Although background activity and cooling responses did not differ between groups. Interestingly, the cooling threshold, the temperature at which HT-CRs respond, was reduced in the pDC-depleted group (22.53 + 0.54°C vs 27.97 + 0.46°C).

**Conclusion:** These findings demonstrate that corneal pDCs are crucial for both the function and maintenance of corneal nerves. The structural and functional alterations following pDC depletion, as well as rescue of corneal innervation by pDC supernatant, may have relevant consequences for ocular diseases in which sensory abnormalities predominate.
13. Development of a Moxifloxacin Eluting Hydrogel Patch for Sealing Ocular Lacerations

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**Purpose:** Ocular injuries render the eye susceptible to infection and vision loss and are particularly difficult to manage in low resource/emergency settings which typically require extensive surgical skill with high frequency post-operative eye drop regimens. To address this issue, we have developed a nanoparticle (NP) hydrogel composite system with the potential to be used as a rapid repair sealant that allows for quick stabilization of the globe for laceration-type injuries while concurrently permitting sustained drug delivery of an antibiotic to prevent infection.

**Methods:** Moxifloxacin (MXF) nanoparticles were synthesized by desolvation method and characterized using dynamic light scattering (DLS) to determine particle diameter (PD), polydispersity index (PDI), and surface charge, and the encapsulation efficiency (EE) was determined. The hydrogel sealant, composed of gelatin methacryloyl (GelMA) and glycidyl methacrylate-hyaluronic acid (HAGM), was characterized for degree of methacrylation (DM) and in vitro biocompatibility using human corneal epithelial cell. Lastly, free MXF and MXF NPs were loaded into the hydrogel, and drug release profiles were determined via dialysis. The MFX NP hydrogel was further assessed in vitro for sealant capabilities via burst pressure testing and for antimicrobial efficacy by agar disk diffusion tests.

**Results:** MXF NPs demonstrated a PD of 221 ± 11 nm, PDI of 0.21 ± 0.02, surface charge of around -5.00 mV, and EE of 93.8 ± 8.2%. GelMA and HAGM exhibited 61% and 11% DM, respectively, and the hydrogel demonstrated no signs of cytotoxicity. The drug release profiles showed a 74.7 ± 2.4% and 29.7 ± 2.7% burst release within 2 h, with remaining MXF released within 1 day and 5 days respectively for hydrogels loaded with free MXF and MXF NPs. The MXF NP hydrogel can withstand high levels of pressure with a burst pressure of 40.8 ± 4.2 kPa. Lastly, the MXF NP hydrogel demonstrated high antibacterial efficacy with a zone of inhibition> 30 mm against Pseudomonas Aeruginosa (gram –) and > 28 mm for Staphylococcus Aureus (gram +) SA over 5 days.

**Conclusion:** The hydrogel sealant is highly adhesive and capable of successfully loading NPs containing antibiotics. Our drug-eluting sealant has the potential to address critical factors in emergency ocular injury care by rapidly restoring the globe and concurrently allowing for efficacious levels of antibiotics to prevent infection.

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**Purpose:** Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis (SJS/TEN) are two ends of a spectrum of severe cutaneous reactions typically incited by medications and characterized by mucositis and epithelial sloughing. The eyes are among the most commonly affected non-cutaneous areas and may develop debilitating chronic sequelae. Studies have shown a predisposition to developing SJS/TEN with certain human leukocyte antigen (HLA) class I genes. This study seeks to investigate the association between HLA class I genes and SJS/TEN with or without severe ocular complications (SOC) in an American cohort of patients of different ethnicities.

**Methods:** Patients with history of SJS/TEN were enrolled between January 2017 and August 2020. Healthy volunteers from families of the patients with SJS/TEN served as controls. Data collection included demographics, inciting agent, HLA genotype, ocular manifestations of SJS/TEN, and treatment. Analyzed genotypes included HLA-A, HLA-B, HLA-C, HLA-DR, and HLA-DQ.

**Results:** Seventy-four SJS/TEN patients and 49 controls were included. In the SJS/TEN cohort, 60 patients had known inciting agents; all others did not have known medication etiologies. Most common inciting agents included: lamotrigine, trimethoprim-sulfamethoxazole, ibuprofen, penicillin, and allopurinol. Age, sex, or race had no effect on the development of SOCs in those with SJS/TEN based on a linear regression model.

**Conclusion:** HLA-A*68:01 was positively associated with disease for those with lamotrigine-associated SJS/TEN. HLA-B*58:01 was positively associated with disease for those with allopurinol-associated SJS/TEN. HLA-DQB1*03:01 was negatively associated with SOC in the SJS/TEN cohort. Several additional candidate genes may have a relationship with SOCs and may be elucidated with larger samples in the future. Demographics did not appear to have a relationship on the development of SOCs.
15. Conjunctival Goblet Cells Stimulated with an Allergic Mediator Histamine Secrete Extracellular Vesicles that Exhibit Paracrine Secretagogue Activity

Changirm Lee, Darlene A. Dartt

**Purpose:** Goblet cell functions, especially secretion of mucins, are relatively well studied owing to their protective role on the ocular surface; however, little attention is given to the other secretory products, such as extracellular vesicles (EVs). The purpose of this study is to examine the existence and the function of goblet cell secreted EVs and test whether EVs produced by inflammatory stimuli will cause distinct actions compared to those produced by healthy (baseline) cells on recipient goblet cells.

**Methods:** First, serum-starved primary human conjunctival goblet cells (HCGCs, grown from conjunctiva explants) were incubated for 4 h with and without the allergic mediator histamine (His, 10-3 ~ 10-5 M) to induce inflammation or to indicate normal cells, respectively. EVs isolated from each treatment were denoted as EVs-H (Histamine) and EVs-B (Basal), respectively. Second, a nanoparticle tracking analyzer and western blotting were used to analyze the size and the molecular identity of the isolated EV particles. Third, to examine the secretagogue activity of EVs, freshly prepared recipient HCGCs were treated for 4 h with either EVs-H or EVs-B diluted to 1, 10, 100, and 1000 ng/mL in Hank’s balanced salt solution (HBSS). The amount of secreted high molecular weight glycoproteins (HMWP) in the cell culture media during this 4 h period was measured using an enzyme-linked lectin assay (ELLA). Fourth, to test the paracrine and endocrine actions of HCGC EVs, fluorescent Ca2+ indicator Fura-2 loaded HCGCs and primary human corneal epithelial cells were spiked with EVs-H or EVs-B and observed for changes in intracellular Ca2+ concentration (delta[Ca2+]).

**Results:** HCGCs stimulated with His secreted a 2-fold higher amount of high molecular weight glycoproteins (HMWP) as well as a 7-fold higher number of cell-derived nano-scale particles (EVs-H) into the media compared to the control group, which confirmed that His stimulation increases both mucin secretion and EV release in primary HCGCs. The mean diameter of isolated EVs-H and EVs-B was 204.4 and 170.9 nm, respectively. Isolated EVs were enriched with CD9, ALIX, and CD81 but negative for GM130 (Golgi marker) and calnexin (ER marker), showing comparable biophysical and molecular characteristics to the standard set forth by the international community (MISEV 2018). When 10 ng/mL of EVs-H were incubated with recipient HCGCs, HMWP secretion was increased by 14-fold compared to HBSS treated HCGCs (negative cntl). This increase was also 6-fold and 4-fold higher compared to the 10 ng/mL EVs-B treated HCGCs and 10-5 M His treated HCGCs (positive cntl), respectively. An increase of 2~3-fold was observed at other EV concentrations (1, 100, and 1,000 ng/mL); however, all were lower than the activity seen in 10 ng/mL EVs-H. EVs-H also induced an increase in intracellular Ca2+ concentration ([Ca2+]) not only in HCGCs but also in primary human corneal epithelial cells. In HCGCs, 100 ng/mL EVs-H increased [Ca2+][i] by 7-fold compared to mock treatment (negative cntl) and this increase was comparable to 10-5 M His (positive cntl) treatment. In corneal epithelial cells, only 10 and 100 ng/mL EVs-H showed positive delta[Ca2+][i] while all other doses showed negative values for delta[Ca2+][i] upon addition. EVs-H’s ability to mobilize intracellular Ca2+ in both HCGCs and corneal epithelial cells suggests that they are capable of inducing intracellular signaling in paracrine and endocrine manner on the ocular surface.

**Conclusion:** We conclude that EVs secreted from inflammatory mediator stimulated HCGCs (EVs-H) have differential secretagogue activity and intracellular Ca2+ mobilization capacity when compared to EVs secreted from the basal HCGCs (EVs-B). Despite the indispensable role of HCGC EVs in regulating goblet cell secretion and intracellular signaling at the extracellular level, their contributions to the ocular surface homeostasis have been almost neglected until today. The results presented here warrant in-depth investigation into their molecular properties, mechanism of action, and their contributions to the overall ocular surface health.
16. Myeloid-derived suppressor cells promote corneal allograft survival by preventing dysfunction of regulatory T cells in high-risk corneal transplantation

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Purpose: Regulatory FoxP3+ T cells (Tregs) are critical in maintaining corneal allograft tolerance. The immunoregulatory function of Tregs is gravely altered in inflamed or vascularized recipients. Emerging evidence describes the functional crosstalk between CD11b+Gr-1+ myeloid-derived suppressor cells (MDSCs) and Tregs, but little is known about the impact of MDSCs on the rescue of inflammation-induced Treg dysfunction. The purpose of this study is to determine the rescue effect of MDSCs on IL-6-mediated Treg dysregulation. We further evaluate the in vivo role of sub-conjunctively injected MDSCs in Treg rescue, prolonging the corneal graft survival in the setting of high-risk corneal transplantation.

Methods: BALB/c bone marrow cells were isolated and cultured in complete RPMI supplemented with IL-6 (20ng/ml) and GM-CSF (20ng/ml) for 4 days to differentiate MDSCs. MDSCs were isolated using CD11b+Gr-1+ MDSC MACS kits. IL-10 KD MDSCs were generated by IL-10 siRNA (100nM) transfection for 24 hours. Tregs were isolated from BALB/c splenocytes using CD4+CD25+ MACS kits. Tregs (2.5x10^5) and co-cultured either with MDSCs or with IL-10 knockdown (KD) MDSCs at a ratio of 1:1 in the absence and presence of IL-6 (40ng/ml) for 48 hours. Tregs were co-cultured with MDSCs in the presence of IL-6 for 48 hours after which their suppressive capacity was evaluated by co-culture with differentiated Th1 cells. Allogenic corneal transplantation was performed on inflamed host beds in 8-week-old female BALB/c mice using donor tissues from C57BL/6 mice. Immediately following transplantation mice (N=10/group) received a subconjunctival injection of MDSCs (50,000 cells) or vehicle (phosphate buffered saline). Following in vitro and in vivo experiments, flow cytometry, immunoblotting, and PCR were performed to quantify FoxP3 and T-bet expression and IL-10 and IFN-γ secretion of Tregs. Their suppressive capacity was evaluated by measuring proliferation of Th1 cells through Carboxy Fluorescein Succinimidyl Ester (CFSE) labeling as well as Th1 expression of T-bet and IFN-γ.

Results: Flow cytometric quantification showed Tregs cultured with IL-6 had significantly reduced expression of FoxP3 and IL-10, and a reduced suppressive capacity over differentiated Th1 cells compared to control. Addition of MDSCs to culture led to significantly higher Treg expression of FoxP3 (32.93±1.9%, p=0.0035) and IL-10 (60.03±13.3%, p=0.0238). Tregs co-cultured with IL-10 KD MDSCs exhibited significantly reduced FoxP3 (23±1.8%) and IL-10 (55.93±4.4%) compared to control MDSCs (p=0.0403 and p=0.0015 respectively). Tregs pre-treated with IL-6 and MDSCs showed significantly lower expression of T-bet (1.0±0.1%, p<0.0001) and IFN-γ (2.0±0.3%, p=0.0013) compared to pre-treatment with IL-6 only (3.6±0.1% and 3.6±0.1% respectively). Preclinical evaluation depicted prolonged graft survival in the group treated with MDSCs (N=9/10). Accepted mice had a higher expression of FoxP3 (49.8±3.3%, p=0.0001) and a lower expression of T-bet (5.8±0.1%, p=0.0002) compared to those from rejected mice (17.2±2.3% and 7.7±0.2% respectively). Tregs from accepted mice suppressed the proliferation of CFSE-labeled Th1 cells more than those from rejected mice.

Conclusion: Our data demonstrate that ex vivo generated MDSCs are effective in preventing inflammatory cytokine-induced impairment of Treg function, which can be explored as a novel therapeutic approach in a clinically relevant model of high-risk corneal transplantation.
17. Clinical Outcomes in Patients with Peripheral Ulcerative Keratitis at a Tertiary Academic Center

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**Purpose:** Peripheral ulcerative keratitis (PUK) consists of a group of inflammatory disorders that results in peripheral corneal thinning and possible corneal perforation without aggressive use of immunosuppressive therapies. We evaluated the clinical course of patients with PUK to determine risk factors for perforation.

**Methods:** Retrospective study of 66 consecutive PUK patients (132 eyes) seen by 4 physicians at the Division of Ocular Immunology at the Wilmer Eye Institute from January 2003 to August 2022. Data collected included demographic information, immunosuppressive medications and doses, and whether perforation occurred.

**Results:** The median age was 54 years; 55% were male. Caucasians comprised 48% of subjects, and the median duration of ocular symptoms prior to presentation was 3 months. A systemic disease was present in 39 patients (59%), with the most prevalent being rheumatoid arthritis. Of the 132 eyes examined at baseline, 55 (42%) had corneal thinning at presentation, and 8 (6%) had perforated prior to presentation. During follow-up, 5 eyes (4%) perforated in a median time of 82 days from presentation. Surgical management for perforations consisted of a patch-graft (PG) in 3 of the 5 eyes, and penetrating keratoplasty (PK) for 3 of the 5 eyes. Two eyes required multiple surgical interventions: one with a PG then subsequent PK, and one requiring 2 successive PKs. At the time of perforation, 3 of 5 patients were on systemic corticosteroids, with a median dose of 60mg. Only one patient was taking a systemic immunosuppressive medication (mycophenolate mofetil dosed at 1 gram daily), but without concomitant corticosteroid use. In patients who experienced perforation in at least one eye prior to presentation or during follow-up, 58% (N=7) had an underlying systemic illness.

**Conclusion:** The percent of patients with PUK requiring PG or PK decreases with aggressive initiation and continuation of immunosuppressive therapy. High dose prednisone at induction and combination immunosuppressive therapy may decrease the likelihood of perforation occurring in patients with PUK. Long term immunosuppressive therapy is especially useful in patients with PUK to reduce the risk for perforation, especially in those with an underlying autoimmune disorder.
18. Neurokinin-1 Receptor: A Novel Pharmacological Target for Ocular Pain

Amirreza Naderi

**Purpose:** Corneal pain is a common symptom of dry eye disease (DED). Moreover, many patients experience dry eye-like pain (DELP) following refractive surgery. Substance P (SP) is a well-known modulator of pain. Elevated SP levels in tears of post-LASIK patients have been associated with DED symptoms. Recently, SP was shown to modulate nociception in intact murine cornea. In this pre-clinical study, we determine the contributions of SP to DED pain and DELP through antagonism of its preferred receptor, neurokinin-1 receptor (NK1R).

**Methods:** To induce DED, 6-week old C57BL/6 female mice were housed under desiccating stress in a controlled environment chamber for 14 days. To model DELP, the central epithelial and anterior stroma of male and female 6-week old C57BL/6 mice was mechanically removed using Alger Brush II. Eye wiping test (EWT) was performed to evaluate hyperalgesia after instillation of hypertonic saline (2M NaCl) as noxious stimulus on days 0, 4, 7, 14 and 21 (only DELP group). On the same days, allodynia was assessed by quantifying palpebral ratio (PR) via an automated video analysis of the animals’ eyes after instillation of a saline solution iso-osmolar to tears (0.9 % NaCl) as innocuous stimulus. L-733,060 (1μg/μl), an NK1R antagonist, or vehicle was administered topically twice daily for the length of the study. Corneas and trigeminal ganglions (TG) were harvested to measure SP levels via ELISA on the last days. One-way ANOVA followed by Tukey’s post hoc test or student t-test was used.

**Results:** Eye wipe behavior was significantly higher in vehicle groups compared to normal mice. Treatment significantly decreased the wipe count in both DED (days 4 and 14) and DELP models (days 7 and 14). PR was significantly lower in DELP and DED models compared to normal mice. Treatment significantly increased PR in both DED (days 4 and 14) and DELP (days 4, 7 and 14). In DED model, SP levels peaked on day 4 and treatment significantly reduced it in both cornea and TG. Interestingly, SP levels reached maximum on day 14 in corneas of DELP model but decreased in TG. Treatment had no significant effect on SP levels of neither cornea or TG in DELP model.

**Conclusion:** Treatment with NK1R antagonist leads to a decrease in nociception. Furthermore, the reduction of SP following the inhibition of its receptor suggests a negative feedback loop. In conclusion, NK1R antagonists show promise for the treatment of ocular pain.
19. Alpha-Melanocyte-Stimulating Hormone (α-MSH) suppresses corneal angiogenesis

Sheyda Najafi, Elsayed Elbasiony, Asmaa A. Zidan, Shudan Wang, Jia Yin

Purpose: Trigeminal neurons and corneal nerves express high levels of the neuropeptide alpha-melanocyte-stimulating hormone (α-MSH). α-MSH has been reported to regulate ocular surface inflammation. However, there is little knowledge on the effect of this neuropeptide on corneal angiogenesis.

Methods: To investigate the effect of α-MSH on angiogenesis in vitro, Human Retina Endothelial Cells (HRECs) tube formation and migration were evaluated following 6- and 16-hours of α-MSH treatment (100 nM) respectively. Vascular Endothelial Growth Factor (VEGF) (100 nM) was used as positive control. The expression of α-MSH receptors in the cornea harvested from naïve Balb/c mice was analyzed using RT-PCR. In addition, the localization of α-MSH receptor type I (MC1R) was investigated in corneas harvested from naïve Balb/c mice using immunofluorescence. Corneal angiogenesis was induced by intrastromal placement of a figure-8 suture in Balb/c mice and the protein expression of α-MSH was quantified in trigeminal ganglia after 14 days using ELISA. To explore the anti-angiogenic effect of α-MSH, mice were treated with α-MSH (100 µM) 3 times daily for 2 weeks immediately after suture placement. In order to assess the corneal neovascularization area, slit lamp images of the sutured eye were captured and analyzed using imageJ.

Results: Treatment of HRECs with α-MSH resulted in significant inhibition of migration and tube formation compared to the control (**p<0.01). However, treatment with VEGF (100 nM) reversed the effects of α-MSH on tube formation and migration. α-MSH receptor expression analysis in the cornea showed high expression of MC1R and immunofluorescence staining of MC1R in the cornea confirmed robust localization of MC1R to the cornea endothelial cells. The expression of α-MSH in the trigeminal ganglia was significantly increased at day 14 following suture placement, as compared to unsutured controls (*p<0.05). Moreover, α-MSH treatment significantly reduced corneal neovascularization compared to PBS-treated mice (*p<0.05).

Conclusion: Our results demonstrate that α-MSH reduces corneal angiogenesis following intrastromal suture placement.
20. The Involvement of Th1-mediated Alloimmunity in Graft Rejection in a Mouse Model of Endothelium Keratoplasty

Akitomo Narimatsu, Ayta Musayeva, Shima Dehghani, Katayoon Forouzanfar, Seokjoo Lee, Tomas Blanco, Reza Dana

Purpose: Given the considerable shift in clinical practice from endothelial keratoplasty (EK) to penetrating keratoplasty (PK), and the relative dearth of knowledge surrounding the mechanisms associated with graft rejection and failure in EK, we sought to investigate the mechanisms involved in host’s alloimmunity and graft rejection in our recently established mouse model of EK.

Methods: EK was performed using C57BL/6 donor grafts and BALB/c recipients. The central endothelium and Descemet membrane was removed from the recipient cornea, and a 1.5-mm posterior lamellar donor graft was made adherent to the recipient cornea with a small amount of viscoelastic. Graft opacity was assessed (1-5) over a 6-week period, then corneas were divided into two groups: low-grade opacity (group 1, <2) and high-grade (group 2, >2). Lymph nodes were extracted 6 w after EK and CD4+ T cells were sorted by magnetic-activated cell sorting and cocultured with syngeneic or allogeneic antigen-presenting cells (APC) to evaluate the expression of IFN-γ by alloreactive Th1 cells (ELISPOT). Direct (donor) and indirect (host) pathways of sensitization were then compared.

Results: Low-grade opacity was observed in 55% while high-grade opacity was observed in 45%. IFN-γ+ positivity was not detected in any mouse with a low-opacity score; however, the number of IFN-γ+ spots significantly increased in the high-grade opacity scored mice (p<0.01). Remarkably, in this group, directly sensitized Th1 cells secreted significantly more IFN-γ+ than the indirectly sensitized (p<0.01).

Conclusion: This work shows for the first time that alloreactive Th1 cells could mediate alloimmune rejection after EK, which opens new ways to investigate graft failure after EK.
21. Effect of ROCK inhibitor on cell migration in Fuchs Endothelial Corneal Dystrophy
Mohit Parekh, Annie Miall, Neha Deshpande, Ula V Jurkunas

Purpose: Fuchs endothelial corneal dystrophy (FECD) is a progressive loss of corneal endothelial cells (CECs) that are post-mitotically arrested with limited proliferative capacity. Therefore, wound healing is mainly achieved through cell enlargement and migration. Inhibition of Rho-kinase, a key regulator of cytoskeletal reorganization has been shown to promote cellular migration. The purpose of this study was therefore to investigate the effect of a novel Rho-associated coiled-coil-containing protein kinase (ROCK) inhibitor, ripasudil (K-115) in promoting CEC cell migration on Descemet’s membrane in FECD ex vivo.

Methods: Normal and FECD Descemet’s membrane-CEC tissues were obtained from cadaveric corneas or patients undergoing Descemet’s membrane endothelial keratoplasty (DMEK) surgeries. The tissues were preserved in Optisol-GS and after washing in PBS were stained with Hoechst 33342 for 30 sec and attached to the plastic plates by air drying for 3 min with the endothelial cell side facing up. The tissues were treated with 1uM of K-115 drug and monitored using live cell imaging microscope (Leica DMi8) for 5 hours. Controls were treated with Chen’s media without the drug. The images from three biological and three technical replicates were collected per group, and the velocity and displacement of the cells were analyzed using the TrackMate plugin in ImageJ. Mann-Whitney and one-way Anova tests were used for statistical analysis with p<0.05 being statistically significantly different.

Results: Mean velocity (pixels/hour±SD) of CECs without the drug was 0.45±0.11 in normal and 0.64±0.21 in FECD tissues; and increased to 0.65±0.20 (p<0.05) and 0.82±0.39 (p<0.001) with K-115, respectively. Mean displacement (pixels/hour±SD) of the cells from the normal and FECD tissues without the drug was 4.33±2.19 and 6.63±5.8, which increased to 13.49±10.32 (p<0.001) and 15.02±13.10 (p<0.001) respectively with K-115. Although the fold change in mean displacement between normal and FECD cells did not change significantly following the treatment with K-115, a significantly higher mean velocity (p<0.01) was observed in FECD compared to normal cells.

Conclusion: FECD cells migrate faster on the Descemet’s membrane following the treatment with K-115 compared to the normal cells, which further provides a promising therapeutic approach for the treatment of FECD using ripasudil after Descemetorhexis without endothelial keratoplasty (DWEK).
22. Investigation into the Antifungal Mechanism of Propranolol through Fungal Keratitis causing Pathogen *Fusarium solani*

Chetan Pavuluri, Cecilia Gutierrez-Perez, Kevin K. Fuller, Robert A. Cramer, Michael E. Zegans

**Purpose:** With few novel antifungal treatments against fungal keratitis, our primary aim was to better define the fungicidal activity of the beta-blocker propranolol against *F. solani*.

**Methods:** *Fusarium solani* with propranolol alone or in combination with antifungals (including voriconazole, caspofungin, and amphotericin B) or stressors such as sorbitol, pH modification, and CaCl\(_2\) was grown on Potato Dextrose Agar (PDA) growth medium. Plates were inoculated with 1000 fungal conidia, and radial growth was measured after 72 hours. To determine propranolol’s dose-dependent fungicidal activity, spores were first grown in an RPMI MIC assay for 48 hours and then transferred to propranolol-free PDA plates for 72 hours.

**Results:** Among the three major classes of antifungals tested, polyenes, azoles, and echinocandins, co-treatment with voriconazole demonstrated the greatest propranolol potentiation as measured by decreased relative radial growth (*p*<.0001). Increasing the pH of the PDA media to 8 also increased propranolol’s relative inhibition (*p*<.0001). Calcium supplementation significantly prevented propranolol’s inhibitory activity (*p*<.0001). Propranolol exhibited a dose-dependent effect, and no growth was observed upon replating onto PDA at the MIC value.

**Conclusion:** Our preliminary data shows that propranolol exhibits fungicidal activity against *F. solani*. Further research is necessary to determine the role of ion pathways and if calcium-mediated fungicidal-blocking is a consequence of the drug’s mechanism or unrelated calcium-dependent cell processes. Exploring propranolol co-treatment with voriconazole or a calcium chelator such as EDTA could be considered as strategies to improve propranolol’s efficacy as a fungal keratitis medication. Identifying the fungal targets underlying propranolol’s antifungal activity may allow more efficacious, tolerable, and bio-available analogs to be developed.
23. A Machine Learning Platform for Noninvasive Smartphone-Based Assessment of Corneal Epithelial Integrity

Jayanth Pratap

Purpose: Fluorescein examination is critical for diagnosing corneal epithelium (CE) breakdown. However, the current clinical standard requires visual examination via a slit lamp microscope and a trained clinician, which poses challenges in low-resource environments. To provide an accessible and objective method to assess CE health, we developed and validated a low-cost, noninvasive, and quantitative CE evaluation pipeline using a custom smartphone attachment and convolutional neural networks (CNNs).

Methods: A custom 3D-printed smartphone adapter and placido disk illumination module was attached to a OnePlus 7 Pro smartphone. In a pilot clinical trial, 26 smartphone-acquired images were obtained from 15 subjects, comprising a dataset including healthy eyes and corneal epitheliopathies of Oxford I-V. A regression CNN was trained to estimate placido disk center coordinates for image preprocessing. A classifier CNN was then trained to identify areas of suspected epithelial disruption. Results were compared with fluorescein examination of the same subjects for visual correspondence between disrupted areas and clinical Oxford grading.

Results: The preprocessing regression network successfully learned to estimate placido disk centers with an average train set error of 2.5 pixels and test set error of 24 pixels on 500 × 500 pixel input images. Our patch-based convolutional neural network used to grade CE had a binary testing accuracy of >90% with respect to the annotated image patches. Qualitatively, there was significant agreement between areas of CE disruption identified by our smartphone-based technique and those revealed by fluorescein staining, as verified by an expert ophthalmologist.

Conclusion: Our technique for smartphone-based CE imaging and automated analysis is a low-cost, noninvasive method to quantitatively evaluate the CE. This tool can be used to evaluate CE disruption indicative of ocular surface disease in low-resource regions and prompt further investigation of suspected corneal epitheliopathies.
24. Adoptive Transfer of Plasmacytoid Dendritic Cells Promotes Allograft Survival in a Murine Allogeneic Corneal Transplantation

Fangfang Qiu, Brendan M. Kenyon, Cecilia Chao, Deshea L. Harris, Olivia Esteireiro, Pedram Hamrah

**Purpose:** Graft rejection remains the leading cause of allograft failure in corneal transplantation. Plasmacytoid dendritic cells (pDCs) are implicated in the maintenance of immune tolerance in other models. Herein, we tested the hypothesis that adoptive transfer of pDCs can promote allograft survival via inducing immune tolerance, using an experimental allogenic corneal transplantation model.

**Methods:** Allogeneic corneal transplantation was established using BALB/c recipients and C57BL/6 donors. Adoptive transfer of sorted splenic allo-pDCs or saline were performed using fibrin sealant at d 1 post-surgery. Anergic T cells, regulatory T cells (Tregs), Th1, and Th17 cells in draining lymph nodes (dLNs), and infiltrating leukocytes and T cells in corneas, were detected via flow cytometry at d14 post-transfer. Grafts was assessed twice weekly for 70 days with slit-lamp to score graft neovascularization (NV, 0-8+ scale), opacity (0-5+ scale), and rejection (graft opacity≥ score 3). Rejection-free graft survival was evaluated by Kaplan-Meier survival curves followed by log-rank test; unpaired t-test was used for comparisons.

**Results:** pDCs induced anergic CD4+ T cells (CTLA-4+: 1.6-fold, PD-1+: 1.8-fold, LAG-3+: 4.6-fold, compared to the saline group; n=3, all p<0.05), and expanded the Foxp3+CD4+ Tregs compartment (2.5-fold; n=3, p<0.001), while they had no effect on IFNγ+CD4+ Th1 and IL17+CD4+ Th17 (1.2-fold and 1.1-fold respectively; n=3, both p>0.05) in dLNs. In addition, pDCs ameliorated infiltration of CD45+ leukocytes and CD3+ T cells in corneal beds (CD45+: 87.5%, CD3+: 79.0% decrease compared to saline group; n=3, both p<0.05) and grafts (CD45+: 75.8%, CD3+: 71.9% decrease; n=3, both p<0.01). Moreover, pDCs suppressed corneal NV from d7 until d70 (score 2.8±0.34 SEM to 5.4±0.36, n=12 for pDCs transfer group vs 4.0±0.23 to 6.4±0.22, n=11 for saline control group; p<0.05 for all timepoints). Further, pDCs alleviated graft opacity from d14 until d70 (score 0.4±0.15 to 1.5±0.33, n=12 vs 1.1±0.21 to 3.5±0.45, n=11; all p<0.05), and improved survival rate of grafts (83.3%, n=12 vs 36.4%, n=11; p<0.01) through the end of 70 days.

**Conclusion:** Adoptive transfer of pDC promote survival of corneal allograft, which is associated with T cell tolerance, suggesting the translational potential of cell-based pDC therapy use in corneal transplantation.
25. Machine learning used to quantify recruitment among cells involved in the corneal wound healing response

Kristen L. Segars

**Purpose:** When the corneal epithelium is injured, calcium signaling events occur in cells near the injury in response to ATP in the local environment. These signaling events have been observed to propagate between cells, and this propagation is necessary for cellular motility and wound healing. We have developed computational analysis programs to characterize these signaling events and have identified high-signaling cells in the wound response that recruit their neighbors into “clusters” of signaling events. We have also shown that signaling clusters and cell-cell recruitment occur not only at the wound but also distant from it, in the corneal-limbal interface.

**Methods:** Live cell imaging was performed on both ex vivo globes from male C57Bl6 mice and cultured Human Corneal Limbal Epithelial (HCLE) cells using the Zeiss LSM 880 confocal microscope. Globes were pre-incubated with CellMask DeepRed and Fluo-4AM, and stabilized using a 3D printed holder. Images were collected after injury on the corneal epithelium and corneal-limbal regions. SiRNA knockdown was performed on HCLE cells prior to staining with SiR actin and Fluo-4AM, wounding and imaging. Machine learning analysis was performed using Zen, Matlab and ImageJ.

**Results:** Recruitment of cells into signaling clusters is a phenomena observed both at the wound in cell culture models and at the corneal-limbal interface in ex vivo organ culture models. Recruitment is initiated by a functional subpopulation of high-signaling cells that can be identified mathematically using hierarchical clustering analysis and graphically using summed topographical maps of cellular intensity at the wound over time. The P2X7 receptor and Pannexin-1 ion channel play a role in the development of a conductor cell phenotype.

**Conclusion:** The results of our experiments indicate that some cells participating in the wound healing response have different roles than their neighbors, characterized by differences in calcium signaling profile and tendency to recruit neighboring cells into a signaling cascade. Future studies will focus on the hypothesized loss of this functionally distinct cellular phenotype with age and disease, conditions where wound healing in the cornea becomes inefficient.
26. Mesenchymal stem cells-derived interleukin-11 inhibits activation and proliferation of T cells

Aastha Singh, Sharad Mittal, WonKyung Cho, Yilin Guan, Elsayed Elbasiony, Sunil Chauhan

**Purpose:** Uncontrolled T cell activation can result in pathological conditions including autoimmunity and graft rejections. Our group has previously shown that mesenchymal stem cells (MSCs) inhibit activation of innate immune cells including neutrophils and macrophages. Here, we investigate whether MSCs regulate T cell responses by secreting interleukin-11 (IL-11).

**Methods:** Human bone-marrow derived MSCs (hMSCs) were purchased and phenotypically characterized for their expression of CD45- CD34- CD73+ CD90+. CD4+ CD25- T cells (purity: >95%) were magnetically sorted from human peripheral blood mononuclear cells for the MSC-T cell co-culture assays. Expression and secretion of IL-11 by hMSCs were evaluated using real-time PCR and ELISA, respectively. Expression of IL-11 receptor was confirmed in activated CD4+ CD25- T cells using flow cytometry. CD4+ CD25- T cells stimulated with anti-CD3/CD28 beads (1:1 ratio) were co-cultured with MSCs at 1:1 ratio for 24 hours (for early activation) and 66 hours (for proliferation) in the presence and absence of hIL-11 neutralizing antibody (20 µg/ml). Early T cell activation was assessed by evaluating expression of CD40L and CD69 (Median Fluorescence intensity; MFI) using flow cytometry. For proliferation, CD4+ CD25- T cells were stained with CFSE prior to co-culture and their proliferation was quantified by measuring CFSE dilution via flow cytometry.

**Results:** hMSCs constitutively express high levels of IL-11 at both mRNA and protein levels. Naïve CD4+ CD25- T cells showed the expression of IL-11 receptor, which was upregulated by 2-fold following CD3/CD28 stimulation. hMSCs significantly suppressed early activation of naïve T cells, as indicated by an approximate 70% reduction in expression of both CD69 (p=0.025) and CD40L (p=0.011). This MSC mediated reduction in early T cell activation was not observed following the neutralization of IL-11. Furthermore, our CFSE dilution assay demonstrated that hMSCs significantly prevented the proliferation of CD4+ CD25- T cells (p=0.006); however, this MSC-mediated suppression of proliferation was abrogated following IL-11 neutralization.

**Conclusion:** IL-11 secretion by human mesenchymal stem cells is critical for inhibition of early T cell activation and proliferation.
27. Activated innate immune response by low and high doses of AAV8 among species

Zhenwei Song, Chengwen Li

**Purpose:** To evaluate the long-term AAV transduction in cornea with low and high dosed of AAV8 vectors.

**Methods:** Corneas from Human, Monkey, Rabbits, Mice and pigs were injected with 2 doses of AAV8 vectors and cultured for 14 day to determine the transgene expression as well as IFNb.

**Results:** Donor-specific dose response were observed and low dose tended to benefit the long-term transduction with minor activation of innate immune response.

**Conclusion:** Low dose rather than high dose could be a better choice for introstromal injection with AAV therapy.
28. Netarsudil use following descemetorhexis without endothelial keratoplasty in patients with Fuchs endothelial cell dystrophy

Jennifer A. Tran, Michael M. Lin, Ula V. Jurkunas

Purpose: To describe the outcomes of three cases whereby the topical Rho-kinase inhibitor netarsudil was initiated after descemetorhexis without endothelial keratoplasty (DWEK) in the management of Fuchs endothelial cell dystrophy (FECD).

Methods: This is a retrospective chart review case series of three patients diagnosed with FECD treated with netarsudil following DWEK at an academic medical center.

Results: All patients in the case series underwent DWEK for symptomatic FECD and were prescribed netarsudil in the post-operative period. The first patient was a 72 year-old female who underwent two consecutive cataract extraction/intraocular lens placement/DWEK procedures and began using netarsudil on post-operative day 1 for each eye. Both corneas were clear by week 6. The second patient was a 77-year-old male who was unable to obtain the medication for four days and started it on post-operative day 5, after which time he developed rho-kinase-associated reticular corneal epithelial edema (rhoedema). He continued netarsudil and achieved corneal clearance by post-operative week 7. The third patient was a 70-year-old female who was started on netarsudil on post-operative day 1 and developed rhoedema that became persistent after 5 weeks. Netarsudil was held and the edema gradually resolved over the next month, ultimately resulting in central corneal clearance by post-operative month four. The latter two patients developed reticular corneal epithelial edema, which appeared as collections of moderate sized superficial epithelial bullae arranged in a reticular pattern resembling a honeycomb.

Conclusion: This case series demonstrates the differential outcomes of netarsudil use in patients with pre-existing endothelial cell dysfunction. While netarsudil can cause a reversible rhoedema, it has also demonstrated utility in aiding corneal clearance following DWEK in FECD.
29. Allo-primed Effector T Cells Promote Fibrosis in Corneal Transplantation Failure

Shudan Wang, Sharad Mittal, El-Sayed Elsabiony, Tomas Blanco, Hamid Alemi, Hayate Nakagawa, Jia Yin, Sunil Chauhan, Reza Dana, Thomas H. Dohlman

**Purpose:** To evaluate whether fibrosis contributes to corneal transplant failure and to determine whether effector CD4+ T cells, the key immune cell in corneal transplant rejection, play a direct role in fibrosis formation.

**Methods:** Allogeneic corneal transplantation was performed in BALB/c mice. Graft opacity was evaluated by slit-lamp biomicroscopy and fibrosis score was assessed by in vivo confocal microscopy every 2 weeks. Expression of α-smooth muscle actin (α-SMA) in both accepted and failed grafts was quantified by real-time PCR and immunohistochemistry. Frequencies of macrophages, neutrophils and CD4+ T cells were assessed using flow cytometry. MK/T-1 corneal fibroblasts were co-cultured with CD4+CD25- effector T cells and interferon gamma (IFN-γ) was neutralized. The expression of α-SMA in MK/T-1 cells was measured by real-time PCR and ELISA.

**Results:** The majority of failed grafts demonstrated clinical signs of fibrosis which became most evident at week 6 post corneal transplantation. Failed grafts showed high expression of α-SMA as compared to accepted grafts. Flow cytometry analysis showed that CD4+ T cells are the predominant graft-infiltrating immune cell at the time of fibrosis development. Co-culture of allo-primed CD4+CD25- effector T cells with corneal fibroblasts led to an increase in α-SMA expression by fibroblasts and neutralization of IFN-γ suppressed this increase.

**Conclusion:** Fibrosis contributes to graft opacity in corneal transplant failure and allo-primed effector CD4+ T cells directly contribute to fibrosis via IFN-γ.
30. Calcitonin gene-related peptide promotes the healing of Corneal epithelial-stromal injury

Asmaa A. Zidan, Shuyan Zhu, Jia Yin

Purpose: Calcitonin gene-related peptide (CGRP) is an anti-inflammatory neuropeptide abundantly expressed by corneal nerves. Yet, its effects on corneal wound healing, inflammation, and opacity have not been studied before. In this study, we aim to examine the effects of CGRP on corneal epithelial stromal injury in mice.

Methods: Corneal epithelial-stromal injury was induced in C57BL/6 mice by removing the epithelium and anterior portion of the stroma using a hand-held Algerbrush II. Following injury, mice were topically treated either with CGRP or PBS three time daily for 14 days. Corneal epithelial healing, stromal opacity, and thickness were examined using slit lamp photography and OCT. Histological analysis of the tissues was performed by H&E staining. Corneal endothelial cells integrity, alpha-smooth muscle actin (α-SMA) and TGF-β1 expression were assessed by zonula occludens (ZO-1), α-SMA, TGF-β1 immunostaining, respectively. Leukocyte infiltration was determined using flow cytometry.

Results: CGRP topical application led to accelerated epithelial wound healing from 44.6±3.2% to 82.5±8.2% at 24 hrs., P=0.001, and significantly reduced corneal opacity (P=0.001). At day 14, CGRP reduced corneal edema compared to PBS treatment (central corneal thickness 159.7±29.2µm in PBS group vs 85.2±1µm in CGRP group, P=0.013), and preserved corneal endothelial cell integrity. H&E analysis showed decreased fibrous tissue formation, and preserved tissue integrity in CGRP treated corneas compared to the PBS treatment. This result was confirmed by immune staining which showed decrease in α-SMA and TGF-β1 expression. Additionally, CGRP treated group showed a decrease in CD45+ cell infiltration into the cornea from 10.2±1.2% to 6.1±0.3% (P=0.03).

Conclusion: CGRP promotes the healing of epithelial-stromal injury by accelerating epithelial wound closure and preventing corneal opacification and edema. Potential mechanisms include inhibiting TGF-β1 signaling and the production of α-SMA, reducing leukocyte infiltration, and preserving corneal endothelial cells.
31. Transient Receptor Potential Ion-Channels are Required for Contact Lens-Induced Corneal Parainflammation and Modulation of Resident Corneal Immune Cell Numbers

Ananya Datta, Ji Hyun Lee, Orneika Flandrin, David J. Evans, Suzanne M. J. Fleiszig

Purpose: Contact lens wear can predispose the human cornea to sight-threatening infectious keratitis. Previously, using a contact lens wearing murine model of Pseudomonas keratitis, we showed that lens wear was associated with corneal para-inflammation involving increased numbers of CD11c+ cells and γδT cells after 24 h and Ly6G+ cells (neutrophils) and γδT cells after 5-6 days. We have also recently shown that transient receptor potential (TRP) ion-channels associated with corneal nerves are required for CD45+ and CD11c+ cell responses in the murine cornea correlating with protection against Pseudomonas colonization. Here, we explored the role of TRP nociceptors in contact lens-induced corneal parainflammation.

Methods: Contact lens-wearing C57BL/6 wild-type (WT) mice were compared to lens-wearing gene-knockout mice in TRPA1 (-/-) or TRPV1 (-/-) or mice depleted of TRPV1 receptors by subcutaneous injection of Resiniferatoxin (RTX). One eye of each mouse was fitted with a custom-made murine contact lens for 24 h or 6 days and contralateral eyes served as no-lens wear controls. After lens removal, mice were euthanized, enucleated eyes were fixed in ice-cold methanol or 2% paraformaldehyde, and corneal expression of MHC Class II+, CD45+, γδT or Ly6G+ cell responses detected using antibody labeling and imaging. In other experiments without fixation, corneal expression of genes encoding transcriptional profile of select proinflammatory cytokines was assessed. Student’s t-test and One-way ANOVA were used for statistical analysis. P < 0.05 was considered significant.

Results: Lens-wearing corneas of WT mice showed a significant increase in MHC Class II+, CD45+ and γδT cells after 24 h, and Ly6G+ cells after 6 days, versus no-lens wear controls. Corneas remained free of visible pathology. While lens-wearing corneas of TRPA1 mice also showed a significant increase in MHC Class-II+ cells at 24 h vs. no-lens wear controls, this response was absent in TRPV1 (-/-) mice. Interestingly, no-lens wear control corneas of TRPA1 (-/-) or TRPV1 (-/-) mice showed a significant reduction in MHC Class-II+ cells versus WT at 24 h. A similar result was found for corneal γδT cells, but not CD45+ cells. Lens-wearing corneas of TRPA1 (-/-) and TRPV1 (-/-) mice each showed a significant reduction in Ly6G+ cell infiltration versus lens-wearing WT after 6 days. Little or no Ly6G+ cells were observed in 6-day no-lens wear controls. RTX injection reproduced most of the above TRP (-/-) phenotypes in WT mice including reduced baseline numbers of corneal MHC Class II+ cells, loss of lens-induced MHC Class II+ and γδT cell responses after 24 h, and the loss of Ly6G+ cell responses after 6 days. Lens-wearing corneas showed a significant increase in IL-18 (~8.5-fold) & TNF-a (~19.2-fold) gene expression after 24 h versus no-lens-wear controls which were abrogated in RTX-treated mice.

Conclusion: TRP nociceptors are required for contact lens-induced corneal para-inflammarory responses involving significantly increased levels of MHC Class II+ cells and γδT cells after 24 h of wear, and Ly6G+ (neutrophil) cell infiltrative responses after 6 days of wear, but not CD45+ cell responses. TRP nociceptors are also involved in modulating the number of resident MHC Class II+ cells and γδT cells in the cornea. Specific role(s) for TRPA1 and/or TRPV1 in mediating these corneal phenotypes remains to be determined.
32. Fuchs Endothelial Corneal Dystrophy Associations with Comorbid Systemic Disease, Lifestyle, and Nutritional Intake

Aaron R. Kaufman, Francesca Kahale, Myriam Böhm, Pia Leon, Ula V. Jurkunas

Purpose: Contribution of systemic disease, lifestyle, and nutrient intake to the pathophysiology of Fuchs endothelial corneal dystrophy (FECD) is incompletely characterized, but might be potential modifiable risk factors for FECD pathophysiology. The current study aims to identify FECD associations with systemic disease comorbidity, lifestyle, and nutrient intake.

Methods: Retrospective chart review and cross-sectional survey of 50 FECD patients and 50 age- and sex-matched control patients. Inclusion criteria were the clinical diagnosis of FECD disease in the FECD group and absence of corneal disease in the control group. Survey consisted of a validated semiquantitative food frequency questionnaire (SFFQ) and a smoking/exercise questionnaire. Chart review queried demographic information, FECD stage, systemic disease comorbidity, and medication use. Statistical methods were Fisher exact test and Mann Whitney U test.

Results: Comorbid cardiovascular disease had higher occurrence in FECD than in controls (36% vs 16%, p= 0.039). Diabetes had similar rate of occurrence (8% FECD vs 8% control, p =1.000). No difference was observed for having ever smoked (FECD 56% vs control 38%), but FECD patients had higher current daily smoking behavior (8.64 vs 4.92 packs/day, p = 0.046), duration of smoking (13.10 vs 8.52 p = 0.048), and calculated total smoking exposure (11.24 vs 6.12 pack-years, p = 0.017). There was no difference in BMI (FECD 26.54 vs 26.17, p=0.879) or rates of exercise (FECD 4.66 vs control 4.56 hours/week, p =0.840). FECD patients had higher dietary supplement use than controls (66% vs 42%, p = 0.027). Differences in nutritional factors included: sodium (2036.12 mg vs 436.22 mg, p = 0.021), insulinogenic load (711 vs 667.79, p=0.077), total fat (67.00 g vs 71.00g, p = 0.036).

Conclusion: This pilot investigation suggests that it might be prudent to counsel FECD patients about the importance of cardiovascular disease control, reducing excessive salt intake, and cessation of smoking. However, causation is not confirmed by the associations found, and further work using a larger sample size is necessary to confirm a possible cardiovascular disease phenotype in FECD and a dose-dependent effect of smoking behavior on FECD pathophysiology.