Dynamic changes of iridocorneal angle morphology during accommodation in healthy children

Moritz C. Daniel, Becky MacPhee, Patricia Ibanez, Nadia Venturi, Adam M. Dubis, Maria Theodorou, Gillian G. Adams, Maria Papadopoulos, John Brookes, Peng T. Khaw, Annegret H. Dahlmann-Noor. Department of Paediatric Ophthalmology and Strabismus, NIHR Moorfields Biomedical Research Centre at Moorfields Eye Hospital and UCL Institute of Ophthalmology, London, United Kingdom; 2Eye Center, Medical Center, University of Freiburg, Freiburg, Germany; 3BRC at Moorfields Eye Hospital and UCL-Institute of Ophthalmology, London, United Kingdom; 4Department of Paediatric Ophthalmology and Strabismus, Moorfields Eye Hospital, London, United Kingdom; 5Moorfields Eye Hospital, London, United Kingdom; 6Glaucoma Service, Moorfields Eye Hospital, London, United Kingdom; 7Orthoptics, Moorfields Eye Hospital, London, United Kingdom.

Purpose: The dynamics of the iridocorneal angle (ICA) during accommodative effort have not previously been explored, though anatomy would indicate that Schlemm’s canal (SC) morphology may be affected by ciliary muscle (CM) contraction. This work aims to describe changes in ICA morphology in children, a population with a naturally large range of accommodation.

Methods: We acquired anterior segment OCT images (Tomey SS-1000 CASIA, Nagoya, Japan) of ICA and CM of the right eye of 50 children age 4 to 16 years with healthy eyes, at 2 levels of accommodation: 2.5D (“relaxed”) and 15D (“intense accommodative effort”). We noted triplicates of semi-automated nasal ICA measurements: angle opening distance at 500/750 μm (AOD-500, -750), trabecular iris space area at 500/750 μm (TISA-500, -750) and trabecular iris angle at 500/750 μm (TIA-500, -750). One observer manually obtained triplicate measurements of SC diameter (SC-D) and cross-sectional area (SC-CSA) and CM width at 1, 2 and 3 mm from scleral spur (CM-1, CM-2, CM-3). Triplicates were summarised as means. Using SPSS24 (IBM, Armonk, USA) we calculated cohort means and standard deviations (SD). Comparisons: 1) all parameters: 2 tailed t-test with cutoff at a) age 10 years and b) spherical equivalent of 0D; 2) SC-D, SC-CSA, CM-1, 2, 3: 2-tailed paired t-test.

Results: Image quality was adequate for analysis in a high proportion (Table 1). SC parameters and CM width were not significantly different in younger versus older or myopic versus emmetropic/hypermetropic children (p>0.05). SC-D and CM-1 significantly increased with intense accommodative effort, whilst CM-3 significantly decreased (Table 1).

Conclusions: This is the first study to demonstrate dynamic changes in SC-D with accommodation in humans.

Table 1. ICA, SC parameters and CM width on standard and high-resolution AS-OCT with relaxed and intense accommodative effort.

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<thead>
<tr>
<th>Parameter</th>
<th>Relaxed accommodation</th>
<th>Intense accommodative effort</th>
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<tr>
<td>SC-D (mm)</td>
<td>0.015 (0.006)</td>
<td>0.027 (0.015)</td>
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<tr>
<td>CM1 (mm)</td>
<td>0.668 (0.075)</td>
<td>0.692 (0.08)</td>
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<td>CM2 (mm)</td>
<td>0.388 (0.084)</td>
<td>0.370 (0.11)</td>
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<tr>
<td>CM3 (mm)</td>
<td>0.216 (0.066)</td>
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Results: Outflow facility was significantly reduced in POAG eyes compared to normal eyes (p<0.01). Eyes with POAG showed a higher percentage of collapsed SC compared to normal eyes (p<0.02). There were no significant differences in GV density or size comparing POAG to normals and no difference was seen between active and inactive flow regions (p>0.15). There was also no difference in either normal or in glaucomatous eyes of GV density or size comparing the active to the inactive flow regions (p>0.25).

Conclusions: GV size and density did not differ in POAG as compared to age-matched normals in eyes perfusion-fixed at 15 mmHg. A lower, more physiological pressure drop (IOP–episcleral venous pressure) may allow differences in glaucomatous vacuoles to be detected. The similarity in vacuolar density and size in active and inactive flow regions suggests that giant vacuoles may not serve as markers for regions of active flow, as was previously suggested (Parc, IOVS, 2000).

Commercial Relationships: Ariel Gelman; Lihua Gong, None; Elliott D. K. Cha, None; Mark Johnson, None; Haiyan Gong, None

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Presentation Time: 3:45 PM–5:30 PM

Transcleral laser, ciliary muscle shortening & outflow pathway reorganization

Murray A. Johnstone, Steven Padilla, Kimika Wen. Ophthalmology, University of Washington, Seattle, WA.

Purpose: Aqueous outflow system configuration (OSC) determines aqueous flow and IOP that becomes abnormal in glaucoma. We describe a pilot study with an ex vivo system using visually guided positioning and real time observation of OSC changes (Δs) in response to transcleral μP laser (Iridex™) pulses.

Methods: M. fasc. primates A (PrA) & B (PrB). PrA: microscope, video recordings, calibration micrometer, 1 mm thick radial limbal segments from 4 quadrants (Q). micromanipulator, single pulse of 810 nm μP laser, duty cycle 31.3%. Paired parameters of stepwise power; range: 500-3000 mw, stepwise duration; range: 125-3,000 msec. Energy range: 0.08-2.35 joules. Clinically, ~1.59 J (CEJ) is applied per single location. Video capture during pulse. PrA: Motion quantitated from still frames with ImageJ. PrB: H&E & trichrome in 4 Q after Rx with CEJ.

Results: PrA - Visible μP effects were confined to longitudinal ciliary muscle (CM) near sclera (Fig. 1) Shortening/Contraction (ShCo) & relaxation was detected at ≥ 0.08 J in the IN & SN Q and at ≥ 0.16 J in the IT and ST Q. The CM facing the AC transiently moved inward & posteriorly at ≥ 0.75 Joules in all Q. A pilocarpine-like Δ (PLΔ) in Schlemm’s canal shape occurred when the scleral spur (SS), and trabecular meshwork (TM) moved posteriorly (Fig. 1); (www. youtube.com/user/ibmurray). CM bundles recovered/relaxed to near pre-μP configuration at low energies with progressive reduction in the recovery response as energy increased (Fig. 2). CM bundles experienced ShCo & turned white at 2.35 J (~ 48% > than CEJ) with a persistent Δ in CB, SS & TM configuration. Ciliary epithelial damage was absent with direct observation (PrA & B) & histology (PrB).

Conclusions: Transcleral μP laser induces contraction of the CM, a well-characterized muscle response to μP lasers. CM shortening caused posterior and inward movement of the SS & TM. Our pilot study reports CM, SS, TM & SC PLΔs following transcleral 810 nm μP laser Rx. Clinically used μP parameters induce OSC Δs generally associated with improved aqueous flow. Our preparation permits systematic assessment of effects of probe location, power vs. duration relationships, total energy and focal depth. Systematic lab studies to determine optimized parameters may improve success rates, reduce morbidity and ideally achieve lasting improvement in aqueous flow & IOP.
Freeze-thaw Decellularization with Preserved Trabecular Meshwork Scaffold in an Ex Vivo Eye Perfusion Model

Susannah Waxman1, Yalong Dang1, Ralitsa Loewen1, Chao Wang1, 2, Adrianna Jensen1, Nils Loewen1. 1University of Pittsburgh, Pittsburgh, PA; 2Xiangya School of Medicine, Central South University, Changsha, China.

**Purpose:** Repopulation of diseased trabecular meshwork (TM) with new, functional cells may provide a new approach to treating open angle glaucomas. An ideal ex vivo model to establish transplantation techniques requires TM architecture and cellularity similar to glaucomatous eyes and the option to remove existing TM cells. This study’s objective was to develop a simple decellularization technique that preserves an extracellular matrix (ECM) scaffold without the requirement of chemicals or viral ablation agents.

**Methods:** Anterior segments of porcine eyes were mounted and cultured in constant-rate perfusion units. After 72 hours of perfusion to stabilize IOP, eyes that received freeze-thaw treatment (F) were thrice cycled from to -80°C for 120 minutes to room temperature for 60 minutes while eyes that received saponin treatment (S) were exposed to 0.02% saponin for 15 minutes followed by an anterior chamber fluid exchange. Eight eyes were assigned each to F, S, and control group (Co). Post-treatment, eyes were perfused with culture media for 180 hours and IOP was measured at two minute intervals. Calcein and PI co-labelling was utilized to evaluate TM viability. H&E stained sections were used for gross histological analysis. After eGFP-FIV transduction of two additional eyes, real-time TM ablation via S or F was visualized with a dissecting fluorescence microscope.

**Results:** Similar baseline IOPs were obtained in F and S (14.75+/− 2.24 mmHg and 14.37+/- 1.14 mmHg, P=0.288). Shown in Figure 1, F and S experienced IOP reductions at 12 hours and reached similar endpoint IOPs at 180 hours (9.51+/− 0.98 mmHg and 11.65+/− 1.34 mmHg, P=0.151) while Co IOP did not decrease. Viability staining assay revealed numerous PI-labeled dead cells and Calcein-labeled viable cells in both the S and Co groups. In contrast, sparse PI-labeled and no Calcein-labeled TM cells were seen in F. Histology revealed that TM cells were absent in F while the angle architecture and ECM were preserved. Shown in Figure 2, TM cells that expressed eGFP were entirely ablated by F but only partially by S.

**Conclusions:** We developed a chemical-free, freeze-thaw method to produce decellularized TM scaffold with well preserved TM extracellular matrix in organotypic perfusion culture.

**Commercial Relationships:** Murray A. Johnstone, None; Steven Padilla, None; Kimika Wen, None

**Program Number:** 3469  **Poster Board Number:** A0092  **Presentation Time:** 3:45 PM–5:30 PM

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1-way ANOVA). Exposure to Y27632 resulted in decreased ECM deposition and lower resistance (5.1±0.9 and 4.6±1 mmHg, p>0.05 1-way ANOVA).

**Conclusions:** HTM/HMVEC 3D outflow co-culture system induces HMVEC to express HSC markers and behave similarly to HSCs. This model may allow higher throughput screening of agents that affect conventional outflow tract and provide insight into HSC development.

**Commercial Relationships:** Karen Y. Torrejon, Glauconix Inc. (E); Ellen Papke, None; Dhruva J. Bharali, Glauconix Inc. (E); Magnus Bergkvist, Glauconix Inc. (F); W Daniel Stamer, Glauconix Inc. (C); Susan T. Sharfstein, None; Yubing Xie, None; Feryan Ahmed, Glauconix Inc. (E); John Danias, None

**Support:** NSF Award 1448900, NEI R01EY025543

**Program Number:** 3472 **Poster Board Number:** A0095 **Presentation Time:** 3:45 PM–5:30 PM

**Histopathological findings in trabeculectomy specimens**

Andres Lisker-Cervantes, Luis Laneri Pusineri, Rafael Castañeda Diez. Asociación para Evitar la Ceguera en México I.A.P., Mexico City, Mexico.

**Purpose:** The anatomic site of the trabeculectomy could give us information about the status of the trabecular meshwork, like the amount of pigment, type of cells and fibrosis, and if the material was obtained from the proper site. Additionally, there is a prognostic factor depending on the site of the cut; for example, materials obtained posterior to the scleral spur, tend to fail sooner than the anterior ones. However, the histopathological study of this material is often set aside. There is no study of trabeculectomy specimens in Mexican population.

**Methods:** Materials obtained from trabeculectomies of patients with primary open angle glaucoma (POAG) and primary angle closure glaucoma (PACG), were fixated in 10% formaldehyde for their histopathological study. The materials were stained with Hematoxin & Eosin, Masson’s trichrome, and periodic Acid-Schiff and observed in a light microscope.

**Results:** To date we have collected 4 samples. In the two samples from POAG patients, we recognize the trabecular meshwork with moderate pigmentation, decreased cell count and a small amount of fibrosis at the anterior edge of the meshwork (figure 1). The other two samples from PACG patients, the trabecular meshwork was identified in one of them with a moderate inflammatory cellular infiltrate and a reduced trabecular cells population, in the last specimen, we could only identify collagen fibers from the cornea with no trabecular meshwork in it.

**Conclusions:** Our results are consistent with the theoretical findings in the POAG. However, we need a larger sample to identify structural changes that can help us understand all the physiopathological mechanisms involved in POAG and PACG. Further studies are needed to recognize the clinical consequences of these findings.

**Commercial Relationships:** Andres Lisker-Cervantes, None; Luis Laneri Pusineri, None; Rafael Castañeda Diez, None

**Program Number:** 3473 **Poster Board Number:** A0096 **Presentation Time:** 3:45 PM–5:30 PM

**Morphologic Changes in Trabecular Meshwork Associated with Thapsigargin Induced Mouse Glaucoma Model**

Ruiyi Ren1,2, Yi ZHOU1, Thuy Duong Le1, Enzhi Yang1, Yiqin Du1, Haiyan Gong1,2. ‘Ophthalmology, Boston University School of Medicine, Boston, MA; 2Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA; 3Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, PA.

**Purpose:** Trabecular meshwork (TM) regeneration by cell-based therapy is a promising new approach for glaucoma treatment. An endoplasmic reticulum (ER) stressor, Thapsigargin (Thap), was previously reported to induce apoptosis and necrosis in cultured TM cells and elevation of intraocular pressure (IOP) in mouse eyes (Yang et al, ARVO 2016). This mouse glaucoma model can be used for further investigation of cell-based therapy for glaucoma. However, the effects of Thap treatment on TM morphology in this mouse model has not been investigated. This study aimed to investigate the morphologic changes in TM of Thap induced mouse glaucoma model.

**Methods:** C57BL/6 mice (7-8 weeks old) were injected with Thap (0.5µg/ml) intracamerally, subconjunctivally, or without any treatment as control (N=3 for each group). After measurements of IOP for 9 weeks, eyes were enucleated and fixed. Each eye was dissected to obtain anterior segment and then further dissected into eight wedges. Four wedges from each eye (one from each quadrant) were randomly selected to generate four radial and four frontal sections. All sections were then dehydrated, embedded, semi-thin (1µm) sectioned and imaged with light microscopy. One radial and one frontal sections from each eye were randomly selected and sectioned (80nm) for transmission electron microscopy (TEM). All sample processing and morphological analyses were performed in a masked fashion.

**Results:** No significant difference in general morphology was found at light microscopic level between Thap injected groups and the control group. At TEM level, dilated rough ER, dissolution of internal structure of mitochondria, and necrosis-like disruption or rupture of cell and organelle membranes were observed in the inner wall of Schlemm’s canal, juxtanaculal tissue and the trabecular beams in both Thap injected groups, but not in control group. There seems no

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significant difference between two Thap treated groups with different injection methods.

**Conclusions:** Thap can induce cellular abnormalities including structural changes in ER, mitochondria, and cell membrane in all three types of cells in the TM. Intracameral and subconjunctival injections of Thap induce similar morphologic changes in the TM. Further study on Thap’s effects on TM matrix may elucidate the mechanisms by which Thap increases IOP.

**Commercial Relationships:** Ruiyi Ren, None; YI ZHOU, None; Thuy Duong Le, None; Enzhi Yang, None; Yiqiu Du, None; Haiyan Gong, None

**Support:** Bright Focus Foundation Grant G2014086, NH Grant EY025643, NH Grant EY022634, The Massachusetts Lions Eye Research Fund

**Program Number:** 3474 Poster Board Number: A0097

**Presentation Time:** 3:45 PM–5:30 PM

**Treatment of refractory glaucoma using high-intensity focused ultrasound cycloablation**

Chiara Del Noce, Alessandro Bagnis, Michele M. Iester, Carlo E. Traverso. DiNOGMI Clinica Oculistica, IRCCS Azienda Ospedaliera Universitaria San Martino - IST, Genoa, Italy.

**Purpose:** To evaluate the clinical efficacy and safety of ultrasound cyclophotocoagulation in lowering intraocular pressure (IOP) in refractory glaucoma patients.

**Methods:** Twenty-one glaucomatous patients (age: 74.85 years +/- 34.75) were enrolled in this observational study. Five patients had neovascular glaucoma, 7 primary open angle glaucoma and 9 chronic angle closure glaucoma.

The main inclusion criterion was the diagnosis of glaucoma with a baseline IOP > 21 mmHg while on maximum topical and systemic medical hypotensive treatment. All underwent ultrasound cycloablation treatment with the EyeOP1 device with 8 activated transducers lasting 4 s (group 1), 6 s (group 2) or 8 s (group 3), respectively.

Patients were assessed before and at 1, 7, 14, 30, 90, 180 days and 12 months days after the procedure. Primary outcomes were the mean IOP reduction in the overall population and in groups 1, 2 and 3.

**Results:** No patient experienced pain during the procedure. The mean preoperative IOP was 31.95 mmHg and decreased to 21.71 mm Hg (p = 0.001) at one year after the procedure. At last follow-up the mean number of drops was 1.95 per day, corresponding to a significant reduction of 24.12% of the quantity of drops taken (p = 0.002). Only 6 patients continued acetazolamide tablets after 1 year in an average amount of 0.9 tablets per day, showing a reduction of 40% of the therapy (p < 0.001) while all the other patients could discontinue any oral therapy. Group 3 patients showed a greater IOP reduction than the other two groups (mean IOP reduction of 38.12% for group 3 vs. 29.05% for group 2 and 28.57% for group 1; p = 0.322 and p < 0.001, respectively).

According to the three different types of glaucoma treated we found the best results in IOP lowering among angle closure glaucoma patients (mean IOP reduction of 39.11%; p < 0.05), followed by the group of open angle glaucoma (mean IOP reduction of 32.98%; p = 0.02) and the neovascular glaucoma (mean IOP reduction of 25%; p = 0.006).

**Conclusions:** Ultrasound cyclophotocoagulation is a simple, effective and safe procedure. This technique is a good alternative to other cycloablation methods and is well tolerated by patients who show less postoperative eye pain and inflammation.

**Commercial Relationships:** Chiara Del Noce, None; Alessandro Bagnis, None; Michele M. Iester, None; Carlo E. Traverso, None

**Support:** Bright Focus Foundation Grant G2014086, NH Grant EY025643, NH Grant EY022634, The Massachusetts Lions Eye Research Fund

**Program Number:** 3474 Poster Board Number: A0097

**Presentation Time:** 3:45 PM–5:30 PM

**Clinical outcomes of combined cataract surgery with iStent implantation**

Anna Djoungarian1, Allison Angelilli1, 2, Robert Rothman1, 2, Craig H. Marcus1, 2, Daniel Hayes2, 3. Ophthalmology, Northwell, Great Neck, NY; 1Glaucoma Consultants of Long Island, Bethpage, NY.

**Purpose:** Treatment of open angle glaucoma (OAG) involves reduction of intraocular pressure (IOP), either by reducing aqueous humor production or by improving facility of aqueous humor outflow. This is commonly done with topical medications, laser trabeculoplasty, or incisional surgery. Newer options, such as the iStent trabecular microbypass stent (Glaukos; San Clemente, CA), can be implanted during cataract extraction (CE) to further reduce both IOP and medication usage in patients with mild to moderate OAG. The purpose of this study is to determine the effect of combined CE and iStent on IOP reduction and medication use.

**Methods:** A retrospective review was conducted at a large glaucoma practice for patient visits between September 2013 and September 2016. Inclusion criteria were patients with mild to moderate OAG who underwent combined CE and iStent during this time. Exclusion criteria included patients with any other type of glaucoma or prior incisional glaucoma surgery. Statistical analysis was conducted with Stata v. 13 (Houston, TX). The assumption of normality was not violated, as assessed by Shapiro-Wilk’s test (p = 0.05). A paired samples t-test was used to determine if there was a statistically significant difference between the pre-operative mean IOP and the number of medications used as compared to 3, 6, 9, 12, and 24 months post-operatively.

**Results:** There were 54 eyes of 42 patients. Twenty-one were women and 21 were men. Average age was 77 years old, ranging from 62 to 92 years old. Average follow-up was 14.78 months, ranging from 3 to 24 months. When compared to their pre-op IOP values, patients had significantly decreased post-op IOP at 3 months (17.04 mm Hg ± 0.53 SEM vs. 14.81 ± 0.35; n=54; p≤0.0005), 6 months (17.95 ± 0.65 vs. 14.9 ± 0.48; n=40; p≤0.0005), 9 months (18.00 ± 0.63 vs. 15.27 ± 0.58; n=37; p≤0.0005), 12 months (17.63 ± 0.64 vs. 15.75 ± 0.43; n=36; p≤0.0005), 18 months (18.00 ± 0.84 vs. 16.60 ± 0.72; n=25; p≤0.05) and 24 months (18.05 ± 1.00 vs. 15.70 ± 0.96; n=17; p≤0.05). When compared to pre-op number of medications used, patients had significantly less usage at 12 months (1.77 ± 0.15 vs. 0.91 ± 0.19; n=36; p≤0.0005) and at 24 months post-op (1.76 ± 0.21 vs. 0.70 ± 0.22; n=17; p≤0.05).

**Conclusions:** Combined CE with iStent implantation results in significantly reduced IOP and number of medications used.

**Commercial Relationships:** Anna Djoungarian, None; Allison Angelilli, None; Robert Rothman, None; Craig H. Marcus, None; Daniel Hayes, None

**Program Number:** 3476 Poster Board Number: A0099

**Presentation Time:** 3:45 PM–5:30 PM

**Safety and Efficacy of iStent Insertion Performed by Resident Surgeons**

Kevin Mays1, Keri F. Aller2, Bethany B. Markowitz2, Shawn Iverson2. 1University of South Carolina School of Medicine, Columbia, SC; 2Department of Ophthalmology, Palmetto Health - University of South Carolina Medical Group, Columbia, SC.

**Purpose:** To compare resident complication rates and IOP lowering efficacy of iStent and phacoemulsification to phacoemulsification alone.

**Methods:** Retrospective review of all resident phacoemulsification cataract extraction with or without iStent between October 2015 and
Aqueous humor dynamics were assessed by digital Shiotz tonography, fluorophotometry, and pneumatonometry. Uveoscleral outflow was calculated using Goldmann’s equation with an assumed episcleral venous pressure of 10 mmHg.

**Results:** Thirty-two healthy volunteers, 32 patients with uveitis with normal IOP and 26 cases with hypertensive uveitis were recruited. The participants’ characteristics are shown in Table 1. The mean tonographic outflow facility (C) in hypertensive uveitis cases was 0.20±0.14 μl/min/mmHg. This was significantly lower than controls (0.23±0.09 μl/min/mmHg, p=0.03) as well normotensive with uveitis (0.26±0.13 μl/min/mmHg, p=0.05). However, the aqueous flow rate (Ft) was similar across all groups (2.7±0.72 μl/min, 2.1±0.89 μl/min, and 2.5±0.88 μl/min). Additionally, the uveoscleral outflow (Fu) was comparable between all groups (Table 2).

**Conclusions:** 1. Aqueous humor production is not significantly affected by several attacks (≥3) of uveitis in eyes with or without raised IOP.

2. The cause of raised IOP in hypertensive uveitis is related to the reduction in trabecular outflow.

3. As none of the eyes were actively inflamed at the time of the study, cells and debris obstruction of the trabecular meshwork is unlikely.

4. One can assume that repeated episodes of uveitis and perhaps previous exposure to topical corticosteroids lead to permanent structural alteration of the trabecular meshwork in those eyes with hypertensive uveitis.

**Table 1. Patients characteristics**

<table>
<thead>
<tr>
<th>Healthy volunteers</th>
<th>P value</th>
<th>95% CI</th>
<th>Uveitis with normal IOP</th>
<th>P value</th>
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<th>Hypertensive uveitis</th>
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**Table 2. Aqueous humor parameters of participants**

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**Clinical Trial:** NCT02765308

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Program Number: 3478 Poster Board Number: A0101
Presentation Time: 3:45 PM–5:30 PM
Effect of continuous eye perfusion on rat aqueous humor dynamics
Christopher L. Passaglia1, 2, Kayla Ficarrotta1, 3, Chemical and Biomedical Engineering Department, University of South Florida, Tampa, FL; 2, Ophthalmology Department, University of South Florida, Tampa, FL.
Purpose: Current methods for inducing glaucoma in animals rely on altering the trabecular meshwork to elevate IOP. We have developed an in-vivo eye perfusion technique for chronically elevating IOP, which causes glaucomatous patterns of retinal ganglion cell loss without experimentally targeting the trabecular meshwork. The aim of this study was to characterize the long-term effects of perfusion-induced ocular hypertension on aqueous humor dynamics.
Methods: A fine polyimide tube was implanted in the anterior chamber of one eye of Brown-Norway rats and passed subdermally to a skull mount, which was connected via a tether system to an external reservoir filled with artificial aqueous humor. IOP was continuously measured with an in-line pressure transducer and checked by tonometry. IOP was elevated by 10 mmHg for 9 weeks in six implanted animals. At two-week intervals, the animals were anesthetized with isoflurane and the conventional outflow facility of the implanted eye was measured using a modified constant-pressure perfusion technique with the tethered cannula line connected to a perfusion pump. At the end of the 9-week period, the animal was anesthetized and a 3G needle was inserted into each eye. The cannula line was cut and sealed to prevent leakage, and conventional outflow facility was measured for both eyes using the modified constant-pressure perfusion technique.
Results: Conventional outflow facility did not significantly change in the implanted eye over the 9-week period of IOP elevation. At experiment end, the outflow facility of implanted and control eyes was 0.022 ± 0.002 and 0.024 ± 0.001 µl/min/mmHg, which was not statistically different (p=0.094). Baseline IOP at the start and end of the experiment was not measurably different for implanted and control eyes, further attesting to a lack of permanent or significant damage to the trabecular meshwork.
Conclusions: While chronic IOP elevation can cause glaucomatous damage to the retina, it alone does not appear to impact aqueous outflow pathways. For the conditions tested in rats, no signs of pressure- or flow-induced changes in the biomechanical properties of trabecular meshwork were detected.
Commercial Relationships: Christopher L. Passaglia; Kayla Ficarrotta, None
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Program Number: 3479 Poster Board Number: A0102
Presentation Time: 3:45 PM–5:30 PM
Aqueous Angiography Based Comparison of High- and Low-Flow Regions for Alpha-Smooth Muscle Actin (α-SMA) and Versican in Post-Mortem Human Eyes
Sindhu Saraswathy1, Thania Bogarin1, 2, Chirayu Mohindroo1, 2, Brian A. Francis1, James C. Tan1, 3, David R. Hinton1, Robert N. Weinreb1, 4, Alex S. Huang1, 2, 3, 4, 5, 6, Ophthalmology Department, University of Southern California, Los Angeles, CA; 2, Pathology, University of Southern California, Los Angeles, CA; 3, University of California, San Diego, La Jolla, CA; 4, Shiley Eye Institute, San Diego, CA.
Purpose: Here, aqueous angiography was used to identify low- and high-flow regions to enrich for biological protein differences that were probed using various methods.
Methods: Post-mortem eyes (San Diego and One Legacy eye bank) were obtained with no history of glaucoma, within 48 hours of death. The eyes were perfused with Balanced Salt Solution (BSS) followed by tracer (fluorescein [2.5%] or indocyanine green [0.4%]) diluted in BSS with a pressure of ~10 mm Hg. After angiographic imaging with the Spectralis HRA+OCT (Heidelberg Engineering), eyes were fixed with 4% paraformaldehyde or prepared for western blot. Wedges including the angle were cut from angiographically positive and negative regions, paraffin embedded, and processed for sectioning (~5 microns). The sections were immunostained and viewed under a Keyence BZ-X700 digital imaging microscope (IF). Separately, the TM from positive and negative regions were dissected and processed for western blot (WB). Antibodies were used against α-SMA and versican with GAPDH as a WB control.
Results: α-SMA levels were increased in low-flow regions by WB (3.23-fold from 2 eyes) and IF (4.49-fold; n = 8 sections of low- and high-flow from 2 eyes; p < 0.001). By WB, in a small sample (n = 2 eyes) versican was also increased (5.46-fold).
Conclusions: Immunofluorescence and WB analyses of target proteins after aqueous angiography-based segregation of high- and low-flow AHO regions demonstrated increased protein levels of α-SMA and versican. Elevated levels of α-SMA have been implicated in glaucomatous trabecular meshwork and increased levels of versican have been seen in low-flow trabecular meshwork regions in micro-bead based outflow studies.

Figure 1. TM from positive and negative regions were dissected out and processed for western blot analysis against antibodies specific for α-SMA with GAPDH as a WB control. α-SMA levels were increased in low flow regions by 3.23 fold by WB. LF = low-flow and HF = high-flow.
Commercial Relationships: Sindhu Saraswathy, None; Thania Bogarin, None; Chirayu Mohindroo, None; Brian A. Francis, Infocus (F), BVI Endoptiks (C), Lumenis (F), Aquesys (F), Diopsys (F), Allergan (F); James C. Tan, None; David R. Hinton, None; Robert N. Weinreb, Heidelberg Engineering (F), Aerie Pharmaceutical (C), Alcon (C), Carl Zeiss Meditec (F), Quark (F), Perspective Vision S Sensimed (C), Topcon (F), Optovue (F), Eyenovia (C), Bausch & Lomb (C), Genentech (F), Allergan (C), Unity (C); Alex S. Huang, Heidelberg Engineering (F), Allergan (C).
Support: Supported by National Institutes of Health, Bethesda, Maryland (K08EY024674 [ASH]; AGS Young Clinician Scientist Award 2015 [ASH]; Research to Prevent Blindness Career Development Award 2016 [ASH]; and an unrestricted grant from Research to Prevent Blindness (New York, NY).
Steroid-Response in Primary Culture Scleral Cells, Implications for Distal Outflow Pathology

Thania Bogarin1, 4, Sindhu Sarawathy1, Elizabeth Tannous1, Chi Zhang1, Jose Gonzalez1, James C. Tan4, 4, David R. Hinton2, Robert N. Weinreb3, 4, Jie J. Zheng1, Alex S. Huang1, 4.
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Purpose: Steroid-response glaucoma is thought to be due to trabecular meshwork (TM) changes in response to steroid exposure either after topical administration in intact eyes or after drug exposure in situ using cell culture models. Continued steroid-response can be observed with steroid delivery even after trabecular bypass surgeries suggesting distal aqueous humor outflow (AHO) pathway steroid-response mechanisms as well. This project proposes to study steroid-response in primary scleral cultures to understand distal outflow pathway pathology.

Methods: Scleral primary cultures were generated from published protocols using donor corneo-scleral rings after corneal transplantation via scleral mid-scleral depth excised explants. Preliminarily, scleral cells were grown until 70% confluent in chambered slides (n = 2) under 3 treatment groups for 7 days, 100 nM dexamethasone (DEX), DMSO (vehicle), and MEDIA (control; DMEM/F12 with 10% FBS). Cells were prepared for western blot for myocilin expression; or were fixed with 4% paraformaldehyde, blocked with 5% fetal bovine serum, and permeabilized with 0.25% Triton-X-100. Fixed cell were incubated in Phalloidin (Alexa Fluor-568; Life Technologies) with 1% fetal bovine serum overnight at 4°C. Cells were washed, stained/mounted with DAPI (Vector Labs), and viewed under BZ-X700 digital imaging microscope (Keyence). Western blot was processed by standard procedure with GAPDH as a control. Band pixel intensities were quantified (ImageJ).

Results: DEX treated scleral cells exhibited the development of cross-linked actin networks (CLANS). By western blot, myocilin expression was increased (DEX = 2.05, DMSO = 0.95, and MEDIA = 0.78; arbitrary intensity units normalized to GAPDH). Alternatively, DMSO and MEDIA treatment did not show increased myocilin protein or CLAN formation but instead typical uniform and parallel F-actin fiber organization.

Conclusions: Increased CLANS and myocilin expression are known hallmarks of steroid-response as described in TM cell-culture. DEX treatment of primary scleral cultures in a small sample size can lead to a steroid-response like phenotype with CLAN and increased myocilin protein formation.

Figure 1. Cross-linked actin network (CLAN) formation (Phallloidin Alexa Fluor-568; arrows) in (A) Dex, (B) vehicle, and (C) media-treated scleral cells.

Figure 1

Commercial Relationships: Thania Bogarin, None; Sindhu Sarawathy, None; Elizabeth Tannous, None; Chi Zhang, None; Jose Gonzalez, None; James C. Tan, None; David R. Hinton, None; Robert N. Weinreb, Heidelberg Engineering (F), Aerie Pharmaceutical (C), Alcon (C), Carl Zeiss Meditec (F), Quark (F), Foresight Vision V Sensimed (C), Topcon (F), Optovue (F), Eyenovia (C), Bausch & Lomb (C), Genentech (F), Allergan (C), Unity (C); Jie J. Zheng, None; Alex S. Huang, Heidelberg Engineering (F), Allergan (C)

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Differential miRNA Expression in Response to Mechanical Stress in Human Trabecular Meshwork Cells

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1Cellular Biology and Anatomy, Augusta University, Augusta, GA; 2Ophthalmology, Duke University Medical Center, Durham, NC; 3Medicine, Duke University Medical Center, Durham, NC.

Purpose: miRNAs have been shown to play important roles in regulating trabecular meshwork (TM) function and aqueous outflow. We aimed to identify differentially expressed miRNAs in human TM (HTM) cells in response to long-term mechanical stress.

Methods: Six strains of primary HTM cells were isolated from cadaveric eyes with no history of eye disease and cultured under serum-starved condition in the presence or absence of 15% mechanical stretch, 1 cycle/second, for 24 hours using the computer controlled Flexcell Unit. Total RNA was isolated using Ambion mirVana miRNA Isolation kit. Expression of 800 human miRNAs was profiled using NanoString nCounter Human miRNA Assay kits. After quality check and normalization, differentially expressed miRNAs in the stretched condition were identified using Bioconductor Limma package. Droplet digital PCR was used to validate the differential expression of selected miRNAs.

Results: 373 miRNAs were detectably expressed in the cultured HTM cells. Of these miRNAs, 74 miRNAs (10 down- and 64 up-regulated) were differentially expressed in stretched cells (p≤0.05). The top 5 most significantly expressed miRNAs were miR-32-5p, miR-4286, miR-135-5p, miR-136-5p, miR-93-5p (p value= 1.2x10^-5, 1.3x10^-5, 4.5x10^-5, 7x10^-4, 2x10^-4 respectively). The miRNAs with the greatest fold change in response to stretch included miR-4286, miR-32-5p, miR-136-5p, miR-93-5p, miR-135-5p, miR-21-5p, miR-19b-3p with log2 fold change ranging from 1.3 to 0.98. Consistent with previous reports, miR-100-5p, miR-27a-3p, miR-27b-3p, miR-24-3p, miR-16-5p, let-7f-5p and let-7i-5p were significantly upregulated in stretched versus control HTM cells. Ingenuity Pathway Analysis showed that these miRNAs are mostly associated with cellular functions related to proliferation, development, cell cycle regulation and cellular movement. Remarkably, the highly expressed miRNAs are predicted regulators of CDKN2A, a glioma-related gene shown to be upregulated in a rat glioma model.

Conclusions: Our miRNA profiling suggests a potential role of miRNAs in homeostatic responses of HTM cells to in vivo physiological mechanical stress. Further characterization of these miRNAs may advance our understanding of HTM-mediated regulation of aqueous outflow resistance and generate possible therapeutic targets to regulate intraocular pressure.

Commercial Relationships: Inas Helwa, None; Michelle Dreway, None; William M. Johnson, None; W. Michael Dismuke, None; Inas F. Aboobakar, None; Kristin M. Perkumas, None; R. R. Allingham, None; Michael A. Hauser, None; W. D. Stamer, None; Yutao Liu, None

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Role of FHOD1, EDNRA and LPAR1 on the intraocular pressure lowering effects of miR-200c
Pedro Gonzalez, Megan Parker, Coralia C. Luna. Ophthalmology, Duke University, Durham, NC.

Purpose: We have previously reported that miR-200c mimic induced strong inhibition of contraction and decreased cell traction forces in human trabecular meshwork (HTM) cells and showed reduction of intraocular pressure (IOP) in rats. Here we investigate the role of some of the miR-200c targets relevant to TM cell contraction (FHOD1, EDNRA and LPAR1) and its effects in rats IOP.

Methods: Primary HTM cells were transduced with adenovirus expressing FHOD1, EDNRA, LPAR1 and null virus and evaluated for changes in gene expression by real time Q-PCR; in protein expression by western blot; and effects on cell contraction by using a three-dimensional collagen gel (1.5 mg/mL COL1A1) contraction assay. Sprague Dawley rats were injected with these viruses in the anterior chamber using a Hamilton syringe and IOP was measured during 15 days using a Tonolab rebound tonometer. Anterior chamber morphology was evaluated by paraffin sections.

Results: HTM cells transduced with EDNRA, FHOD1 and LPAR1 adenoviruses increased gene expression by 2.2 (p 0.016), 124.47 (p 1.15E-06) and 2.6 (p 0.0008) fold respectively. All three genes induced significant contraction of HTM cells on collagen gels compared to controls (15 to 64% less area). Rats injected with FHOD, LPAR or EDNRA showed a slight but significant increase in IOP around days 6 to 12. Morphology of the anterior chamber looks normal after injection of the viruses.

Conclusions: Mir-200c is a post-transcriptional inhibitor of genes relevant to TM cell contraction. FHOD, LPAR and EDNRA are some of the target genes of miR-200c and its individual overexpression increase HTM cell contraction and induce a small increase in IOP confirming that part of the effects of miR-200c are mediated by these genes.

Mechanotransduction
Joshua Hirt, Paloma B. Liton. Ophthalmology, Duke University - Albert Eye Research Institute, Durham, NC.

Purpose: Our previous studies reported the activation of autophagy in TM cells in response to high pressure and elongation. Here, we investigate the role and the mechanistic pathway triggering stretch-induced autophagy.

Methods: Human TM cells were subjected to biaxial cyclic mechanical stress (20% elongation, 1 cycle/sec) for up to 16 hours. Blockage of autophagy or chaperon assisted-selective autophagy (CASA) was achieved using siRNA targeting Atg5 and Atg7 (siAtg5/7) or BAG3 (siBAG3), respectively. Scrambled siRNA was used as control (siNC). Autophagy was evaluated by monitoring the LC3-I to LC3-II conversion by WB and via confocal imaging using the tFLC3 assay. Protein expression levels of autophagy related proteins were quantified in nuclear and cytosolic fractions. Whole-transcriptome expression profiling and alternative splicing analyses were performed using Affymetrix Clariom D arrays and analyzed with Transcriptome Analysis Console and Partek software.

Results: Microarrays analysis revealed 240 genes and 131 genes differentially expressed (> 2-fold, p<0.05, n=3) in TM cells with inhibited autophagy or CASA, respectively. Among the genes commonly found under both conditions (total = 22) we highlight intraflagellar transport 27 (IFT27), a core component of the intraflagellar transport complex implicated in mechanosensation. Other genes showing common differential expression with siAtg5/7 and siBAG3 include the actin-binding proteins tropomyosin 4, phosphatase and actin regulator, and phospholipase C. Very interestingly, we observed for the first time the nuclear expression of the autophagosome marker LC3, as well as Atg7, p62 and BAG3 in TM cells. No endosomal or lysosomal markers were detected in the nuclear fractions. Moreover, the levels of nuclear LC3, in particular

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Role of Calponin in Regulating Contractile Activity of Trabecular Meshwork Cells

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1Ophthalmology, Duke University School of Medicine, Durham, NC; 2Pharmacology and Cancer Biology, Duke University School of Medicine, Durham, NC.

Purpose: Hypercontractility of the trabecular meshwork (TM) has been demonstrated to influence conventional aqueous humor (AH) outflow and intraocular pressure (IOP). Hence, it is important that we explore and thoroughly understand the regulation of TM contractile activity in the context of glaucoma pathobiology. Towards this objective, in this study we investigated the role of calponin (CNN), an actin-binding, smooth muscle abundant contractile protein, in TM cells. Serum starved TM cells treated with lysophosphatidic acid (5 μM/6 hr), dexamethasone (0.5 μM/24 hr), angiotensin II (2μM/24 hr) and endothelin-1 (2μM/24 hr) showed a significant increase in both CNN 1 and 3 protein levels compared to controls. Similarly, the levels of phosphorylated CNN 3 were increased in TM cells under the above described treatment conditions. Pharmacological inhibition of Rho kinase and protein kinase C using M/18 hr) and GF109203X (20 μM/6 hr), respectively, led to significant decreases in the levels of phosphorylated CNN 3 in TM cells.

Conclusions: Taken together, these results reveal that both the expression and phosphorylation-status of CNN which are known to influence the contractile activity of smooth muscle, are responsive to various external factors and intracellular signaling molecules in TM cells. This study also identifies CNN as one of the molecular targets for Rho kinase inhibitors in TM cells and its potential role in AH outflow and homeostasis of IOP.

Elevated Hydrostatic Pressure Selectively Alters TGF-β2, ET-1, and CTGF Gene Expression in Human Trabecular Meshwork Cells

Jonathan Lautz1,2, Evan B. Stubbs2,3.
1Program in Neuroscience, Loyola University Chicago, Hines, IL; 2Research, Edward Hines Jr VA Hospital, Hines, IL; 3Ophthalmology, Loyola University Chicago Health Science Center, Maywood, IL.

Purpose: Primary open-angle glaucoma (POAG) is a progressive optic neuropathy characterized by loss of peripheral vision secondarily associated with elevated IOP. Transforming growth factor (TGF)-β2 is markedly elevated in the AH of patients with POAG. We and others have previously shown that TGF-β2 increases IOP, in part, by inducing expression and release of endothelin-1 (ET-1) and connective tissue growth factor (CTGF) within the trabecular meshwork (TM). ET-1 and CTGF may ultimately lead to increases in IOP by enhancing TM cell contractility and ECM deposition. Despite these advancements, the direct effect of elevated IOP on TM cell responsiveness remains unknown. Here we determined whether elevated hydrostatic pressure (HP) can selectively alter gene expression in a transformed human TM cell line.

Methods: Confluent cultures of transformed human TM cells were serum-starved x 24h and subsequently cultured in the absence or presence of elevated HP (15, 30, or 45 mm Hg above ambient) for up to 24h. Changes in the expression and/or release of TGF-β2, ET-1, CTGF, and select ECM constituents were quantified by qRT-PCR, Western immunoblot, or ELISA. Cell viability and integrity was assessed by quantifying MTT reduction and LDH release.

Results: Transformed human TM cells exposed to elevated HP exhibited a transient increase in TGF-β2 and ET-1 mRNA expression and protein secretion compared to ambient controls. Exposing cells to elevated HP for up to 12h did not significantly affect cell viability, as quantified by LDH release or MTT reduction. In contrast, elevated HP elicited a sustained increase in CTGF mRNA and protein secretion over 24h and elicited a time-dependent change in select ECM gene expression. By comparison, elevated HP had no effect on the expression of 14 other genes implicated in the pathogenesis of POAG. Modest changes in HPs, as low as 15 mm Hg, were found to elicit significant changes in TGF-β2, ET-1, and CTGF mRNA expression.

Conclusions: Cultured human TM cells exposed to elevated HP exhibit marked and selective changes in the expression of genes associated with the pathogenesis of POAG. We propose that pressure-dependent changes in TM cell gene expression represent a feed-forward mechanism that exacerbates TGF-β2 associated increases in TM cell contractility and altered ECM synthesis and deposition in affected POAG patients.

Commercial Relationships: Arumugam Ramachandran Muralidharan, None; Vasanth Rao, None
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Commercial Relationships: Jonathan Lautz, None; Evan B. Stubbs, None
**Program Number:** 3487 **Poster Board Number:** A0110  
**Presentation Time:** 3:45 PM–5:30 PM  
**Inhibition of TGFβ2 using CRISPR interference in the trabecular meshwork**  
Weiming Mao1, Hannah Webber1, Alvin Nguyen2, Daniel Goan3.  
1North Texas Eye Research Institute, UNT Health Science Center, Fort Worth, TX; 2Texas College of Osteopathic Medicine, University of North Texas Health Science Center, Fort Worth, TX.  
**Purpose:** In primary open angle glaucoma (POAG) patients, glaucomatous intraocular pressure (IOP) elevation is due to pathological changes in the trabecular meshwork (TM). Our recent publication showed that there is histone hyperacytation in the glaucomatous TM which may lead to elevated TGFβ2. However, gene specific histone modification has been technically challenging. Recently, the novel CRISPR interference technology has made this modification possible. The dCas9-KRAB system employs the mutant Cas9 enzyme (dCas9) which is able to bind specific DNA regions with the help of sgRNA but lacking DNA cleavage capability as well as the KRAB domain which is fused to dCas9 and is able to deacetylase histone proteins. We determined whether the CRISPRi/dCas9-KRAB system is useful in lowering TGFβ2 levels in the TM.  
**Methods:** Three dCas9-KRAB expression systems were used: 1) a one-vector system containing the ubiquitin (UBC) promoter-dCas9-KRAB cassette and the U6 promoter-sgRNA cassette; 2) a two-vector system containing a vector with the EF1α promoter-dCas9-KRAB cassette and a vector with the U6 promoter-sgRNA cassette; 3) a two-vector system containing a vector with the SFFV promoter-dCas9-KRAB cassette and a vector with the U6 promoter-sgRNA cassette. sgRNA was designed using an online software targeting regions close to the transcriptional start site. Four sets of sgRNA were subcloned downstream of the U6 promoter. Vectors were transfected or cotransfected into the transformed GTM3 cell strains. Four days after transfection, cells were harvested for qPCR analysis and Western immunoblotting. The expression level of TGFβ was normalized to GAPDH using the delta-delta Ct method.  
**Results:** The expression of dCas9-KRAB and/or sgRNA did not show toxicity to GTM3 cells. qPCR analysis showed that the one vector system with one of the four sgRNAs has mild repression of TGFβ2 expression. In contrast, the 2 two-vector systems showed a dramatic repression of TGFβ2 in GTM3 cells.  
**Conclusions:** The CRISPRi/dCas9-KRAB mediated inhibition of TGFβ2 expression is sgRNA as well as promoter dependent. Further studies are required to determine the specificity and suitability of this technology in other genes and primary human TM cells.  
**Commercial Relationships:** Weiming Mao; Hannah Webber, None; Alvin Nguyen, None; Daniel Goan, None

**Program Number:** 3488 **Poster Board Number:** A0111  
**Presentation Time:** 3:45 PM–5:30 PM  
**Effects of Wnt/beta-catenin and SMAD/TGF-beta crosstalk on cadherins in the trabecular meshwork**  
Hannah Webber, Weiming Mao, Abbots F. Clark. North Texas Eye Research Institute, University of North Texas Health Science Center, Fort Worth, TX.  
**Purpose:** We have shown cross-inhibition of the primary open angle glaucoma (POAG)-associated Wnt/beta-catenin and SMAD/TGF-beta signaling pathways in TM but the downstream effects of this crosstalk are unknown. Wnt transcription factor and cadherin accessory protein, beta-catenin, plays a major role in cadherins junction stability. We studied the role of Wnt/beta-catenin and SMAD/TGF-beta signaling on cadherins junctions in the TM and studied TM cell adhesion using the Acea iCelligence system.  
**Methods:** Confluent NTM cells were treated for 2 or 24 hours with or without 100ng/mL Wnt3a or 5ng/mL TGF-beta2 and their membrane-bound protein, conditioned medium, and total protein were isolated for western immunoblotting. Samples were probed for fibronectin (FN), PAI-1, p-Smad3, beta-catenin, GAPDH, Pan-, K-, or OB-cadherin. Some NTM cells were grown to 80% confluence then transfected with 0.3nM or 0.5nM K-cadherin, OB-cadherin, or non-targeting siRNA. Forty-eight or 72 hours after transfection, cells were harvested for western immunoblotting or were fixed for immunofluorescence. NTM cells were plated for the Acea iCelligence system to determine cellular impedance with data collected every 30 minutes to establish baseline. After 24 hours, culture medium was replaced and cells were transfected with siRNA. Cell impedance was continuously measured for an additional 96 hours. Bright field images of the cells were taken.  
**Results:** Wnt3a treatment resulted in increased K-cadherin expression. Co-treatment of Wnt3a and TGFβ2 decreased the expression of K-cadherin. Three days after transfection with 0.5nM K-cadherin siRNA decreased K-cadherin expression was observed in both whole cell lysate and membrane-bound fractions. Transfection with K-cadherin siRNA decreased NTM cellular impedance compared to non-targeting siRNA control.  
**Conclusions:** Crosstalk between Wnt/b-catenin and SMAD/TGF signaling pathways in the TM may regulate the expression of cadherins as well as NTM cell adhesion.  
**Commercial Relationships:** Hannah Webber, None; Weiming Mao, None; Abbott F. Clark, Reata Pharmaceuticals (F), Western Commerce (C), Nicon Research Institute (F)

**Program Number:** 3489 **Poster Board Number:** A0112  
**Presentation Time:** 3:45 PM–5:30 PM  
**The effect of dexamethasone on myocilin, AXIN2 and sFRP1 expression in primary human trabecular meshwork cells**  
Jie J. Zheng, Sarah D. Akhemade, Elizabeth Tannous, Chi Zhang, Department of Ophthalmology, Stein Eye Institute, UCLA, Los Angeles, CA.  
**Purpose:** To investigate how dexamethasone (Dex) affects the expression of myocilin mRNA, AXIN2 mRNA and sFRP1 mRNA, over 10 days of treatment in primary human trabecular meshwork (TM) cells.  
**Methods:** Primary human trabecular meshwork cells cultured from corneal scleral rims, were treated with 100 nM Dex or DMSO vehicle every day for 10 days. The RNA was isolated each day and quantitative PCR analysis was subsequently carried out for the expression of myocilin mRNA, AXIN2 mRNA and sFRP1 mRNA.  
**Results:** Myocilin mRNA, AXIN2 mRNA and sFRP1 mRNA were all significantly up regulated in Dex treated cells compared to their vehicle counterparts. The increase of myocilin expression in Dex treated cells started at day 2; AXIN2 expression elevated around day 3, and sFRP1 expression increased around day 7. The highest point of expression of sFRP1 was observed between days 8 and 9 and this correlated with decreased AXIN2 expression.  
**Conclusions:** : During the course of Dex treatment, there was increased expression of myocilin, AXIN2 and sFRP1 respectively in the TM cells. Myocilin is known to activate Wnt signaling in TM cells; expression of Axi2n is an established biomarker of the Wnt/b-catenin signaling; and sFRP1 is a negative regulator of the Wnt signaling pathway. We therefore propose that in the prolonged presence of Dex, heightened myocilin expression may over activate Wnt and the resultant increase in sFRP1 expression we also observed is suggestive of a regulatory mechanism against this uncontrolled Wnt signaling.

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Normal confluent human trabecular meshwork (HTM) cells

A peptide that disrupts fibronectin fibril assembly also affects collagen, fibrillin, and laminin extracellular matrix (ECM) assembly in human trabecular meshwork (HTM) cells

Purpose: To determine if disrupting fibronectin (FN) fibril assembly, which is a critical mediator of the ECM, affects the assembly of other matrix proteins in HTM cells.

Methods: FN fibril formation was disrupted using a recombinant FN-binding peptide, derived from the Streptococcus pyogenes F1 adhesin protein, called FUD (pU4R). A mutated, inactive FUD peptide was used as a control. HTM cells were treated ± FUD for 1-14 days depending upon the experiment. In some experiments, FN expression was induced with dexamethasone (DEX) during FUD treatment. Both newly confluent cells lacking a mature ECM and cell cultures possessing a preformed, mature matrix were treated with FUD. FN fibril formation was determined using a differential extraction of cells with deoxycholic acid in an On-cell western (OCW) assay. Assembly of FN, fibrillin (FBN), type IV collagen (Col IV) and laminin (LN) into matrices was assessed using immunofluorescence (IF) microscopy.

Results: HTM monolayers treated for 2 days with Alexa Fluor®-488-tagged FUD demonstrated a matrix of FN fibrils that co-localized with tagged FUD. By 4 days, however, FUD-treated cultures demonstrated a striking loss of FN fibrils. This was observed in both newly confluent cultures and cultures with a mature ECM. FUD also caused a reduction in FN fibrils in HTM cultures treated with DEX. OCW analysis showed that FUD decreased DEX-induced FN fibrils by 59% (100 nM DEX) and 77% (500 nM DEX). By IF microscopy, FUD also appeared to disrupt Col IV, FBN and LN matrices in newly confluent cultures. However in cultures with a mature ECM, FUD had no effect on these other matrices even though FUD effectively removed de novo and existing FN fibrils from these cultures.

Conclusions: FN plays an important role in mediating the assembly of the ECM within the TM. Disruption of pre-existing and de novo FN fibril assembly by FUD in vitro also affected the de novo assembly of other proteins like FBN, Col IV and LN into the TM ECM. This suggests that under in vivo conditions, FUD would disrupt FN fibrils and de novo assembly of other ECM proteins but not existing ECM protein matrices. These data also suggest that targeting FN fibril assembly may be a viable treatment for POAG and possibly other glaucomas.

Commercial Relationships: None

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Program Number: 3490 Poster Board Number: A0113
Presentation Time: 3:45 PM–5:30 PM

Controlling Fibronectin Fibrillogenesis May Also Control IOP

Jennifer Faralli1, Mark S. Filla1, Donna M. Peters1,2
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Purpose: An increase in the deposition of extracellular matrix (ECM) proteins is thought to be involved in the reduction of outflow facility and elevation in intraocular pressure (IOP) observed in primary open angle glaucoma. Since fibronectin (FN) often serves as a template for ECM formation, we examined if disruption of FN fibril formation can effect IOP.

Methods: Normal confluent human trabecular meshwork (HTM) cells were treated with 2ng/ml TGFβ2 for 2 days to induce an increase in ECM formation. Some cells were also incubated with a peptide derived from the functional upstream domain (FUD) of Streptococcus pyogenes adhesin F1 protein, which is a known inhibitor of FN fibrillogenesis or a mutated, inactive FUD. Cells were then extracted with a 1% deoxycholate (DOC) lysis buffer and FN fibrils were detected by immunofluorescence microscopy and/or an On-cell western (OCW) assay. To determine if FUD could affect IOP, a mouse model of ocular hypertension and a porcine organ cultured anterior segment (POCAS) model were used. In the POCAS model, cultured anterior segments (AS) were treated with TGFβ2 +/− FUD and outflow facility was measured for 24 hr. Some AS were paraffin embedded and sections were labeled for FN. In a mouse model of ocular hypertension, Balb/c mice were injected intravitreally with the AdSCMV-TGFβ2 (226/228) virus. Two weeks after injection, some mice were then injected intracamerally with FUD or a mutated FUD. IOP was measured weekly in mice anesthetized with a ketamine/xylazine mix using a rebound tonometer (TonoLab).

Results: As expected, TGFβ2 increased the deposition of FN into the matrix of HTM cells and FUD prevented this increase. In POCAS treated with TGFβ2 + FUD, 7 out of 9 POCAS had an increase in outflow facility compared to baseline. Immunofluorescence microscopy indicated that TGFβ2 + FUD treated AS had less FN labeling than TGFβ2 only treated AS. Expression of constitutively active TGFβ2 in mice caused a significant increase in IOP 2 weeks post injection. Two days after injection of FUD, however, the TGFβ2 induced IOP increase was significantly decreased. The decrease, however, was temporary and IOP returned to pre-FUD injection levels four days after FUD injection, presumably because the FUD was washed out. In contrast, injection of the mutated FUD had no effect on IOP.

Conclusions: These studies suggest that targeting the assembly of fibronectin fibrillogenesis may represent a way to control IOP.

Commercial Relationships: Jennifer Faralli, None; Mark S. Filla, None; Donna M. Peters, None

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Matricellular Protein Cysteine-Rich Angiogenic Inducer 61 (CCN1/CYR61) Attenuates Fibrogenic Response In Trabecular Meshwork Cells

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Purpose: Well-documented corroborations link actin cytoskeleton, extracellular matrix (ECM) proteins in trabecular outflow pathway...
and intraocular pressure (IOP). This study investigates the role of CCN1, a secreted matricellular protein, that binds to and signal via integrins, in the regulation of trabecular meshwork (TM) contractile properties and aqueous humor outflow homeostasis.

**Methods:** Using immunofluorescence and immunoblotting analyses, we assessed a) the cellular distribution, basal expression and secretion of CCN1 in primary human TM (HTM) cells and in culture media, b) the regulation of CCN1 by lysophosphatidic acid (LPA), dexamethasone, transforming growth factor-β2 (TGFβ2), and vascular endothelial growth factor (VEGF), c) the effects of recombinant human CCN1 (rhCCN1)-induced- and lentiviral shRNA mediated knockdown of CCN1 using shCCN1- on changes in actin cytoskeletal organization and fibrogenic markers including α-smooth muscle actin (α-SMA) and collagen Iα. All experiments described had sample sizes greater than three. Results before and after treatments were compared by paired Student’s t-test. Results were significant if p<0.05.

**Results:** Immunoblotting analysis confirmed CCN1 (40kDa) expression in HTM cells and its secretion in conditioned media. Cellular distribution of CCN1 assessed by immunofluorescence showed predominantly cytoplasmic localization. Serum-starved HTM cells when treated with TGFβ2 (10ng/ml) and VEGF (20ng/ml) significantly increased intracellular CCN1 expression (2.15 folds) within 5h whereas LPA (10μM) for 24h and dexamethasone (500nM) for 5 days did not. Further, treatment with rhCCN1 (20ng/ml) for 5h led to decreased actin stress fibers and expression of the fibroctic markers - α-SMA and collagen Iα, as visualized by immunofluorescence; and a 2.6 fold decrease in α-SMA compared to 0.1% BSA treated control HTM cells as detected by immunoblotting. Additionally, TGFβ2-mediated induction of α-SMA was significantly decreased (1.6 fold) by rhCCN1 treatment. On the other hand, knockdown of CCN1 using shCCN1 led to a significant increase in α-SMA levels (1.7 fold).

**Conclusions:** Taken together, it is evident that CCN1 decreases TM cytoskeletal integrity and plays an important role in regulating the fibrogenic responses in TM. This suggests that CCN1 can decrease outflow resistance making it a potential therapeutic target.

**Commercial Relationships:** Ishita Gupta, None; Padmanabhan P. Pattabiraman, None

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**Methods:** A lentivirus carrying the short hairpin RNA targeting human parvin-a (shparvin-a) was constructed using the pLKO.1 vector system. shparvin-a was then used to suppress parvin-a in primary cultures of human TM endothelial cells using an MOI of 10. Selected ECM proteins from cell lysates and conditioned media were analyzed by immunoblotting.

**Results:** Immunoblot analysis showed that treatment of lentishparvin-a suppressed the expression of parvin-a as compared to shcontrol in TM cells. Cells with suppressed parvin-a showed significantly attenuated levels of Fibronectin (n=5, p=0.0154), Collagen I (n=3, p=0.0240), Collagen VI (n=4, p=0.0135) and Laminin (n=4, p=0.0293). SPARC (n=3, p=0.4784), AP1 (n=2, p=0.9284) and Rac1 (n=2, p=0.6608) levels were unchanged by parvin-a when compared with shcontrol.

**Conclusions:** Suppression of parvin-a causes a decrease in ECM proteins such as Collagen IV, Collagen I, Collagen VI, Fibronectin and Laminin, suggesting that parvin-a may play a regulatory role in the downstream signaling of SPARC-ILK.

**Commercial Relationships:** Alexandra G. Castillejos, None; Min H. Kang, None; Douglas J. Rhee, None

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**Program Number:** 3494 **Poster Board Number:** A0117

**Presentation Time:** 3:45 PM–5:30 PM

**Tissue transglutaminase mediated ocular hypertension and effects of a small molecule crosslinking modulator**

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**Purpose:** Transforming Growth Factor-β2 (TGF-β2) induces expression of the crosslinking enzyme tissue transglutaminase (TGM2) in human trabecular meshwork (TM) cells. We studied (1) whether TGM2 overexpression in the TM can increase intraocular pressure (IOP) and decrease aqueous humor (AH) outflow facility (C) in mice and (2) whether a small molecule TGM2 inhibitor can decrease ECM crosslinking in vitro.

**Methods:** 2μl of the expression vector Ad5.CMV.TGM2 (1-50 × 10⁶ pfu) was injected intravitreally (OS) in BALBc/J (n=18) or C57BL/6J mice (n=9) while contralateral eyes served as un.injected controls. Daytime conscious IOPs were measured (Tonolab) twice/week. C was measured following IOP elevation in BALBc/J (n=6) and C57BL/6J (n=3) mice. In vitro, primary human glaucoma TM cells (GTM 125, GTM 60 A and GTM 46) were treated with a small molecule TGM2 inhibitor (5nM).

**Results:** Ad5.CMV.TGM2 injection significantly elevated IOP, where BALBc/J showed maximum IOP at Day 19, [15.86 mmHg (injected)] vs. 10.7 mmHg (control), p = 0.0001, ANOVA] and C57BL/6J showed maximum IOP at Day 17 [(17.09 mmHg (injected)) vs. 12.01 mmHg (control), p<0.05, ANOVA]. In BALBc/J, C in transduced eyes (0.013 μl/min/mmHg) was significantly lower than un.injected eyes (0.021 μl/min/mmHg), p = 0.01, paired Student’s t-test. In C57BL/6J, C in injected eyes (0.012 μl/min/mmHg) was significantly lower than un injected eyes (0.019 μl/min/mmHg), p = 0.046, paired Student’s t-test. In GTM cells, primary GTM cells showed high endogenous ECM crosslinking, and treatment with a TGM2 inhibitor (5nM) reduced fibronectin expression and crosslinking but did not affect TGM2 expression as seen with immunocytochemistry and western immunoblotting.

**Conclusions:** TGM2 overexpression in the mouse TM significantly elevates IOP and decreases the AH outflow facility. A TGM2 inhibitor decreased crosslinking in TM primary cells. In future, we will study the role of TGM2 and the TGM2 small molecule inhibitor in TGFβ2 induced ocular hypertension.

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Gene

EY011906; EY026220

Biomedical Engineering, Georgia Institute, None

NIH Grants EY018590, EY025096 and P30-EY-005722

adMSCs (Lonza) were labeled with 20nm diameter


Purpose: Cellular contractility and adhesion, mechanotransduction and extracellular matrix (ECM) organization of the trabecular meshwork are all presumed to interdependently influence aqueous humor outflow and intraocular pressure. This study uses mechanistic approaches to explore whether ECM protein phosphorylation plays a role in modulating these events

Methods: Experimental studies have been designed using human TM cell primary cultures to quantitatively assess the effects of vertebrate lonesome kinase (VLK), a secretory tyrosine kinase on ECM protein phosphorylation, the influence of ECM protein phosphorylation on TM cell focal adhesion kinase (FAK) activity, focal adhesions and actin stress fibers, and the effects of pharmacological inhibition of FAK in TM cells using siRNA, pharmacological agents, immunoblot and immunofluorescence analyses.

Results: siRNA-mediated suppression of VLK expression in human TM cells led to a dramatic decrease in ECM protein tyrosine phosphorylation, and was associated with a significant decrease in actin stress fibers and focal adhesions. Importantly, the effects of ECM protein phosphorylation on TM cell focal adhesions and actin stress fibers were found to be regulated partly through activation of FAK activity. Moreover, pharmacological inhibition of FAK in the TM cells triggered cellular changes that were identical to those caused by suppression of VLK-mediated ECM phosphorylation

Conclusions: Taken together, these observations reveal novel and close mechanistic and functional interactions between VLK-regulated ECM protein tyrosine phosphorylation and FAK activation, and a collective influence of these two events on the regulation of TM cell adhesive activity, mechanotransduction and contractile response, all of which are expected to impact aqueous humor outflow and intraocular pressure. These results also support the conclusion that VLK may prove to be a potentially useful therapeutic target for lowering of intraocular pressure in glaucoma patients.

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Program Number: 3497 Poster Board Number: A0119

Presentation Time: 3:45 PM–5:30 PM

Different transduction efficiencies between single- and double-stranded Adeno-associated viruses (ssAAV & dsAAV) in the human trabecular meshwork extend across seven serotypesLaura Rodriguez Estevez, Priyadarshini Asokan, Terete Borras. 1. Ophthalmology, Univ of NC at Chapel Hill, Chapel Hill, NC; 2. Gene Therapy Center, Univ of NC at Chapel Hill, Chapel Hill, NC.

Purpose: Our long term goal is to increase transgene transduction in the TM to lower dose and toxicity in gene therapy. Previously, we demonstrated that ssAAV2 viral transduction in the human TM was very inefficient while that of the ds form (scAAV2) was very high. Our goal here was to investigate whether this ss/ds characteristic extended to serotypes 1, 2, 5, 6, 8 & 9, to determine the rank of their efficiency, and to explore whether a capsid mutated scAAV2 virus could significantly enhance gene transduction in the HTM cells.

Methods: A primary HTM cell line from a single individual was used at passage 3-5. AAV- & scAAV.GFP high titer stocks were made at the UNC vector core. A tyrosine mutated AAV2 capsid (Y444F) was generated by synthesizing a 767bp DNA fragment containing a single nucleotide change in codon TAC (Y) to TTC (F), and swapping it for the corresponding wild-type in the pXr2 plasmid (BsiWI-XcmI sites). Viral infections were performed at moi 100-1000 pfu on subconfluent cells. Quantification of intracellular viral DNA (24h) and RNA (72h) was conducted on total cell DNA and cDNA by TaqMan PCR using a GFP probe. Single-copy RPPH1 and 18S probes were used for respective normalizations. Quantification of fluorescence (transduction) was conducted on images captured on a monochrome camera (DP70) at the same exposure and threshold using the Metamorph software (Olympus).

Results: In all serotypes, there was a high difference in transduction (cDNA fold-changes and fluorescence intensity units) between each scAAV and its corresponding AA. In contrast, the number of intracellular AAV viral genomes was higher than the scAAV in serotypes 2.5, 1, 5 & 2 (11-6 fold), and just slightly lower in serotypes 9, 6 & 8 (0.31-0.65 fold). The highest transduction efficiency of the scAAV serotypes ranked 2, 6 & 5 followed by a distant, negligible transduction of 9, 8, 1 & 2.5. The capsid mutated scAAV2.Y444F virus showed a large transduction enhancement compared to scAAV2 (14.2-fold).

Conclusions: In the human TM, all seven serotypes showed a large transduction difference between the AAV’s ds and ss forms. The serotype 2 capsid mutation Y444F conferred a marked transduction enhancement to the human TM. Determining the optimal conditions for the human TM transduction is key for the development of gene therapy of glaucoma.

Commercial Relationships: Laura Rodriguez Estevez, None; Priyadarshini Asokan, None; Terete Borras, None

Support: EY011906; EY026220

Program Number: 3497 Poster Board Number: A0120

Presentation Time: 3:45 PM–5:30 PM

Visualizing stem cell delivery to trabecular meshwork using ultrasound/photoacoustic imaging with nanoparticles

C. R. Etherie, Eric Snider, Kelsey Kubelick, Heechul Yoon, Stanislav Emelianov. 1. Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA; 2. Biomedical Engineering, Emory University, Atlanta, GA.

Purpose: To reduce trabecular meshwork (TM) cellularity in glaucoma, stem cells offer potential therapeutic benefits if delivery and engraftment into TM occurs. Here, we utilize ultrasound and photoacoustic (US/PA) imaging to track nanoparticle-tagged adipose-derived mesenchymal stem cells (adMSCs) during delivery into the anterior segment and to the TM.

Methods: adMSCs (Lonza) were labeled with 20nm diameter gold nanospheres (AuNS) and fluorescently tagged (CFSE) for histology. Labeled adMSCs were injected into the anterior chamber of whole enucleated porcine eyes or into an anterior segment porcine organ culture model while pressure was hydrostatically clamped at physiologic levels. Cell injections and delivery to the TM were monitored in real-time using US/PA imaging (Vevo 2100/LAZR). Whole eyes and organ-cultured anterior segments were fixed 4 hours and 1 week (respectively) after adMSC injection for more detailed US/PA imaging and delivery confirmation by en face TM fluorescent micrographs.

Results: US/PA imaging visualized key anatomical landmarks due to PA signal from pigmented tissue, e.g. iris and TM (Fig 1A).
Following labeled-adMSC injection, cells were evident in the anterior segment (Fig 1B) and their movement could be tracked over time. The unique spectral signature of AuNS allowed unmixing of AuNS signal from that of pigmented tissues; after injection, cells were evident in the TM, iris and lens regions (Fig 2A-D). TM delivery of adMSCs was evident 4 hrs (Fig 2E) and 1 week post-injection (Fig 2F) from fluorescent micrographs.

**Conclusions:** US/PA imaging can track adMSC injection and subsequent delivery to the TM. Next steps will optimize adMSC injection protocol and labeling to increase TM engraftment and generate greater PA signal. Further, longitudinal US/PA imaging will confirm engraftment can be tracked for multiple weeks.

Fig 1: (A) representative US/PA image of porcine anterior globe with relevant anatomical landmarks identified, (B) US/PA image immediately following adMSC injection into anterior chamber.

Fig 2: PA images taken before (A, B) and 4 hours after (C, D) adMSC injection into porcine eyes. Signals from AuNS (A, C) and background (B, D) were spectrally unmixed. adMSC delivery was confirmed at 4 hours (E) and at 1 week (F) by fluorescence microscopy. Panels A-D are from the same eye but image plane in C, D is slightly different to A, B.

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attractants on the bottom. Percentage of cell number migrated down through the insert membrane was compared in different conditions. Chemokine expression of TMSCs and TM cells were detected by qPCR. Mouse chemokine expression on mouse TM tissue with and without laser photocoagulation was determined by qPCR. TMSCs were intracamerally injected into partially laser-treated mice and TMSC distribution was detected by wholemount staining.

**Results:** Human TMSCs have higher affinity to the TM cells than TM cells and fibroblasts to the TM cells. TM cells with increased expression of SDF1 are the highest attractant to TMSCs. TMSCs with reduced expression of CXCR4 have less affinity to TM cells. After laser treatment, the mouse TM cells had increased expression of CXCR4 and SDF1. Injected TMSCs specifically homed to laser damaged mouse TM region and reduced intraocular pressure (IOP).

**Conclusions:** TMSCs have higher expression of CXCR4 while TM cells have higher expression of SDF1. Chemokines CXCR4-SDF1 play important roles in TM stem cell homing for TM regeneration and IOP regulation.

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