231 Nanotechnology and tissue engineering
Monday, May 04, 2015 11:00 AM–12:45 PM
1EF Mile High Blrm Paper Session
Program #/Board # Range: 1673–1678
Organizing Section: Nanotechnology and Regenerative Medicine Group

Program Number: 1673
Presentation Time: 11:00 AM–11:15 AM
3D Natural Biopolymer Scaffold for *In Vitro* Modeling of the Trabecular Meshwork
Sarah Bernier[1], Melissa Krebs[1], Mina B. Pantcheva[2]. [1]Chemical and Biological Engineering, Colorado School of Mines, Golden, CO; [2]Ophthalmology, University of Colorado Denver, Aurora, CO.
Purpose: To develop a 3D scaffold using natural biopolymers that mimics the trabecular meshwork (TM) structure to help improve our understanding of TM cell biology and the intraocular pressure system and aid in the screening and development of pharmacological and biological agents targeting the trabecular outflow facility.
Methods: Using a unidirectional freezing and lyophilization process, we engineered anisotropic porous scaffolds of two polymers naturally present in the TM extracellular matrix. This technique allowed us to develop collagen-glycosaminoglycan (GAG) scaffolds with uniaxially aligned pores. The scaffolds were characterized with scanning electron microscopy (SEM) and dynamic mechanical analysis. We assessed the ability of our scaffolds to support primary porcine TM (pTM) cells' viability, proliferation, and migration using fluorescent imaging, a cell proliferation assay, and histology.
Results: Fabricated scaffolds displayed anisotropic, unidirectional pore alignment when imaged with SEM (Fig.1). The average pore diameter was 13.69±5.2 μm, and the average pore density was 2028 pores/mm^2. The storage modulus of hydrated scaffolds was 38.23±6.1 kPa. Total GAG content of scaffolds was measured with a quantification assay showing that 66% of the GAG was retained after fabrication and sterilization. pTM cells seeded on the surface of scaffolds and cultured for 2 weeks showed a 60% increase in metabolic activity over the time period. Fluorescent viability stains were used to confirm the presence of live cells in the scaffolds up to two weeks after initial seeding (Fig.2A). Histological sections stained with H&E revealed cell attachment and migration into the interior of the scaffolds (Fig.2B).
Conclusions: Our results confirm that pTM cells grown on collagen-GAG scaffolds demonstrate high viability and are capable of migrating into the scaffold structure. Further development will allow this cell culture system to be used to study the physiology and pathology of glaucoma and to aid in the screening and development of new therapeutic agents.

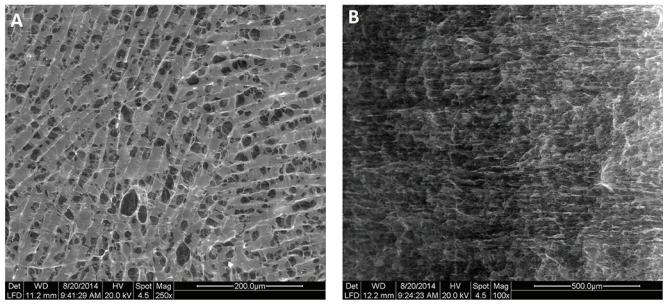

Fig. 1 SEM images of a collagen-GAG scaffold **A.** Top surface **B.** Cross section (top of the scaffold at the right of the image)

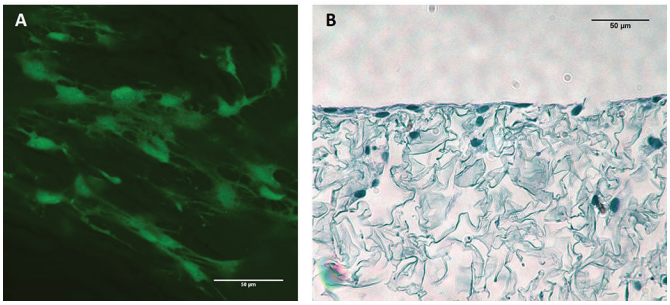

Fig. 2 Images of pTM cells on collagen-GAG scaffolds at 14 days **A.** Confocal microscopy of pTM cells labeled with a live/dead fluorescent dye **B.** Light microscopy image of an H&E histological section
Commercial Relationships: Sarah Bernier, None; **Melissa Krebs**, None; **Mina B. Pantcheva**, None

Program Number: 1674
Presentation Time: 11:15 AM–11:30 AM
An ultra-thin amniotic membrane as carrier in corneal epithelium tissue-engineering
Wei Li[1, 2], Liying Zhang[1, 2], Dulei Zou[1, 2], Yangluowa Qu[1], Sanming Li[1], Tingting Liu[1], Juan Li[1, 2], Zuguo Liu[1, 2]. [1]Eye Institute of Xiamen University, Fujian Provincial Key Laboratory of Ophthalmology & Visual Science, Xiamen, China; [2]Xiamen University affiliated Xiamen Eye Center, Xiamen, China.
Purpose: Amniotic membrane (AM) is widely used as a carrier for limbal epithelial stem cell ex vivo expansion. However, the thick stroma of AM affects the postoperative corneal transparency. In this study, we developed an ultra-thin AM (UAM) and evaluated its biomaterial characteristics and efficacy in corneal epithelium tissue-engineering.
Methods: Cryo-preserved AM was treated with 0.02% EDTA, after that AM epithelium was removed by scraper. Stromal side of the denuded AM (DAM) was then digested with type IV collagenase at 37 for different durations to generate UAM. The characteristics including optical transmittance, thickness, decellularization and microstructure were evaluated. Intact AM (IAM) and DAM were served as control. The components of UAM were analyzed by immunostraining of collagen I, III, IV, VII, perlecan and Laminin 5. Rabbit limbal epithelial cells were cultured on UAM and DAM, cell proliferation and differentiation markers such as p63, Ki67, K3, K12, K14 were detected by immunofluorescent staining or Western blot. The engineered corneal epithelium was transplanted to the ocular surface of total limbal stem cells deficiency (LSCD) rabbit model, and the cornea was observed by slit-lamp microscope postoperatively.
Results: Compared with IAM and DAM, UAM showed favorable optical transmittance in both moist and dry form, ultra-thin, accellular, compact and regularly collagen fibrils arrangement. The rabbit corneal limbal epithelial cells seeded on UAM formed a multilayered epithelial structure and showed better cell polarity than those on the DAM. K14 and P63 were highly expressed in UAM based tissue-engineered corneal epithelium than those in DAM at protein level. Postoperatively, the UAM based tissue-engineered corneal epithelium showed higher transparence in rabbits with total LSCD. Histology of cornea after transplantation showed better stratification of UAM based grafts compared with DAM based grafts.
Conclusions: UAM preserves compact layer of the amniotic membrane and maybe an ideal carrier for corneal epithelium tissue-engineering.

Commercial Relationships: Wei Li, None; **Liying Zhang**, None; **Dulei Zou**, None; **Yangluowa Qu**, None; **Sanming Li**, None; **Tingting Liu**, None; **Juan Li**, None; **Zuguo Liu**, None

Program Number: 1675
Presentation Time: 11:30 AM–11:45 AM
Tissue Engineered Model of the Inner Neural Retina
Karl E. Kador[1], Praseeda Venugopalan[1, 2], Monisha Malek[1], Jeffrey L. Goldberg[1]. [1]Shiley Eye Center, UC San Diego, San Diego, CA; [2]Neuroscience Graduate Program, University of Miami, Miami, FL.
Purpose: The lack of regeneration found in the adult mammalian retina following optic nerve injury has led to the study of cell and tissue engineered cell delivery methods. However when designing these delivery devices, it is necessary to orient axon growth and cellular organization to mimic that of the nerve fiber layer as well as stimulate dendrite growth and synapse formation with the cell binding partners in the host retina.
Methods: RGC layer scaffolds were produced by electrospinning medical grade PLA using a radial collector which orients fiber formation from the outer edge to a central point. Fibrous scaffolds were reacted using a gradient filter and UV initiated photo crosslinker to immobilize Netrin-1, polarizing RGC growth. RGC-seeded scaffolds were then cultured in a 3D model with amacrine cells or transplanted to explanted rat retinas. Following co-culture and transplantation models, samples were fixed and evaluated for synapse formation by immunostaining as a measure of integration.
Results: RGCs seeded on radial scaffolds mimicked the organization of the mammalian retina with 81% of axons orienting radially. When scaffolds were immobilized with a radial-in netrin gradient, the seeded cells were further polarized to >50% growing axons towards the scaffold center. RGCs formed synaptic connections to amacrine cells in in vitro models and to explanted rat retinas.
Conclusions: Using this scaffold in both a 3D model and ex vivo transplantation systems we have observed the formation of synapses by immunostaining, a step towards the goal of RGC transplantation.
Commercial Relationships: Karl E. Kador, None; **Praseeda Venugopalan**, None; **Monisha Malek**, None; **Jeffrey L. Goldberg**, None
Support: NEI P30-EY022589, NEI RC1-EY020297

Program Number: 1676
Presentation Time: 11:45 AM–12:00 PM
Imaging Novel Ruthenium bipyridine-based Nanophotoswitches in Retina
Lan Yue[1], Steven Walston[2], Mingyi S. Lin[3], Melanie Pribisko[4], Dennis Doughurty[4], Robert Grubbs[4], Harry Gray[4], Robert Chow[3], Mark S. Humayun[1]. [1]Ophthalmology, USC Eye Institute, University of Southern California, Los Angeles, CA; [2]Biomedical Engineering, University of Southern California, Los Angeles, CA; [3]Zilkha Neurogenetic Institute, University of Southern California, Los Angeles, CA; [4]Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA.
Purpose: Nanophotoswitches (NPSs) offer a new tool for optical stimulation of neuronal activity, in vitro and also potentially in vivo. Our group previously reported a ruthenium bipyridine (Rubpy)-based NPS that inserts into the plasma membrane and upon visible illumination generates an electrical dipole, triggering action potentials in adrenal chromaffin cells. We have recently demonstrated that after intravitreal injection of this NPS into the eyes of blind rats, illumination of the eye elicited electrical activity in the contralateral superior colliculus. To better understand the site of action of the NPS in retina, we examined the distribution of the molecules in different retinal layers after intravitreal injection.
Methods: Rubpy molecules can be visualized by their luminescence (610 nm) upon visible wavelength illumination (460 nm). To resolve the luminescence from different retinal layers, a rapid-scan two-photon imaging system (LaVison) was used (Ti:Sapphire laser tuned to 900 nm). Intravitreal injection (1 mM, 4 μL Rubpy-based NPSs in BSS), followed by eye removal and retina isolation 2-5 hrs after, was performed on young RCS rats. Luminescence images of the wholemount retina were captured by an EM-CCD camera (Andor).
Results: At 2 hrs after intravitreal injection and with continuous superfusion of Ames medium, luminescence was confined near the injection site. Luminescence was observed localized to surface membranes of axons and somata of retinal ganglion cells (RGC), demonstrating the impermeability of the cell membrane to the NPS molecules. The outer retina did not show significant luminescence. After 3 additional hours, luminescence was more diffused within the RGC layer and still did not extend to the outer retina.
Conclusions: This study shows marked staining of RGC layer by intravitreally injected Rubpy-based NPS molecules, consistent with the hypothesis that the photoactivated NPS molecules induce electrical activity in the superior colliculus by acting on the RGCs that deliver electrical signals to the visual pathway outside the eyes. Distinct from other nano-scale optical cellular modulating approaches using optogenetics or azobenzene-based photoswitches, the NPS approach obviates the need for gene manipulation or toxic UV illumination, highlighting its potential in generating high-acuity prosthetic vision in patients blinded by retinal degenerative diseases.
Commercial Relationships: Lan Yue, None; **Steven Walston**, None; **Mingyi S. Lin**, None; **Melanie Pribisko**, None; **Dennis Doughurty**, None; **Robert Grubbs**, None; **Harry Gray**, None; **Robert Chow**, None; **Mark S. Humayun**, None
Support: NSF CBET-1404089; Research to Prevent Blindness; USC Eye Institute; Institute of Biomedical Therapeutics

Program Number: 1677
Presentation Time: 12:00 PM–12:15 PM
Regeneration of retinal neurons and glia by Lgr5^{+} amacrine cells in adult mice
Hongjun Liu[1], Shenghuo Tian[1], Nathan Glasgow[2], Gregory Gibson[3], Xiaoling Yang[1], Christen Shiber[2], James L. Funderburgh[1], Simon Watkins[3], Jon Johnson[2]. [1]Department of Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, PA; [2]Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA; [3]Center for Biologic Imaging, University of Pittsburgh, Pittsburgh, PA.
Purpose: Retinal cells that possess the capacity to regenerate lost neurons hold great therapeutic potential for vision restoration in people who suffer from blindness associated with retinal degeneration. The purpose of this study is to identify adult mammalian retinal cells that have regenerative capacity.
Methods: To identify these cells, we adapted a genetic strategy. We first examined the expression of the adult stem cell marker, leucine rich repeat containing G-protein coupled receptor 5 (Lgr5), in the retina of the *Lgr5$^{EGFP\text{-}Ires\text{-}CreERT2}$* mice, and determined the identity of Lg5^{+} cells with established retinal cell markers. We then labeled Lgr5^{+} cells with the *Rosa26-LacZ* reporter in the *Lgr5$^{EGFP\text{-}Ires\text{-}CreERT2}$; Rosa26-LacZ* mice, and further determined if new retinal cells could be generated from labeled cell at later times.
Results: We observed that Lgr5 was expressed in the inner nuclear layer of the retina and marked a population of amacrine cells in adult mice. Nevertheless, *LacZ*$^{+}$ retinal cells could be detected at later times in new locations, including the ganglion cell layer under physiological condition and the photoreceptor layer in response to injury.

Conclusions: These findings suggest that Lgr5^{+} amacrine cells may function as an endogenous regenerative source and contribute to retinal homeostatic maintenance. The identification of such cells in the mammalian retina may provide new insights into neuronal regeneration and points to therapeutic opportunities for debilitating retinal diseases.
Commercial Relationships: Hongjun Liu, None; **Shenghuo Tian**, None; **Nathan Glasgow**, None; **Gregory Gibson**, None; **Xiaoling Yang**, None; **Christen Shiber**, None; **James L. Funderburgh**, None; **Simon Watkins**, None; **Jon Johnson**, None

Program Number: 1678
Presentation Time: 12:15 PM–12:30 PM
Nanoceria are not toxic at maximum dosage in murine retina
Xue Cai[1], Sudipta Seal[2], James F. McGinnis[1, 3]. [1]Ophthalmology, Univ of Oklahoma Health Sciences Center, Oklahoma City, OK; [2]Advanced Materials Processing Analysis Center, Mechanical Materials Aerospace Eng., Nanosci. and Tech. Ctr., University of Central Florida, Orlando, FL; [3]Cell Biology & Oklahoma Center for Neuroscience, University of Oklahoma Health Sciences Center, Oklahoma City, OK.
Purpose: We have previously shown that cerium oxide nanoparticles (nanoceria) catalytically scavenge Reactive Oxygen Species (ROS) and can long-term inhibit retinal degeneration in *tubby* mice and inhibit/regress neovascularization in *vldlr*$^{-/-}$ mice. However, the toxicity of nanoceria retention in the retina was unknown. The current study is proposed to address this point.
Methods: Wild type (C57BL/6J) mice at P30 were intravitreally injected with increasing doses of nanoceria (0.1mM, 0.3mM, 1mM, 3mM and 10mM) in 1μl saline. Saline injection and uninjected mice served as controls. Retinal structure, ONL (outer nuclear layer) thickness and cellular infiltration were analyzed using H & E stained slides at post injection (PI) 7h, 3d, 7d, 15d and 30d. Retinal function was evaluated with electroretinography at PI 30d. The fundus abnormality and angiogenesis were assessed with fundoscopy and fluorescence angiography. The expression of inflammatory cytokines and markers of activation of microglia/macrophages were analyzed by qRT-PCR or western blots at PI 7h.
Results: Intravitreal injection of all concentrations of nanoceria did not cause changes in retinal morphology and ONL thickness compared to untreated and saline injected groups indicating that no photoreceptor death was induced. No cellular infiltration was seen in the histological slides. Decrease of retinal function was not found. There is no elevation in the expression of TNFα, IL-1β, MIF and GFAP.
Conclusions: High concentrations of nanoceria do not cause any damage to retinal structure and function. No cellular infiltration and no increases in inflammation responses were found in the eyes. Our data indicate that nanoceria are safe to use for treatment of a variety of eye diseases.
Commercial Relationships: Xue Cai, None; **Sudipta Seal**, OUHSC/UCF (P); **James F. McGinnis**, OUHSC/UCF (P)
Support: NIH: P30-EY12190, COBRE-P20 RR017703, R21EY018306, R01EY018724. FFB C-NP-0707-0404-UOK08. NSF: CBET-0708172. Funds from PHF and RPB. Thanks also go to the NEI/DMEI Image and Animal Core facilities.

280 Stem cells, nanotechnology and tissue engineering
Monday, May 04, 2015 3:45 PM–5:30 PM
Exhibit Hall Poster Session
Program #/Board # Range: 2253–2269/B0158–B0174
Organizing Section: Nanotechnology and Regenerative Medicine Group

Program Number: 2253 **Poster Board Number:** B0158
Presentation Time: 3:45 PM–5:30 PM
Characterization of the early cellular response in a regenerating zebrafish retina
Rose M. DiCicco, Brent A. Bell, Joe G. Hollyfield, Bela Anand-Apte, Brian D. Perkins, Alex Yuan. Ophthalmic Research, Cleveland Clinic-Cole Eye Inst, Cleveland, OH.
Purpose: The zebrafish retina regenerates following injury. Previous studies showed Müller cells become retinal progenitors to repopulate the damaged areas. However, little is known about the role of microglia during regeneration. We hypothesize microglia are the earliest responders to injury. To test this hypothesis we characterized the early cellular response following targeted injury to photoreceptors in the adult zebrafish retina.
Methods: Focal lesions, localized to the photoreceptor layer, were created in fish retina using our optical coherence tomography (OCT)-guided laser injury model (DiCicco et al., IOVS 2014). Each laser-injured retina was imaged with both OCT and confocal scanning laser ophthalmoscopy (cSLO) at intervals starting at 0 days post lesion (dpl) and continuing through 6 weeks post lesion (wpl) to monitor the progression of the lesions *in vivo*. At each interval, fish were euthanized and the eyes cryosectioned. Immunohistochemistry was used to identify microglia (4c4), apoptotic cells (TUNEL), and proliferating cells (PCNA) within the injury site and surrounding tissue.
Results: Following laser injury, there was a rapid (1 dpl) migration of microglia to the outer nuclear layer (ONL) and outer segment zone (OSZ) from their baseline locations. There was also focal microglia migration to the inner plexiform layer (IPL) adjacent to the injury site. Before injury the microglia were found mainly in the outer plexiform layer (OPL) and inner retina. Apoptosis followed the migration of microglia and was predominantly found in the ONL and OSZ with some cells in the INL. Apoptosis increased through 3 dpl then declined. Proliferating cells were located mainly in the INL and ONL, and increased through 3 dpl then declined. A few proliferation cells were also found in the IPL and GCL.
Conclusions: Laser injury to the zebrafish retina produces a rapid migration of microglia to the lesion site. This response from the microglia occurs prior to the increase in cellular proliferation and apoptosis, and thus supports our hypothesis that microglia are the earliest responders to injury in zebrafish. Cellular proliferation and apoptosis begin to increase after the appearance of the microglia within the injury zone.
Commercial Relationships: Rose M. DiCicco, None; **Brent A. Bell**, None; **Joe G. Hollyfield**, None; **Bela Anand-Apte**, None; **Brian D. Perkins**, None; **Alex Yuan**, None
Support: NEI 1K08EY023608-02, RPB unrestricted grant, FFB center grant

Program Number: 2254 **Poster Board Number:** B0159
Presentation Time: 3:45 PM–5:30 PM
Controlling retinal cell fate using nanotopography and neurotrophic factors
Sebastian Johansson[1], Marina Zalis[1], Fredrik Johansson[2], Ulrica Englund Johansson[1]. [1]Ophthalmology, Clinical sciences in Lund, Lund, Sweden; [2]Functional Zoology, Department of Biology, Lund, Sweden.
Purpose: Bioscaffolds, supporting survival and guiding axonal growth, holds great promise for the advancement of cell-based therapies for retinal neurodegenerative diseases. Increased knowledge is required on the effect of nanotopographies, extracellular matrix (ECM) proteins and neurotrophic factors on retinal cell survival, cell fate and axonal guidance. Hence, we here investigated the influence of nanotopography, laminin (ECM protein) and neurotrophic factors on retinal ganglion cells (RGC), photoreceptors (PR) and glial cells.
Methods: Post-natal day 4 mouse retinas were isolated and dissociated into single cells and cultured for 7 days *in vitro*, at either Poly-L-Lysine-coated chamber slides or electrospun polycaprolactone (PCL) fiber substrates with random or aligned orientation. Non-coated and laminin-coated substrates were used. Either basic (DMEM-F12, B27 supplement) or the enriched Full-SATO (Neurobasal, CNTF, BDNF, Forskolin, Insulin) medium was used. Analysis parameters: survival, quantification and morphology assessment of RGC (RBPMS+, NeuN+, β-tubulin III+)-, PR (rhodopsin+) and glial (GFAP+) cells, and neurite length and orientation.
Results: Nanotopography *per se* significantly affected the formation of retinal cells morphologies, exemplified by larger fractions of multipolar cells found on flat surfaces and random fibers and uni- and bipolar cells on aligned fibers. Addition of laminin and use of Full-SATO medium in all three substrates promoted both RGC and PR maturation, demonstrated by complex neuronal morphologies and extended neurite outgrowth. Notably, laminin induced cell neurites to grow along the fibers and not perpendicular to their orientation, as occurred on non coated fibers. Preliminary results also suggest increased numbers of RGC, PR and glial cells in cultures with laminin and Full-SATO medium.
Conclusions: With a future cell-based retinal therapy in mind, we here provide further in-depth knowledge on control of retinal cells fate using electrospun PCL fibers, ECM-guiding proteins and a supportive enriched culture media. Controlled neural development and axonal guidance was improved by using laminin-coated aligned fibers and Full-SATO medium.
Commercial Relationships: Sebastian Johansson, None; **Marina Zalis**, None; **Fredrik Johansson**, None; **Ulrica Englund Johansson**, None

Program Number: 2255 **Poster Board Number:** B0160
Presentation Time: 3:45 PM–5:30 PM
The combination of neurotrophic factors and laminin substantially promote retinal ganglion cell axon outgrowth after dissociation
Marina Zalis, Sebastian Johansson, Ulrica Englund Johansson. Ophthalmology, Lund University, Lund, Sweden.
Purpose: Laminin, present in the optic nerve, promotes regrowth of injured retinal ganglion cell (RGC) axons in lower species. However, a permissive substrate alone is not enough to promote axonal regeneration in adult mammals. Hence, we wanted to explore the regenerative potential of dissociated retinal cells on a laminin functionalized substrate and a RGC-specific enriched medium as a first step towards cell therapy in retinal degenerative disease.
Methods: Isolated post-natal day 4 mouse retinas were dissociated to single cell suspension. Cells were cultured on 4-well-chamber slides coated with Poly-L-Lysine (PLL) or PLL + Laminin for 7 days in vitro (DIV). Either basic neuronal medium (DMEM-F12, 2% B27 supplement) or the enriched so called Full-SATO (Neurobasal, CNTF, BDNF, Forskolin, Insulin) medium was used. Immunohistochemistry using cell specific markers were used to identify RGCs (*i.e.* NeuN, β-Tubulin III and RBPMS) and glial cells (GFAP).
Results: Repeated studies comparing the effect of basic neuronal medium and Full-SATO medium on the overall survival of retinal cells cultured on PLL coated slides, revealed no obvious difference up to 7 DIV. Cells cultured with basic neuronal medium on PLL coated slides mainly appeared as single cells, with round cell bodies and short processes, except for a modest GFAP+ cell population that displayed a polygonal morphology. In contrast, Full-SATO medium induced both a significant increase in numbers of GFAP+ and RBPMS+ cells, and more complex cellular profiles. Addition of laminin further increased numbers of GFAP+ and RBPMS+ cells using Full-SATO medium, compared to PLL counterparts. These cells formed clusters, displayed extensive neurite outgrowth (NeuN+, β-Tubulin III+, even GFAP+) and highly complex cell morphologies. In all culture conditions, cells co-expressing GFAP and RBPMS were found, suggesting a progenitor state.
Conclusions: We have shown that the trauma of axotomy due to dissociation can be overcome in vitro using a laminin coated substrate, and an enriched RGC-specific medium, which resulted in substantial axonal outgrowth. Our finding is an important step for understanding the environmental conditions needed in successful cell-based restorative therapies for retinal degenerations.
Commercial Relationships: Marina Zalis, None; **Sebastian Johansson**, None; **Ulrica Englund Johansson**, None

Program Number: 2256 **Poster Board Number:** B0161
Presentation Time: 3:45 PM–5:30 PM
Characterization of Mesenchymal Stem Cells vs. Trabecular Meshwork Cells
Eric Snider[1, 2], Christopher Pride[1], Akash Patil[1], W D. Stamer[3], C R. Ethier[1, 2]. [1]Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA; [2]Biomedical Engineering, Emory University, Atlanta, GA; [3]Biomedical Engineering, Duke University, Durham, NC.
Purpose: Due to reduced trabecular meshwork (TM) cellularity in glaucoma, meshwork repair with suitably differentiated stem cells has potential. This requires characterization methods to detect differences between TM and stem cells. The gold standard for identifying TM cells is dexamethasone (dex) induction of myocilin (MYOC); however, to assess differentiation, additional assessments of function and genetic profile are desirable. Here we evaluate multiple techniques to assess differences between mesenchymal stem cells (MSCs) and TM cells.
Methods: Human adipose-derived MSCs (Lonza; n=3 strains) and primary human TM cells (Stamer lab; n = 4 strains) were used. Three approaches assessed MSC and TM differences. First, qRT-PCR was performed on RNA isolated from cell lysates. Second, induction of myocilin expression after 500 nM Dex treatment for 1 week was assessed using western blots. Finally, cell contractility was determined by measuring area vs. time in free-floating cell laden collagen I gels.
Results: Differentially expressed mRNAs between MSCs and TM cells were observed (subset shown in Figure 1 for 4 different TM strains where 2 strains with more juxtacanalicular [JCT] cells are noted), with *MGP*, *MYOC* and *Plas-Act* message levels higher in most TM strains. In addition, JCT/TM strains differentially expressed lower *CHI3l1* levels compared to TM-only strains. Dex led to c.

20-fold higher MYOC protein expression in TM cells vs. MSCs. TM cells were found to be less contractile than MSCs over 7 – 10 day time scales.
Conclusions: qRT-PCR identified a number of markers upregulated in TM cells vs. MSCs. TM cell and MSC strains were found to be heterogeneous in their expression profile, and subsequent studies should use multiple cell strains to account for cell variability. Dex induction of MYOC was confirmed as a robust but not rapid characterization approach. Differences were also noted in contractile properties of the cell types, with MSCs more contractile than TM cells. Next steps will expose MSCs to relevant biophysical stimuli to establish a differentiation protocol to a TM lineage.

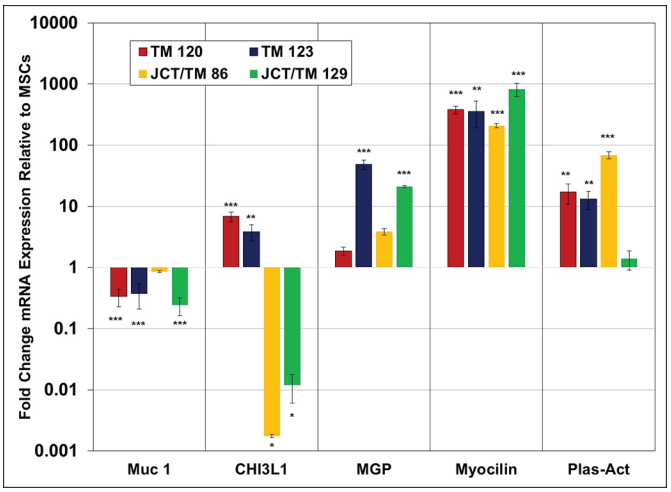

Figure 1: mRNA expression (log scale) in TM cells relative to average expression in three MSC lines. (Mean ± SEM; * $p < 0.01$, ** $p < 0.05$, *** $p < 0.001$).
Commercial Relationships: Eric Snider, None; **Christopher Pride**, None; **Akash Patil**, None; **W D. Stamer**, None; **C R. Ethier**, None
Support: Georgia Research Alliance (CRE), NSF Graduate Research Fellowship (EJS), RPB (WDS) and EY022349 (WDS)

Program Number: 2257 **Poster Board Number:** B0162
Presentation Time: 3:45 PM–5:30 PM
Human Chorionic plate derived mesenchymal stem cells (CPMSC) rescue deterioration of R28 cells exposed to ischemia by expression of GAP-43 and HIF-1α.
Helen Lew, Seungsoo Rho, Mira Park. Ophthalmology, CHA University, Seongnam, Korea (the Republic of).
Purpose: Different damage factors are known to promote cell death in optic nerve cells. Mesenchymal stem cells are potent candidates in traumatic optic nerve injury due to their ability to secrete protective anti-inflammatory cytokines and recovery factors. We investigated the neuroprotective effects of human chorionic plate derived mesenchymal stem cells (CPMSC) using an established ischemic model of R28 cells which were subjected to induced inflammation and exposed to hypoxia.
Methods: Under optimal conditions, 2×10^5 CPMSCs, added in a trans-well system, conferred neuroprotection to R28 cells subjected to inflammation insult of TNF-α (10ng/ml) and hypoxic insult of $CoCl_2$ (50μM/ml). R28 cell death, measured by dehydrogenase activities in cells (Cell Counting Kit-8 assay). Since neuroprotection is a prominent function of the cytokine GAP-43 and HIF-1α, we investigated the expression of GAP-43, HIF-1α and activated caspase3 using western blot under injured or control conditions. And the expression was evaluated upon CPMSC co-culture conditioned media as well as pretreatment with GAP-43 antagonist glutamate N-methyl-D-aspartate (NMDA) receptor blockade and HIF-1α antagonist PX478, respectively.
Results: R28 cell death was reduced by CPMSCs or by conditioned medium derived from CPMSCs exposed to inflammation or hypoxia, suggesting the active release of factorial components. GAP-43 protein level was recovered and activated caspase3 were also reduced by co-culture of R28 with CPMSCs under ischemic compared to control conditions.
Conclusions: Therefore, CPMSCs induced neuroprotection in ischemic R28 cells may be partially explained by GAP-43 and HIF-1α expression. These findings may also explain for the therapeutic effects seen in optic nerve injury in vivo studies after treatment with these cells.
Commercial Relationships: Helen Lew, None; **Seungsoo Rho**, None; **Mira Park**, None
Support: NRF-2014R1A2A2A01002617

Program Number: 2258 **Poster Board Number:** B0163
Presentation Time: 3:45 PM–5:30 PM
Human umbilical tissue-derived cells (hUTC) promote synapse formation and neurite outgrowth via Thrombospondin family proteins: A potential mechanism for hUTC-based therapy in retinal-degenerative diseases
Sehwon Koh[1], Namsoo Kim[2], Nadine S. Dejneka[3], Ian R. Harris[3], Henry H. Yin[2], Cagla Eroglu[1]. [1]Cell Biology, Duke University, Durham, NC; [2]Psychology & Neuroscience, Duke University, Durham, NC; [3]Janssen Research & Development, Spring House, PA.
Purpose: Human umbilical tissue-derived cells (hUTC) were previously shown to preserve vision in a rodent model of retinal degeneration; however, it is not clear how the cells were able to achieve this effect. We hypothesize that hUTC may improve vision by promoting neuronal survival, neurite outgrowth and synaptogenesis.
Methods: We utilized a purified rat retinal ganglion cell (RGC) culture system. In this *in vitro* system we studied the effects of hUTC-conditioned media on RGC survival, neurite outgrowth and synapse formation.
Results: We found that hUTC promote neuronal survival and enhance neurite outgrowth. Interestingly, hUTC strongly induce excitatory synaptogenesis between RGCs as determined by immunolabeling of synaptic markers and electrophysiological recordings. The synaptogenic factors are larger than 100kDa and can be blocked by the antiepileptic drug Gabapentin. Gabapentin is a known blocker of synaptogenic Thrombospondin (TSP) family proteins. We found that hUTC secrete TSPs (TSP1, 2 and 4) and silencing TSP expression in hUTC eliminated the synaptogenic effects of these cells as well as their ability to promote neurite outgrowth.
Conclusions: Our results show hUTC enhance neuronal survival, neurite outgrowth and promote development of functional synapses through trophic mechanisms. We identified TSPs as the major synaptogenic factors secreted by hUTC. hUTC-secreted TSPs also supported neurite outgrowth. Our findings demonstrate that hUTC affect multiple aspects of retinal cell health and connectivity and each of these paracrine effects may individually contribute to the therapeutic function of these cells.
Commercial Relationships: Sehwon Koh, None; **Namsoo Kim**, None; **Nadine S. Dejneka**, Janssen Research and Development (E); **Ian R. Harris**, Janssen Research and Development (E); **Henry H. Yin**, None; **Cagla Eroglu**, None
Support: Janssen Research and Development

Program Number: 2259 **Poster Board Number:** B0164
Presentation Time: 3:45 PM–5:30 PM
BCVA changes after suprachoroideal graft of autologous cells in Dry AMD

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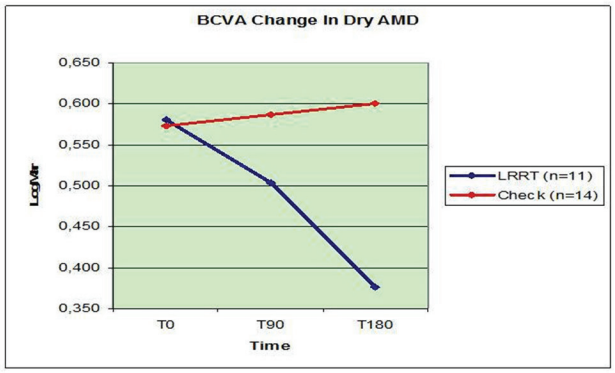
Purpose: Our prior study (ARVO 2014, Medicine© 2014, in press) showed in eyes affected by Dry AMD a significant improvement of ERG data, recorded after the suprachoroidal autologous graft. We used three different types of autologous cells: adipocytes, present in the orbital fat dislocated in the suprachoroidal space, platelets, derivated from Platelet Rich Plasma (PRP) and adipose derived stem-cells (ADSCs), included in tissue adipose's stromal vascular fractions (SVF), both grafted by suprachoroidal lipofilling. The study analyzes whether, in eyes affected by Dry AMD, the suprachoroidal graft of these cells can improve the BCVA over time.

Methods: We studied two groups of patients, both affected by Dry AMD, with BCVA equal to or greater than 1 logMAR.
Group A consisted of 11 eyes of 8 patients (age average: 71.5 years), treated with autologous suprachoroidal graft by Limoli Retinal Restoration Tecnique (LRRT). Group B consisted of 14 eyes of 12 patients (age average: 80.4), used as a control variable. For each patient diagnosis has been verified by SD-OCT (Zeiss Cirrus) and microperimetry (Maia or MP1). Before the graft (T0), BCVA (Logmar) and close up visus (pts), maximal, scotopic and photopic ERG (μV), according to standard ISCEV, have been considered. BCVA was measured also at T90 and at T180.

Results: In Group A, following LRRT, BCVA changed from 0.581 (T0) to 0.504 (T90) to 0.376 logM (T180) (+32.20%). In the control Group, BCVA changed from 0.573 (T0) to 0.587 (T90) to 0.601 logM (T180) (-4.75%). No negatives side effects occured in either case.

Conclusions: In patients with dry AMD who received LRRT treatment, BCVA can increase in six months, compared to the control group. All implanted cells can produce a lot of growth factors (bFGF, VEGF, PEDF, IL, M-CSF, GM-CSF, PlGF PDGF, ecc.), with neurotrophic and angiotrophic properties, ensuring a constant secretion of growth factors in a choroidal flow. From the choroidal layer, growth factors could reach retinal photoreceptors. Interaction with membrane receptors of retinal cells can activate an intracellular pathway. Consequently, we can have in the cell nucleus genic responses. We can obtain an antiapoptotic effect which can explain the neuroenhancement. This suggests a greater potential increase of BCVA in the grafted group.



BCVA changes after 6 months from suprachoroidal autologous graft.

Commercial Relationships: Paolo G. Limoli, None; **Enzo M. Vingolo**, None; **Marcella Nebbioso**, None; **Sergio Zaccaria Scalinci**, None; **Luigi Mele**, None; **Celeste Limoli**, None

Program Number: 2260 **Poster Board Number:** B0165
Presentation Time: 3:45 PM–5:30 PM
Limbal reconstruction using decellularized human corneal lenticule with compressed collagen for treating limbal deficiency in rabbit experimental model

Hong Kyun Kim, Man-Il Huh, Kyoung Min Kang, Ji-Ahn Kim. Ophthalmology, Kyungpook National University School of Medicine, Daegu, Korea (the Republic of).

Purpose: We previously studied decellularized human cornea lenticule (HCL) generated by hypotonic enzymatic solution has great advantages as a scaffold for corneal regeneration. The aim of this study is to make a suitable bio-engineered limbal tissue for severely destructed limbal structure and then to evaluate therapeutic effect of the construct.

Methods: HCLs were decellularized using our standardized method and then gelated collagen was compressed on the decellularized human cornea. The constructs were trephined into 4 mm and examined mechanic and optical properties. Human corneal limbal epithelial cells (HCLECs) were re-seeded on compressed collagen (CC) surrounding decellularized HCL. Limbal deficient experimental model was established by alkali burn with 1N NaOH and construct with HCLECs was transplanted on the limbal deficient cornea. The operated corneas were collected at 1, 3 weeks. Limbal deficiency and invasion of conjunctival cells were examined by histology.

Results: The construct combined CC with decellularized HCL showed better strength than CC only and decellularized HCL only, and histological results showed that it was well established. Immunologic staining showed that HCLECs were successfully reseeded expressing progenitor marker. Numerous goblet cells and several vascular were observed at inside of limbus. Histology of transplanted corneas showed that the construct with HCLECs inhibited conjunctival cell migration into center cornea compare to control, and had more transparent than the other at 2 weeks after operation.

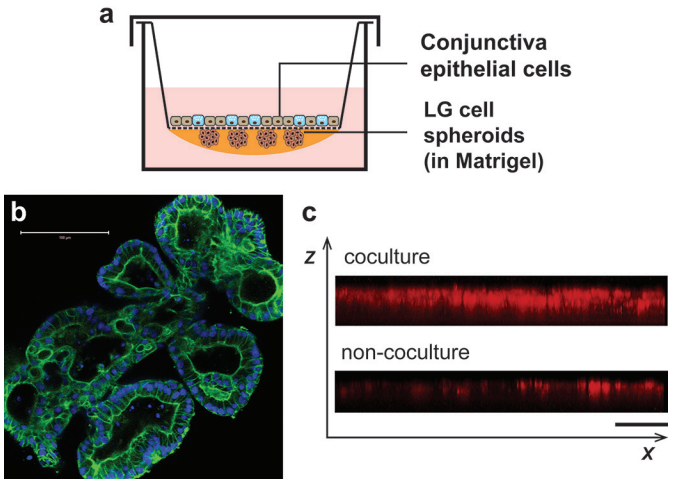
Conclusions: Data in this study showed that combined construct had several advantages for limbal reconstruction ; stronger, easier for handling and and better bio-compatibility than CC only or decellularized HCL only. HCLECs on the bio-engineered limbal tissue were remained and kept its phenotype and its stemness without

any infiltration of inflammatory cells. Collectively, our data suggest that combined construct with HCLECs has a great potential for the reconstruction of limbal niche in highly destructive limbal deficiency.
Commercial Relationships: Hong Kyun Kim, None; **Man-Il Huh**, None; **Kyoung Min Kang**, None; **Ji-Ahn Kim**, None
Support: MI Korea 10048358

Program Number: 2261 **Poster Board Number:** B0166
Presentation Time: 3:45 PM–5:30 PM
PRGF-Endoret® in corneal tissue engineering
Ana Cristina Riestra[1, 2], Natalia Vázquez[3, 1], Manuel Chacón[3, 2], Eva García[6, 4], Alvaro Meana[3, 4], Gorka Orive[5], Eduardo Anitua[5], Jesus Merayo-Lloves[1, 3]. [1]Universidad de Oviedo, Oviedo, Spain; [2]Instituto Oftalmológico Fernández-Vega, Oviedo, Spain; [3]Fundación de Investigación Oftalmológica, Oviedo, Spain; [4]CIBERER U714, Oviedo, Spain; [5]Biotechnology Institute, Vitoria, Spain; [6]Universidad Carlos III, Madrid, Spain.
Purpose: The objective of the present study is to analyze the potential use of PRGF-Endoret® in every step of corneal tissue engineering, supplementing the culture media and as a scaffold for limbal stem cells.
Methods: **PRGF-Endoret® derivatives:** Plasma rich in growth factors (PRGF) eye drop was obtained using the Endoret (PRGF) kit in Ophthalmology (BTI Biotechnology Institute, S.L., Vitoria, Spain). Briefly, blood was collected from healthy donors into 9 mL tubes containing sodium citrate as anticoagulant. Blood was centrifuged at 580g for 8 minutes; whole plasma column was drawn off avoiding the buffy coat, activated with $CaCl_2$ and incubated at 37 0C for one hour until membrane coagulation. The released supernatants were collected, filtered, aliquoted and stored at – 800C until use in the following culture media: **Basal medium (BM)**: DMEM/F12 supplemented with 1% antibiotics and 10% PRGF-Endoret® (BM_{PRGF}) or FBS (BM_{FBS}). **Classic medium (CM)**: DMEM/F12 supplemented with 5mg/ml insulin, 8.33ng/ml cholera toxin, 24mg/ml adenine, 1.3ng/ml triiodothyronine, 0.4mg/ml hydrocortisone, 1% antibiotics and 10% PRGF-Endoret® (CM_{PRGF}) or FBS (CM_{FBS}). **Proliferation assays:** Limbal stem cells were obtained from explants of 2-3mm in diameter of the limbal region and cultured on the media described above. At 7 days, cells were fixed in ice-cold methanol and their proliferation was assessed by quantification of the growth area. **Culture on PRGF-Endoret® membrane:** Human limbal stem cells were cultured on PRGF-Endoret® membranes. Once confluent, membranes were fixed and analyzed by phase contrast microscopy, scanning electron microscopy and immunocytochemistry for p63 and cytokeratin in order to check their immunological markers.
Results: Growth area in cultures in CM_{PRGF} was significantly better ($p<0.05$) than cultures in CM_{FBS}, and, even though all cultures in BM were significantly lower ($p<0.05$) than cultures in CM, BM_{PRGF} shows a significantly better ($p<0.01$) growth area than cultures in $BM_{FBS,}$ being as effective as CM_{FBS}. In the other hand, limbal stem cells seems to attach and proliferate on a PRGF-Endoret® membrane maintaining their cellular markers with PRGF-Endoret®, instead of FBS, as supplement in the culture media.
Conclusions: **PRGF-Endoret®** could be used in corneal tissue engineering, both, **supplementing the culture media**, even in a basal media without any other additives, as well **as scaffold** for the culture of the limbal stem cells.
Commercial Relationships: Ana Cristina Riestra, None; **Natalia Vázquez**, None; **Manuel Chacón**, None; **Eva García**, None; **Alvaro Meana**, None; **Gorka Orive**, Biotechnology Institute (E); **Eduardo Anitua**, Biotechnological Institute (E); **Jesus Merayo-Lloves**, None
Support: Retos Colaboración 2014 RTC-2014-2375-1

Program Number: 2262 **Poster Board Number:** B0167
Presentation Time: 3:45 PM–5:30 PM
EncorStat®: a human donor cornea genetically engineered to resist rejection in high risk patients
Scott Ellis[1], Maria Parker[2], Vicky Kennedy[1], Laura McCloskey[1], Trevor McFarland[2], Matt Hartzell[2], Binoy Appukuttan[3], Tim Stout[4], Kyriacos Mitrophanous[1]. [1]Oxford BioMedica (UK) Ltd, Oxford, United Kingdom; [2]Oregon Health & Sciences University, Portland, OR; [3]Flinders University, Adelaide, SA, Australia; [4]Cullen Eye Institute, Baylor College of Medicine, Houston, TX.
Purpose: Corneal transplantation is one of the most successful transplant procedures because of the relatively immune-privileged status of the eye and the avascularity of the cornea. However in high risk patients, which account for >20% of the 100,000 transplants carried out each year, the rejection rate is very high (50-90%) due to vascularisation of the recipient corneal bed. The prognosis in these patients is poor; some are no longer considered for replacement transplant and are left blind. The main reason for graft failure is irreversible immunological rejection and it is therefore unsurprising that neovascularisation (both pre- and post-grafting) is a significant risk factor for subsequent graft failure. Neovascularisation thus is an attractive target to prevent graft rejection.
EncorStat® is a human donor cornea modified prior to transplant by the *ex vivo* delivery of the genes encoding secretable forms of the angiostatic human proteins, endostatin and angiostatin, by a lentiviral vector which subsequently prevents rejection by suppressing neovascularisation.
Methods: Parameters of the EncorStat® process have been tested (transduction media, incubation time, washing/shedding). Modified rabbit corneas have been evaluated in two different models of corneal rejection, a highly aggressive model in which rejection is driven by the retention of thick graft sutures, and a less aggressive model in which rejection is driven by prevascularising the recipient corneal bed prior to surgery. In this latter model thin sutures are used to secure the graft that are removed 2 weeks following surgery, which is more analogous to the clinical setting.
Results: The process to generate EncorStat® corneas has been optimized to secrete substantial and persistent levels of angiostatic proteins with very little shedding of residual vector. These corneas substantially suppress corneal neovascularization, opacity and subsequent rejection in two rabbit models of cornea graft rejection.
Conclusions: The non-clinical data to be presented supports the evaluation of EncorStat®corneas in a First-in-Man trial. With support from the UK Technology Strategy Board (now Innovate UK), this trial will be conducted in 2015, following completion of non-clinical safety studies and GMP vector manufacture this year. An outline of this clinical trial design will also be presented.
Commercial Relationships: Scott Ellis, Oxford BioMedica (UK) Ltd (E); **Maria Parker**, None; **Vicky Kennedy**, Oxford BioMedica (UK) Ltd (E); **Laura McCloskey**, Oxford BioMedica (UK) Ltd (E); **Trevor McFarland**, None; **Matt Hartzell**, None; **Binoy Appukuttan**, None; **Tim Stout**, None; **Kyriacos Mitrophanous**, Oxford BioMedica (UK) Ltd (E)
Support: TSB Supporting regenerative medicine and cell therapies grant 101622

Program Number: 2263 **Poster Board Number:** B0168
Presentation Time: 3:45 PM–5:30 PM
Engineering of a tear-secreting system by coculturing lacrimal gland and conjunctival epithelial cells
Nicole Qiaozhi Lu[1, 2], Hongbo Yin[2, 4], Osama Al-Sheikh[5], Jennifer Elisseeff[2, 3], Michael P. Grant[2]. [1]Materials Science and Engineering, Johns Hopkins University, Baltimore, MD; [2]Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD; [3]Biomedical Engineering, Johns Hopkins University, Baltimore, MD; [4]Ophthalmology, West China Hospital, Sichuan University, Chengdu, China; [5]Oculoplastics and Orbit Division,, King Khaled Eye Specialist Hospital, Riyadh, Saudi Arabia.
Purpose: Dry eye is a multi-factorial disease, and an *in vitro* ocular surface model is in need for dry eye study and therapeutic screening purposes. In our study, a coculture system composed of lacrimal gland (LG) and conjunctival epithelial cells (CECs) was established.
Methods: Rabbit LGs were digested with collagenase I, hyaluronidase and DNase I at 37 °C. Cell spheroids were formed in a shaking flask on an orbital shaker. CECs were isolated by dispase II. Coculture was established in a transwell insert system as illustrated in figure 1a. The morphology of LG cell spheroids was visualized by phalloidin staining. Cells under coculture conditions were harvested and compared to non-coculture samples. Gene expression was analyzed by qPCR. Mucin secreted by conjunctival goblet cells was stained with texas-red conjugated dextran, and imaged under a confocal microscope. LG secretion was quantified by β-hexosaminidase assay after carbachol stimulation.
Results: LG cells aggregated into spheroids within 2 days, with an average diameter of 108 μm. The spheroids formed 3-dimensional organoids inside Matrigel, as revealed by F-actin staining (FITC, figure 1b). Gene expression analysis of LG spheroids showed that cocultured cells expressed significant higher level of E-cadherin, EpCAM and lactoferrin compared to non-cultured samples. After carbachol stimulation, cocultured LG cell spheroids had more hexosaminidase in both supernatants and cell lysates. For CECs, qPCR results indicated that mucin 5AC and cytokeratin 4 was upregulated, while cytokeratin 7 was downregulated in cocultured cells. After 10 days, cocultured CECs secreted a thinker, more continuous mucin layer than non-cocultured ones (figure 1c).
Conclusions: LG cell spheroids have the ability to develop into lacrimal organoids when embedded in Matrigel, which highly resembles the structure of LG acini. Coculture is beneficial for both LG cell spheroids and CECs, as proven by gene expression and protein secretion results. This coculture system could be further engineered into an *in vitro* model for dry eye study and therapeutic screenings.



(a) Schematics of the coculture system. (b) F-actin staining (FITC) of LG cell spheroids in Matrigel at day 5. Blue: nuclei stained with DAPI. Scale bar: 100 μm. (c) Confocal microscopic images (xz scanning) of the mucin layer secreted by CECs, stained with texas red conjugated dextran. Scale bar: 50 μm.
Commercial Relationships: Nicole Qiaozhi Lu, None; **Hongbo Yin**, None; **Osama Al-Sheikh**, None; **Jennifer Elisseeff**, Collagen Vitrigel (P); **Michael P. Grant**, Stryker CMF (C), Synthes CMF (C)
Support: KKESH -Wilmer Collaborative grants

Program Number: 2264 **Poster Board Number:** B0169
Presentation Time: 3:45 PM–5:30 PM
Curcumin formulations for retinal diagnosis of Alzheimer's disease
George Heaton, Ben Davis, Lisa Turner, Shereen Nizari, Li Guo, M Francesca Cordeiro. Glaucoma and Retinal Neurodegeneration, UCL institute of Opthalmology, London, United Kingdom.
Purpose: Alzheimer's Disease (AD) is a devastating neurodegenerative disease.There is strong evidence to suggest that a gradual process of pathogenic accumulation of amyloid-β peptide, the primary constituent of senile plaques, occurs 10-20 years prior to symptomatic manifestation. Curcumin, the active ingredient in turmeric, is a natural fluorophore that binds with high affinity to amyloid-β and is well tolerated in humans at high concentrations. As such, using curcumin as a robust method for detecting AD associated amyloid changes is an attractive prospect. However, curcumin's extremely low water-solubility (0.0004mg/ml) and rapid intestinal and hepatic metabolism dramatically limit its utility for oral dosage or injection. Nano-carriers such as micelles represent a means to overcome this hurdle. In this poster we present an optimised micelle formulation and freeze-drying protocol. By constructing formulations that permit effective CNS drug delivery, curcumin can bind to neurotoxic amyloid β42 associated with AD pathogenesis. Fluorescing curcumin signals can be subsequently captured through retinal imaging. We report that, following intravenous administration of curcumin-micellar solution, there is a significantly elevated retinal spot count in TASTPM transgenic murine AD model compared to age-matched wt. controls.
Methods: Micelles were prepared using thin-film lipid hydration and characterized using absorbance spectroscopy and dynamic light scattering. TASTPM transgenic mice (n=6) & age-matched 14-month C57 (n=8) were given curcumin systemically. Retinal images were acquired under general anaesthetic using an cSLO and analysed for spots via systematic blinded manual counts.

Results: An optimised protocol produced micelles with encapsulation efficiency of 91.27±3.17%, with z-average: 21.22-23.29 and PDI: 0.032-0.169, formulations appeared homogenous over 24 hours and amenable to freeze-drying with stability at resuspension after 5 weeks. Retinal spot counts reveal a significant difference between TASTPM and age matched wild-type controls.
C57: x= 87.625, σx = 27.266, TASTPM: x= 145.67, σx = 49.842, (P 0.02 > 0.015.)
Conclusions: This data offers a preliminary proof of concept study for non-invasive retinal diagnosis of AD through the use of retinal imaging. Retinal screening may represent a promising avenue towards the solution to the AD biomarker quandary.
Commercial Relationships: George Heaton, None; **Ben Davis**, None; **Lisa Turner**, None; **Shereen Nizari**, None; **Li Guo**, None; **M Francesca Cordeiro**, None

Program Number: 2265 **Poster Board Number:** B0170
Presentation Time: 3:45 PM–5:30 PM
Mechanism of disease selective vision restoration by small molecule photoswitches
Ivan Tochitsky, Victor Meseguer, Zachary Helft, Aleksandra Polosukhina, Richard Kramer. Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA.
Purpose: Degenerative blinding diseases such as retinitis pigmentosa (RP) and age-related macular degeneration (AMD) affect millions of patients around the world. These disorders cause the progressive loss of photoreceptors from the retina, while sparing the remaining amacrine, bipolar and retinal ganglion cells (RGCs). We have developed a series of chemical "photoswitches" that selectively photosensitize retinal ganglion cells in retinas suffering from photoreceptor degeneration, while leaving healthy retinas unaffected. We now identify the mechanism of these photoswitches' disease selective action.
Methods: We have created several small molecule photoswitches that can be used to control the activity of neurons by reversibly blocking native ion channels in response to light. Using imaging and electrophysiological methods, we identify the mechanism of these compounds' disease selectivity.
Results: We have previously demonstrated that chemical photoswitches restore light responses to blind retinas in vitro and also enable innate and learned visual behaviors in blind mice. Here, we characterize the action of four different photoswitches on the retina, which target overlapping but different sets of ion channels. All of the compounds exhibit a disease selective effect, photosensitizing retinal ganglion cells from blind mouse, rat and dog retinas, while leaving healthy retinas unaffected. We find that large-pore ATP (P2X) receptors are responsible for the disease selective action of these photoswitches by mediating photoswitch uptake into RGCs. While the photoswitches quickly accumulate in RGCs from blind retinas, they don't enter RGCs from healthy retinas nearly as well. We confirm these findings by imaging the retinal loading of the large, permanently charged dye molecule YO-PRO, which also requires the activity of large-pore ion channels for permeation into neurons. Like the photoswitches, YO-PRO also preferentially loads into RGCs from the blind retina.
Conclusions: Our findings identify a change in P2X receptor expression or function in RGCs in animal models of RP. The selectivity of our photoswitches for diseased but not healthy retinas implies that our pharmacological therapy could not only restore vision in patients with end stage RP, but also improve vision in less advanced RP and AMD patients, by selectively acting on the parts of the retina undergoing photoreceptor death.
Commercial Relationships: Ivan Tochitsky, None; **Victor Meseguer**, None; **Zachary Helft**, None; **Aleksandra Polosukhina**, None; **Richard Kramer**, None

Program Number: 2266 **Poster Board Number:** B0171
Presentation Time: 3:45 PM–5:30 PM
Biocompatibility of Nano-Lawn Structures for Nano-Modification of Microelectrode Array Systems
Claudia Etzkorn[1], Andreas Jupe[2], Andreas Goehlich[2], Wilfried Mokwa[3], Peter Walter[1], Sandra Johnen[1]. [1]Department of Ophthalmology, University Hospital RWTH Aachen, Aachen, Germany; [2]Fraunhofer Institute for Microelectronic Circuits and Systems, Duisburg, Germany; [3]Institute for Materials in Electrical Engineering I, Aachen, Germany.
Purpose: In patients suffering from retinal degenerative diseases, e.g., retinitis pigmentosa, functionality can be regained by prostheses, where electrical stimulation of surviving retinal cells is induced by microelectrodes that interface with neuronal tissue. With regard to charge transfer capacity and signal-to-noise ratio, microelectrode properties can be optimized by nano modification, e.g., coating with nano-lawn structures. The biological compatibility of nano-lawn structures and their substrate materials was analyzed after direct and indirect cell contact with regard to proliferation, survival and gene expression profile.
Methods: Manufacturing of the nano-lawn structures was carried out by our partner from the Fraunhofer Institute for Microelectronic Circuits and Systems. The nano-lawn structures have been realized as an array of free-standing tubes made from Ruthenium. Growth rates and survival of L-929 and retinal precursor (R28) cells were determined after indirect and direct contact. Indirect contact implies the cultivation in medium pre-incubated with the respective structures using a luminescent cell viability assay. Direct contact was evaluated using a fluorescein-diacetate/propidiumiodide-based life-dead assay. For R28 cells, gene expression was analyzed by quantitative real-time PCR.
Results: Compared to reference materials with defined levels of cytotoxicity, the indirect contact with pre-incubated medium had no significant influence on cell growth rates. Both cell types exhibited good proliferation and morphological properties on the nano-lawn structures and their substrate materials, showing less than 2% death cells. Related to glass, cultivation on the nano-lawn structures indicated no significant differences in the gene expression profile of R28 cells.
Conclusions: With regard to direct and indirect cell contact, the tested nano-lawn structures showed good biocompatibility profiles, demonstrating that this nanotechnology provides a promising tool to improve microelectrodes that connect with retinal tissue.
Commercial Relationships: Claudia Etzkorn, None; **Andreas Jupe**, None; **Andreas Goehlich**, None; **Wilfried Mokwa**, None; **Peter Walter**, None; **Sandra Johnen**, None

Program Number: 2267 **Poster Board Number:** B0172
Presentation Time: 3:45 PM–5:30 PM
The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of xenocorneal transplantation
Mee Kum Kim[1], Hyuk Jin Choi[1], Ivo Kwon[2], Richard N. Pierson III[3], David K.C. Cooper[4], Jean-Paul Soulillou[5], Philip J O'Connell[6], Bertrand Vabres[5], Naoyuki Maeda[7], Hidetaka Hara[4]. [1]Ophthalmology, Seoul National University College of Medicine, Seoul; [2]Department of Medical Education,, Ewha Womans University, School of Medicine, Seoul, Korea (the Republic of); [3]Department of Surgery, University of Maryland Medical Center, Baltimore, MD; [4]Department of Surgery, Thomas E. Starzl Transplantation Institute, Pittsburgh, PA; [5]Service d'Ophtalmologie, CHU Nantes, Nantes, France; [6]Centre for Transplant and Renal Research, Westmead Millennium Institute, Westmead, NSW, Australia; [7]Department of Ophthalmology, Osaka University Medical School, Osaka, Japan.
Purpose: To develop an international consensus regarding the appropriate conditions for undertaking clinical trials in xenocorneal transplantation
Methods: 17 international panel of experts from 7 countries (Korea, Japan, United States of America, United Kingdom, Australia, France, and Belgium) in various professional fields which include ophthalmology, ethics, infection, and international xeno-transplantation society reviewed specific ethical, logistical, scientific, and regulatory issues regarding xenocorneal transplantation, and proposed guidelines for conduct of clinical xenocorneal transplantation trials.
Results: Six topics were discussed through e-mail communications, a pre-workshop and a consensus meeting. Consensus statements were finally made on (1) Ethical requirements and regulatory framework in xenocorneal transplantation, (2) Control of Source pigs for corneal transplantation, (3) Quality control of porcine corneal products, (4) Pre-clinical efficacy and safety data that are required to justify initiating a clinical trial, (5) Strategies to prevent Porcine endogenous retrovirus transmission, and (6) Patient selection for clinical trials
Conclusions: The consensus statement is designed primarily to assist in the development of protocols for clinical trials of pig corneal transplantation.

Commercial Relationships: Mee Kum Kim, None; **Hyuk Jin Choi**, None; **Ivo Kwon**, None; **Richard N. Pierson III**, None; **David K.C. Cooper**, None; **Jean-Paul Soulillou**, None; **Philip J O'Connell**, None; **Bertrand Vabres**, None; **Naoyuki Maeda**, None; **Hidetaka Hara**, None
Support: Korea Healthcare Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (Project No. HI13C0954).

Program Number: 2268 **Poster Board Number:** B0173
Presentation Time: 3:45 PM–5:30 PM
Detecting bacteria colorimetrically on contact lens cases using immobilized gold nanoparticles
Sarah A. LeBlanc[1, 2], Mohit S. Verma[1, 2], Lyndon W. Jones[1, 3], Frank Gu[1, 2]. [1]Chemical Engineering, University of Waterloo, Waterloo, ON, Canada; [2]Waterloo Institute for Nanotechnology, University of Waterloo, Waterloo, ON, Canada; [3]Optometry & Vision Science, University of Waterloo, Waterloo, ON, Canada.
Purpose: Non-compliance in contact lens care practices leads to the growth of bacteria and biofilms in contact lens cases, increasing the wearer's risk of contracting both microbial and infiltrative keratitis. Improving compliance requires a simple method for warning the user of bacterial contamination. We developed a colorimetric biosensor using gold nanoparticles that can be applied to contact lens case surfaces for the detection of bacteria.
Methods: Gold nanoparticles were synthesized using a seed-mediated surfactant-assisted growth method. The sensor surface was created by immobilizing a single layer of gold nanoparticles onto glass coverslips using layer-by-layer electrostatic interactions. To simulate the use of a contact lens case, the sensor surface was submerged in a saline solution of *Staphylococcus aureus* at 10^8 CFU/mL overnight followed by rinsing and drying. The resulting color change was characterized using ultraviolet-visible spectroscopy and hue-saturation-value (HSV) analysis of digital photographs. The interactions of the bacteria with the nanoparticles were explored using scanning electron microscopy (SEM).
Results: The sensor surface produced a visible color change, from blue to purple, after incubation with *Staphylococcus aureus*. In addition to being detectable by the naked eye, the color change was quantified by a significant change in hue using the HSV color scale. Furthermore, a significant decrease in the gold nanoparticle 650/525nm absorbance peak ratio confirmed the visual observation. SEM shows that the local interactions of the immobilized gold nanoparticles with bacteria that deposited on the surface are responsible for the change in the absorbance peak ratio. Using the same procedure, the gold nanoparticles were successfully immobilized onto polypropylene, which is commonly used in contact lens cases.
Conclusions: Immobilized gold nanoparticles can be used as a surface-based colorimetric biosensor for visual detection of bacterial contamination. This biosensor is a promising platform for incorporation within contact lens cases. It can provide a cheap, simple, consumer-level technology allowing users to know when a contact lens case becomes contaminated with bacteria and should be replaced. The technology will thus lead to increased compliance and reduction of contact-lens induced microbial and infiltrative keratitis.
Commercial Relationships: Sarah A. LeBlanc, None; **Mohit S. Verma**, None; **Lyndon W. Jones**, None; **Frank Gu**, None

Program Number: 2269 **Poster Board Number:** B0174
Presentation Time: 3:45 PM–5:30 PM
Development of nano structured lipid carriers of ciprofloxacin for ocular delivery : Characterization, *in vivo* distribution and effect of PEGylation
Sai Prachetan Balguri, Goutham Adelli, Prakash Bhagav, Michael A Repka, Soumyajit Majumdar. Pharmaceutics and Drug Delivery, University of mississippi, Oxford, MS.
Purpose: Ciprofloxacin (CIP) is a synthetic fluoroquinolone anti-bacterial agent usually prescribed for the treatment of corneal keratitis, conjunctivitis and other bacterial infections of the eye. The goal of the present project is to fabricate CIP loaded nanostructured lipid carriers (NLCs), to investigate their potential and feasibility in

terms of topical ocular delivery and disposition, and to compare them with PEGylated CIP-NLCs.
Methods: CIP-NLCs were prepared by hot homogenization method comprising solid lipid (Glyceryl monostearate (GMS) and 1,2-distearoyl-sn-glycero phosphoethanolamine, sodium salt (DSPE) and liquid lipids (oleic acid). N-(Carbonyl-methoxypolyethylene glycol-2000)-DSPE was used, in combination with GMS, to prepare PEGylated CIP-NLCs. Total amount of lipid employed in the NLCs was 6% w/v of which solid lipid constituted 50% and oleic acid made up the remaining 50%. Drug load in the formulations was maintained at 0.3% w/v. Formulations prepared were then characterized and evaluated with respect to *in vivo* ocular tissue distribution 2 h post topical administration in a conscious rabbit model. All animal studies conformed to the tenets of the ARVO statement on the use of animals in ophthalmic vision and research and the University of Mississippi IACUC approved protocols.
Results: CIP content in the formulations were within 95-98% of the theoretical value. Mean hydrodynamic radius, zeta potential, polydispersity index & entrapment efficiency of the CIP-NLCs and PEGylated CIP-NLCs were 154, 55.3 nm; 0.072, 0.5 mV; 0.21, 0.29; and 72.5, 83.66 %, respectively. CIP levels in the cornea attained with the CIP-NLCs and PEGylated CIP-NLCs were 1.91 ± 0.47, 3.73 ± 1.23 µg/g of tissue, respectively. Drug levels obtained in all ocular tissues was nearly 2-3 fold higher with the PEGylated CIP-NLCs.
Conclusions: Results suggest that surface functionalization of NLCs with PEGylated chains could further improve intraocular penetration and retention of the drug molecules and thus enhancing the ocular bioavailability and distribution.
Commercial Relationships: Sai Prachetan Balguri, None; **Goutham Adelli**, None; **Prakash Bhagav**, None; **Michael A Repka**, None; **Soumyajit Majumdar**, None
Support: National Institutes of Health(Grant P20 GM104932)

475 Nanotechnology and drug delivery
Wednesday, May 06, 2015 3:45 PM–5:30 PM
Exhibit Hall Poster Session
Program #/Board # Range: 5024–5040/C0001–C0017
Organizing Section: Nanotechnology and Regenerative Medicine Group

Program Number: 5024 **Poster Board Number:** C0001
Presentation Time: 3:45 PM–5:30 PM
Synthesis and Characterization of Hybrid Nanocomposite via Biomimetic Method as an Artificial Cornea
DAVOOD KHARAGHANI[5], Masoumeh Meskinfam[1, 2], Mozhgan Rezaeikanavi[5, 3], Sahar Balagholi[4, 5], Narges Fazili[5]. [1]Department of Chemistry, Islamic Azad University-Lahijan Branch, Lahijan, Iran (the Islamic Republic of); [2]Bioengineering Department, Biomaterials Laboratory, Politecnico di Milano, Milan, Italy; [3]Ophthalmic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran (the Islamic Republic of); [4]Iranian Blood Transfusion Organization, Tehran, Iran (the Islamic Republic of); [5]Ocular Tissue Engineering Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran (the Islamic Republic of).
Purpose: In this research, synthesis, characterization and evaluation of in vitro biocompatibility of a nanocomposite as an artificial cornea have been carried out.
Methods: A scaffold with transparent center from poly vinyl alcohol (PVA) hydrogel, which is surrounded by modified bacterial cellulose (BC) – porous poly vinyl alcohol - nano hydroxy apatite (nHA) composite, was designed. In situ synthesis of nHA in PVA matrix was carried out via biomimetic method. The whole scaffold was made by casting, freezing and thawing cycles. Composite was characterized by FT-IR, XRD and SEM. Biocompatibility of composite was studied by cultivating retinal pigment epithelial (RPE) cells on the composite. Water content of hydrogels was also tested.
Results: Results from FT-IR and XRD showed the formation of nHA in PVA matrix due to presence of active sites on polymers for Ca^{2+} coordination followed by phosphate bonding and initiating the nHA nucleation. SEM images represented that overall morphology for nHA was regular distribution of spherical nano particles. Inverted microscopy revealed significant growth of RPE cells on the composite, indicating the in vitro biocompatibility of the scaffold. Water content of PVA/BC/nHA hydrogel composites was about 82–84% that was close to human natural cornea (78%).
Conclusions: Considering similar properties of the composite components to normal corneal structure and the results of characterization, in vitro biocompatibility and water content test, it is expected that the designed composite can be suitable for using as an artificial cornea.
Commercial Relationships: DAVOOD KHARAGHANI, None; **Masoumeh Meskinfam**, None; **Mozhgan Rezaeikanavi**, None; **Sahar Balagholi**, None; **Narges Fazili**, None

Program Number: 5025 **Poster Board Number:** C0002
Presentation Time: 3:45 PM–5:30 PM
Novel polymer nanoparticles designed for ocular delivery of atropine
Nicole Mangiacotte, Lina Liu, Graeme Prosperi-Porta, Heather Sheardown. Chemical Engineering, McMaster University, Hamilton, ON, Canada.
Purpose: Release of ocular drugs from nanoparticles should occur at a steady rate over a long period of time in order to increase the drug residence time and to avoid administering toxic doses. This study was performed to produce a novel nanoparticle formulation for delivery of atropine into the eye.
Methods: Eight nanoparticle formulations were prepared using suspension polymerization of 2-hydroxyethyl methacrylate with a small amount of ethylene glycol dimethacrylate for crosslinking and methacrylic acid to produce a slightly charged surface. The volume of the organic phase, surfactant concentration, and monomer concentration were altered to determine their impact on nanoparticle size. Average particle diameter and polydispersity was determined for each of the formulations using dynamic light scattering. Images of the nanoparticles, obtained by transmission electron microscopy, were obtained to observe the particle morphology and confirm the size measurements. Factorial regression was used for statistical analysis. Drug release profiles from three of the formulations were developed using high pressure liquid chromatography with UV detection at 254 nm.
Results: The average nanoparticle sizes and the standard deviations obtained from the formulations were 102.70 nm ± 0.92 nm, 97.83 nm ± 0.46 nm, 125.50 nm ± 0.89 nm, 105.87 nm ± 0.45 nm, 123.33 nm ± 0.87 nm, 119.63 nm ± 1.37 nm, 115.47 nm ± 0.91 nm, and 118.43 nm ± 0.67 nm.
Conclusions: The results display the eight formulations consisting of novel polymer based nanoparticles that are capable of drug release have been produced. Further experimentation will be done to add a mucoadhesive component to the nanoparticles to allow for efficient front of the eye drug delivery.
Commercial Relationships: Nicole Mangiacotte, None; **Lina Liu**, None; **Graeme Prosperi-Porta**, None; **Heather Sheardown**, None

Program Number: 5026 **Poster Board Number:** C0003
Presentation Time: 3:45 PM–5:30 PM
Fabrication of Shape and Size Specific Nanoparticles for Ocular Drug Delivery
Stuart Williams, Sanjib Das, Jeremy Hansen, Janet Tully, Melissa Hernandez, Tyler Pegoraro, Rozemarijn S. Verhoeven, Benjamin Maynor, Benjamin Yerxa. Envisia Therapeutics, Durham, NC.
Purpose: A major challenge in ophthalmic drug delivery is overcoming the rapid and effective clearance of drug from the target site(s) such as the ocular surface, subconjunctival space, or posterior segment. It has been shown that PLGA particles can act as biodegradable, sustained drug delivery systems for the eye, opening up the possibility for longer-lasting, more efficient dosage forms. However, traditional methods for PLGA nano- and microparticle preparation offer poor control over polydispersity and shape/size, which can result in sub-optimal particle reproducibility, product safety, and efficacy. Here, we demonstrate the utility of PRINT micromolding technology to produce monodisperse, shape-specific nano- and microparticles for use in ocular drug delivery. We encapsulate difluprednate in these particles to demonstrate high loadings and encapsulation efficiency.
Methods: Biodegradable nano- and microparticles loaded with difluprednate were fabricated using the PRINT™ technology. Particle morphology and size were determined with Scanning Electron Microscopy (SEM) and Dynamic light scattering (DLS). Drug retention in the particles was monitored at 37C in 1X PBS using an HPLC method.
Results: Polymer-difluprednate micro- and nanoparticles were fabricated with control over shape, size, charge, and drug loading (Figure 1). Particles were fabricated in a range of sizes from 200 nm up to 3 mm. Encapsulation efficiencies up to 95% for Difluprednate in microparticles were demonstrated. The ability of the particles to retain and release difluprednate over time was determined in a PBS pH=7.4 solution.
Conclusions: We have shown that PRINT micromolding can be used to reproducibly formulate polymer/difluprednate into particles with specific size and shape for ophthalmic drug delivery applications, with high loading and high drug encapsulation efficiency. These results demonstrate that PRINT technology is a versatile alternative to traditional methods for nano- and microparticle production that may offer superior control of particle size, loading or other properties.

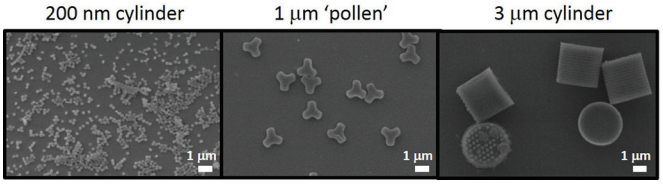


Figure 1. SEM images of (A) 200 nm (dia.) x 200 nm (h) (B) 1 micron 'pollen' shape and (C) 3mm (dia.) x 3 mm (h) polymer-difluprednate particles. Scale bars are 1 mm.
Commercial Relationships: Stuart Williams, Envisia Therapeutics (E); **Sanjib Das**, Envisia Therapeutics (E); **Jeremy Hansen**, Envisia Therapeutics (E); **Janet Tully**, Envisia Therapeutics (E); **Melissa Hernandez**, Envisia Therapeutics (E); **Tyler Pegoraro**, Envisia Therapeutics (E); **Rozemarijn S. Verhoeven**, Envisia Therapeutics (E); **Benjamin Maynor**, Envisia Therapeutics (E), Liquidia Technologies (E); **Benjamin Yerxa**, Envisia Therapeutics (E), Liquidia Technologies (E)

Program Number: 5027 **Poster Board Number:** C0004
Presentation Time: 3:45 PM–5:30 PM
Lipid DNA Nanoparticles as a new type of medication carrier platform for the treatment of retinal diseases
David Simmang[1], Sven Schnichels[1], Jan Willem De Vries[1], Jose Hurst[1], Karl U. Bartz-Schmidt[1], Andreas Hermann[2], Martin S. Spitzer[1]. [1]Center for Ophthalmology, University Eye Hospital, Tübingen, Germany; [2]Department of Polymer Chemistry, Zernike Institute for Advanced Materials, University of Groningen, Groningen, Netherlands.
Purpose: Recently, successful drug delivery via novel lipid DNA-Nanoparticles (NPs) as eye drops to the corneal epithelium was shown (ARVO 2014). Here we tested distribution and delivery of this class of NPs into the back of the eye to treat retinal diseases. A successful delivery via these NPs would offer chances to reduce the concentration of the drugs or reduce the number of injections needed per year.
Methods: NPs toxicity was first tested in 3 different cell lines with MTS, Caspase 3/7 assay and crystal violet (CV) staining. Afterwards fluorescently labelled NPs were injected either into the vitreous body or into subscleral tissue of ex vivo pig eyes obtained from the local slaughterhouse. After 5, 15, 30, 60 minutes of incubation the eyes were embedded into Tissue Tek and frozen in liquid nitrogen. Subsequently, cryo sections of the eyes were prepared and counterstained with DAPI. Using fluorescence microscopy the NP-diffusion and binding to different structures/tissues (retina, sclera, choroidea and pigment epithelium) was investigated. Finally, the NPs were injected into the vitreous body or the subscleral tissue of living rats. At several time points after injection the same parameters were investigated (30 min, 2, 4, 24 h).
Results: No toxicity was observed in the 3 cell lines investigated with any of the three assays. Intravitreal injections into the pig eyes showed good binding to retinal cells, pigment epithelium, choroidal and scleral tissue. Subscleral injections showed similar results with diffusion obviously starting from the sclera and choroid in contrast to intravitreal injections. The in vivo injections into the vitreous body of living rats showed that the NPs bind to most of the tissue in the eye instantly, with residence times lasting up to 24 h.
Conclusions: Our results proof good binding of the NPs to retinal, choroidal and scleral tissue as well as to pigment epithelium with both intravitreal and subscleral injections. Thus DNA-NPs might be a versatile tool to prolong the residence time of intraocularly injected drugs. Further investigations with medical drugs are necessary to confirm these results.
Commercial Relationships: David Simmang, None; **Sven Schnichels**, Novartis (F), University Eye hospital T√ºbingen (P); **Jan Willem De Vries**, University Groningen (P); **Jose Hurst**, None; **Karl U. Bartz-Schmidt**, None; **Andreas Hermann**, University Groningen (P); **Martin S. Spitzer**, Alcon (F), Novartis (F), University Eye hospital T√ºbingen (P)
Support: Intramural Grant: IZKF to DS, EYEnovative Research Prize for MSS & SS

Program Number: 5028 **Poster Board Number:** C0005
Presentation Time: 3:45 PM–5:30 PM
Development, optimization and evaluation of biological activity of tailor-made pentablock copolymer based composite formulation for sustained delivery of protein therapeutics
Vibhuti Agrahari[1], Mary Joseph[1], Sulabh Patel[2, 1], Ravi Vaishya[1], Ashim K. Mitra[1]. [1]Division of Pharmaceutical Sciences, Univ of Missouri Kansas City, Kansas City, MO; [2]Department of Pharmaceutical Sciences, University of Basel, Basel, Switzerland.

Purpose: The objective of this work is to develop, optimize and evaluate pentablock copolymer (PBC) based formulation which can carry large doses, retains biological activity during formulation development, storage and/or release and provide sustained release protein delivery system for the treatment of posterior segment ocular diseases.
Methods: Various ratios and molecular weights of each block were selected for synthesis and optimization of biodegradable and biocompatible PB copolymers for the preparation of nanoparticles (NPs) and thermosensitive gel. PBC were characterized for their molecular weight and purity by NMR, GPC. FTIR and XRD analysis were utilized to determine functional group and crystallinity, respectively. PBC NPs were successfully prepared with double emulsion solvent evaporation method. NPs were characterized for particle size, poly dispersity, entrapment efficiency and drug loading. *In vitro* release studies of protein from NPs alone and composite formulation were performed in PBS 7.4 at 370C. CD spectroscopy, ELISA, cell proliferation and cell invasion techniques were employed to evaluate conformation and binding affinity of released proteins. In order to investigate release mechanisms, release data were fitted to various kinetic models.
Results: NMR, GPC, FTIR and XRD analyses of PBC provided complete characterization of the polymers. Composite formulations demonstrated zero-order release with none or negligible burst release. Optimization of various formulation parameters contributed to increase in %EE from ~25% to ~79% and %DL from ~5% to ~17%. A sustained *in vitro* drug release for more than three months was observed for proteins. CD spectroscopy confirmed retention of structural conformation of proteins. Biological activity of released bevacizumab was confirmed by *in vitro* cell culture. Retention of enzymatic activity of lysozyme and catalase in release samples were also confirmed. Based on the R^2 value, best fit model was identified.
Conclusions: An improvement in drug loading through formulation optimization allowed higher amount of proteins in a smaller injection volume and delivery for extended duration. It is anticipated that these polymers can serve as a platform for ocular delivery of therapeutic proteins, for the treatment of posterior segment chronic indications such as AMD, DR and DME.
Commercial Relationships: Vibhuti Agrahari, None; **Mary Joseph**, None; **Sulabh Patel**, None; **Ravi Vaishya**, None; **Ashim K. Mitra**, None

Program Number: 5029 **Poster Board Number:** C0006
Presentation Time: 3:45 PM–5:30 PM
Polymer Nanoparticle Drug Delivery System Prevent Systemic Absorption of Bevacizumab After Intravitreal Injection
Lingkun Kong[1], Jeroen Pollet[2], Michael J. Heffernan[2].
[1]Ophthalmology, Baylor College of Medicine, Houston, TX; [2]Pediatrics, Baylor College of Medicne, Houston, TX.
Purpose: The major concerns for intravitreal injection of anti-VEGF agent for the treatment of retinal diseases are the systemic absorption of the drug into the bloodstream and fast clearance from the vitreous. We developed a new polymer nanoparticle (NP) intravitreal drug delivery system. We hypothesize that this injectable and biodegradable delivery vehicle for anti-VEGF drugs such as bevacizumab can minimize absorption of the drug into the bloodstream and increase the retention of drug in the vitreous space and retinal tissue.
Methods: Bevacizumab was conjugated to the surface of poly (lactic-*co*-glycolic acid) nanoparticles (PLGA NP), and was injected to the vitreous of adult FVB mice. Blood and vitreous samples were collected before the treatment and at 2 days, 1 week, 2 weeks, and 4 weeks after the treatment to test serum and vitreous bevacizumab levels. Fluorescence imaging was performed with Nile Red-labeled NP-bevacizumab, using an IVIS® Lumina II in vivo imaging system. The tissue distribution and toxicity of the PLGA NP were studied by histology and confocal microscope. Two-tailed student t-test was used for comparing the means of two variables.
Results: Two days after intravitreal injection, serum levels of bevacizumab were 821 ± 176 ng/mL in the free bevacizumab group and 12 ± 1.5 ng/mL in the NP–bevacizumab group. Two weeks after the injection, the serum levels of bevacizumab were 437 ± 137 ng/mL in the free bevacizumab group and undetectable in the NP-bevacizumab group. One week after intravitreal injection, vitreous levels of bevacizumab were similar for the NP-bevacizumab group and the free bevacizumab group (207.8 ± 65.43 ng/mL vs. 184.5 ± 48.20 ng/mL, respectively). At two weeks, vitreous levels of bevacizumab were 55.6 ng/mL in the NP-bevacizumab group and undetectable in the free bevacizumab injection group. The fluorescence intensity persisted in the eye for up to 4 weeks. NP-bevacizumab were distributed in the anterior chamber, vitreous and all layers of retina. No immediate toxicity was found.
Conclusions: The injectable and biodegradable PLGA NP system can potentially serve as intraocular delivery vehicle for anti-VEGF drugs. This study showed that intravitreal injection of PLGA NP conjugated bevacizumab reduced leakage of the drug into systemic blood stream and prolonged the retention of drug in the vitreous.
Commercial Relationships: Lingkun Kong, None; **Jeroen Pollet**, None; **Michael J. Heffernan**, None

Program Number: 5030 **Poster Board Number:** C0007
Presentation Time: 3:45 PM–5:30 PM
Novel Targeted Fluocinolone Acetonide loaded aqueous micelles for the treatment of Posterior Uveitis
Sujay J. Shah, Sulabh Patel, Ashaben Patel, Ashim K. Mitra.
Pharmacy, Univ of Missouri, Kansas City, Kansas City, MO.
Purpose: Diseases of the posterior segment of the eye like posterior uveitis pose a formidable challenge to drug delivery because of the various dynamic and static barriers present in the eye. Current treatment options are intravitreal implants or injections which are not patient compliant. Therefore, the objective of our research project is to develop clear aqueous fluocinolone acteonide (FA) loaded micelles using Vitamin E TPGS (1K), for treatment of posterior uveitis.
Methods: Modified TPGS was synthesized by conjugation of D-α-tocopheryl succinate with mPEG having molecular weight of 2000 (2K). Targeted polymer was synthesized by coupling of folic acid to the polymer. The products was purified by dialysis method. CMC values were calculated using standard pyrene method. Micelles were prepared by thin film hydration technique. Box-Behnken design was used to optimize the formulation to achieve maximum entrapment and solubility of drug. Size and zeta potential of micelles was measured. Quantitative uptake of [(3)H] Folic acid in presence of targeted and non-targeted micelles was studied on D407 retinal cell line with [(3)H] Folic acid as control. Quantitive uptake of fluocinolone acetonide loaded targeted and non-targeted micelles was studied on D407 retinal cell line. Effect of targeted micelles was shown using confocal microscopy.
Results: Modified and targeted polymer was successfully synthesized. The CMC value obtained was 9.44µg/ml which is significantly less than commercially available TPGS 1K. Results showed that solubility of FA maybe increased up to 26 times with newly synthesized 2K polymer. Entrapment efficiency greater than 90% was achieved with 2K polymers. Nanomicelles exhibited very small size (<20nm) and narrow size distribution with both polymers. Uptake of [(3)H] Folic acid in presence of targeted micelles was less than that of control and non-targeted micelles. Confocal microscopy

studies showed increased internalization of targeted micelles as compared to non-taregeted micelles.
Conclusions: Folic acid conjugated Vitmain E TPGS was successfully synthesized. Highly hydrophobic drugs like FA can be formulated into clear aqueous eye drops. Folic acid targeted micelles can be utilized to efficiently deliver drugs to retinal and other ocular tissues.
Commercial Relationships: Sujay J. Shah, None; **Sulabh Patel**, None; **Ashaben Patel**, None; **Ashim K. Mitra**, None
Support: NIH Grant 2 R01 EY010659-12A2

Program Number: 5031 **Poster Board Number:** C0008
Presentation Time: 3:45 PM–5:30 PM
Functionalized nanoparticle technology for enhanced drug delivery
Jai Parekh, David Freilich, Stephanie Youlios, Sima Parekh, Uday Kompella. EyeTrans Technologies, New York City, NY.
Purpose: Due to short residence time of eye drops, drug bioavailability to the eye surface is low, particularly for poorly soluble and poorly permeable drugs, necessitating frequent dosing in treating dry eye and glaucoma. Following intravitreal injections, target specific delivery is currently not feasible for macromolecule and small molecule drugs useful in treating wet age-related macular degeneration and other back of the eye diseases. To address these unmet needs our objective is to develop novel nanoparticle based technologies for topical and intraocular drug and gene delivery.
Methods: Small nanoparticles technologies capable of drug encapsulation and surface modification with hydrophilic cell recognizing components (functionalized nanoparticles) were designed to enhance mucus penetration as well as epithelial surface recognition and uptake. These technologies licensed by EyeTrans are under development for ocular drug delivery and therapy. The nanoparticle technologies utilize a drug carrier, a ligand on the particle surface to recognize cell surface, and other polymers to enhance delivery or stabilize the particle.
Results: This presentation will describe nanoparticle preparation and evidence to date indicating that surface functionalization enhances ocular surface tissue uptake as well as retinal pigment epithelial cell uptake of nanoparticles in ex vivo/in vitro studies. Further, it will describe evidence indicating that dosing with biodegradable functionalized nanoparticles enhances drug delivery to the tissues of the eye in an animal model.
Conclusions: Nanoparticle and drug delivery can be enhanced by using particle surface features that allow tissue recognition and uptake.
Commercial Relationships: Jai Parekh, EyeTrans Technologies (S); **David Freilich**, EyeTrans Technologies (S); **Stephanie Youlios**, None; **Sima Parekh**, None; **Uday Kompella**, EyeTrans Technologies (P), EyeTrans Technologies (S)

Program Number: 5032 **Poster Board Number:** C0009
Presentation Time: 3:45 PM–5:30 PM
Nanowafer Drug Delivery to Treat Corneal Neovascularization
Ghanashyam Acharya, Xiaoyong Yuan, Daniela Marcano, Crystal Shin, Xia Hua, Lucas Isenhart, Stephen C. Pflugfelder. Ophthalmology, Baylor College of Medicine, Houston, TX.
Purpose: Development of a controlled release nanowafer drug delivery system for treating corneal neovascularization.
Methods: The axitinib-nanowafers (Axi-NW) were fabricated *via* the hydrogel template strategy with a few modifications. The nanowafers thus prepared were tested in ocular burn induced murine model. The corneas were subjected to laser scanning confocal imaging and RT-PCR analysis of gene expression.
Results: The nanowafer is a tiny transparent circular disc containing arrays of drug loaded nanoreservoirs (**Figure 1**). The *in vivo* therapeutic efficacy of the nanowafer was demonstrated by treating corneal neovascularization (CNV) in a murine ocular burn (OB) model. In this study, once a day Axi-5-NW treatment was compared with Axi eye drops (0.1%) administered twice a day for its therapeutic effect in inhibiting CNV in OB mouse model. The Axi-5-NW treatment restricted the proliferation of blood vessels to the limbal area and treated eyes very closely resembled the healthy uninjured cornea. However, the OB controls - PVA-NW and Axi eye drop treated corneas exhibited extensive neovascularization (**Figure 2a-e**). In the case of Axi-5-NW treatment, the amount of drug delivered to the cornea was 5 μg per day, and for axitinib eye drop treatment it was 10 μg per day. Although, eye drop treated mice received twice the drug dosage as those treated with Axi-5-NW, still Axi-5-NW treatment was twice as efficacious as the eye drop treatment (**Figure 2f**). The RT-PCR study revealed that Axi-NW was very effective in downregulating the drug target genes VEGF-A, VEGF-R1, VEGF-R2, PDGFR-A, PDGFR-B, TNF-α, bFGF and TGF-β, compared to the untreated OB and Axi-eye drop treatment.
Conclusions: Once a day axitinib delivery by the nanowafer is more efficacious than the twice a day topical eye drop treatment.

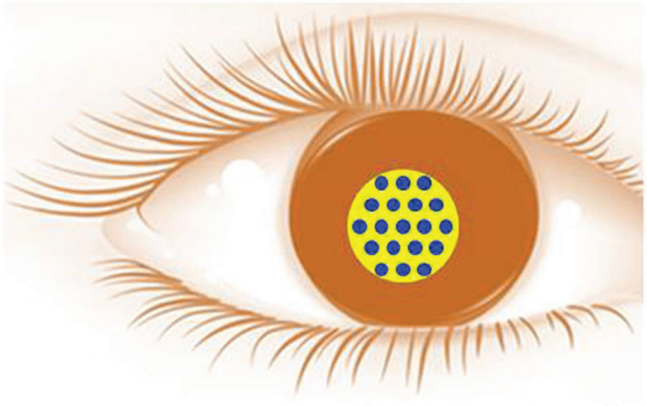


Figure 1. Schematic of the Ocular drug delivery nanowafer instilled on the cornea.

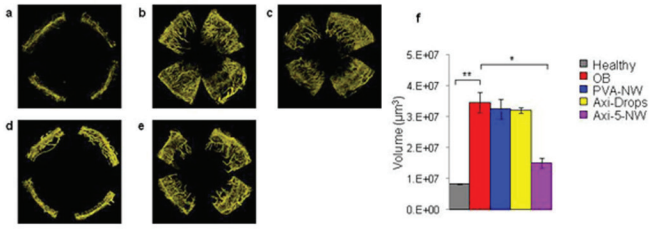


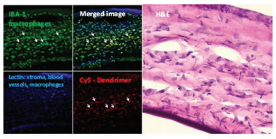
Figure 2. Axitinib-nanowafer is more efficacious than the topical eye drop treatment. Confocal fluorescence images revealing the enhanced therapeutic efficacy of Axi-nanowafer. **a**, Healthy cornea. **b**, OB induced cornea. **c**, PVA-NW. **d**, Axi-NW. **e**, Twice a day Axi-eye drop (0.1%) treatment. **f**, Quantification of corneal neovascularization volume. n = 3 animals,
* P<0.05 vs OB control. All error bars represent standard deviation from the mean.
Commercial Relationships: Ghanashyam Acharya, None; **Xiaoyong Yuan**, None; **Daniela Marcano**, None; **Crystal Shin**, None; **Xia Hua**, None; **Lucas Isenhart**, None; **Stephen C. Pflugfelder**, None

Support: Department of Defense (CDMRP) Award No.1W81XWH-13-1-0146.

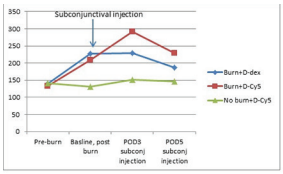
Program Number: 5033 **Poster Board Number:** C0010
Presentation Time: 3:45 PM–5:30 PM
Cubosomes nanoparticles for ocular delivery
Hong Zhang[1, 2], Jonathan P. Wigg[1], Vicki Chrysostomou[1], Terence Hartnett[3]. [1]Ophthalmology, Ctr for Eye Resrch Australia, East Melbourne, VIC, Australia; [2]Eye Hospital, Harbin Medical University, Harbin, China; [3]Department of Chemical and Biomolecular Engineering, University of Melbourne, Melbourne, VIC, Australia.
Purpose: Intraocular injection is presently the only route of administration for large protein therapeutics; however this invasive intraocular procedure needs to be repeated frequently with potential risks. We hypothesized that bicontinuous, lyotrophic liquid crystalline phases and their dispersions (cubosomes) has the potential for the prolonged and safe delivery of large molecules into the eye. We tested this hypothesis in the present study.
Methods: GMO - 1, 2 - dipalmitoyl phosphatidyl serine (DPPS) cubosomes was generated and stabilised using F127 and Pluronic® F108 (F108), a larger and more hydrophilic Pluronic® than F127. Particle size and polydispersity of the dispersions were characterized by dynamic light scattering size (DLS). A laboratory-built humidity-controlled vitrification system was used to prepare the samples for Cryo-TEM. The drug loading efficacy was also tested using the freeze-thaw technique. In vitro safety study was performed in SHSY, PRE and human scleral fibroblast cells (n=3). For in vivo safety testing, cubosomes was injected intravitreally or subconjunctivally in Sprague-Dawley rats (n=18), the functional and structural changes were evaluated by ERG and H&E staining. To test the sustained release profile, fluorescence loaded cubosomes were intravitreally injected into the eyes of Sprague-Dawley rats (n=3) and compared with the control group injected with fluorescence alone.
Results: Stabiliser selection is important as it influences the size and polydispersity of the cubosomes and demonstrate that the stability of these cubosomes is affected by changes in the temperature. In addition, we reveal that the use of F108 as a stabilising agent elicits a reduced toxic response in vitro compared to cubosomes stabilised with Pluronic® F127 ($p<0.05$). Encapsulation efficacy of FITC-BSA is 30 %. Intravitreal and Subconjunctival injections of 100ug/ml cubosome solutions into Rats have shown no evidence of changes to retinal function up to 3 months, nor changes to retinal layer thickness ($p<0.05$). We have also shown evidence of fluorescence labelled cubosomes in the ganglion cell layer after 24 hours after an intravitreal injection, however there was no fluorescence in the control group, suggesting the sustained release of fluorescence by cubosomes.
Conclusions: GMO - 1, 2 - dipalmitoyl phosphatidyl serine (DPPS) cubosomes can safely deliver large molecules into the eye and prolong the retention time of the drugs within the eye.
Commercial Relationships: Hong Zhang, None; **Jonathan P. Wigg**, None; **Vicki Chrysostomou**, None; **Terence Hartnett**, None
Support: NHMRC Project Grant : APP1047603; National Natural Science Foundation of China (Grant No. 81201184) ;2. Cheung Kong Scholars Support Program of Heilongjiang Province 2014-2016

Program Number: 5034 **Poster Board Number:** C0011
Presentation Time: 3:45 PM–5:30 PM
Persistent Pre-conditioning Effects of Nanoceria after Intravitreal Injection—a Microarray Analysis
Lily L. Wong[1], Sudipta Seal[2, 3], James F. McGinnis[1, 4]. [1]Ophthalmology, Univ of Oklahoma Hlth Sci Ctr & DMEI, Oklahoma City, OK; [2]AMPAC, University of Central Florida, Orlando, FL; [3]MSAE/ Nanosci Tech Ctr, University of Central Florida, Orlando, FL; [4]Cell Biology, OCNS, OUHSC, Oklahoma City, OK.
Purpose: Nanoceria are potent catalytic antioxidants. They are effective in slowing retinal degeneration in four rodent models. In vitro studies show that they scavenge reactive oxygen species and possess mimetic activities of two endogenous oxidative enzymes. In healthy rat retinas, nanoceria show no toxic effects apropos retinal function or morphology. In this study, we sought to reveal how the change of the redox environment after a single intravitreal injection of nanoceria altered ocular gene expression.
Methods: We delivered 0.344 ng of nanoceria in 2 μl of saline or saline alone to the vitreous of Sprague Dawley rats at 10 weeks of age. Retinas were collected at 24, 48, and 72 hours post injection. Gene expression levels were compared to uninjected animals. Nanoceria at this same dose inhibited rod degeneration in the light damage on-demand rat model when administered three days earlier or 2 hours after bright light exposure. We used *the Illumina Rat Ref-12 Whole-Genome Gene Expression BeadChips* to interrogate gene expression levels. We used Ingenuity Pathway Analysis (IPA, Qiagen) to find relevant networks and regulators of gene clusters that satisfied the 2.0 fold-change cutoff criterion.
Results: Approximately 4500 ocular genes were identified as significantly expressed at 10% false discovery rate. Over 700 genes were up-regulated after saline injection. Expression levels of these genes returned to uninjected level at 48 and 72 hours. Remarkably, these same genes were up-regulated for all three time points in the nanoceria injected eyes. The IPA analysis identified protein synthesis as the top pathway being up-regulated together with a number of regulators and biological processes that were predicted to be involved according to the gene expression pattern.
Conclusions: We conclude that nanoceria likely act analogous to dietary antioxidants (polyphenols) such as resveratrol, quercetin, etc., by inducing endogenous adaptive cellular stress responses collectively known as “hormesis” to prepare cells for potential insults. We also show that the well documented pre-conditioning effect of intravitreal injection of saline for neuroprotection triggered the same responses but short-lived. Since nanoceria in the rodent retina did not trigger undesirable cellular responses but beneficial ones, they should be deemed safe for treatment of neurodegenerative diseases in humans.
Commercial Relationships: Lily L. Wong, US 7727559 (P), US 8703200 (P); **Sudipta Seal**, US 7727559 and 8703200 (P); **James F. McGinnis**, US 7727559 (P), US 8703200 (P)
Support: NIH: P30EY021725, 1R21EY018306, R01EY018724, R01EY022111; OCAST: HR06-075; NSF: CBET-0708172 and unrestricted funds from RPB and PHF to the Department of Ophthalmology

Program Number: 5035 **Poster Board Number:** C0012
Presentation Time: 3:45 PM–5:30 PM
Subconjunctival dendrimer-drug therapy for the treatment of corneal inflammation
Uri Soiberman, Siva Pramodh Kambhampati, Manoj K. Mishra, Samuel C. Yiu, Abdul Elah Al Towerki, Walter J. Stark, Rangaramanujam M. Kannan. Ophthalmology, Johns Hopkins University, Baltimore, MD.
Purpose: To assess whether subconjunctivally administered dendrimers target activated corneal macrophages, and also whether subconjunctival dendrimer-dexamethasone (D-Dex) conjugate is efficacious in the treatment of corneal alkali burn.
Methods: A rat animal model of corneal alkali burn was used by exposure to 1N NaOH solution. 48 hours following the alkali burn, subconjunctival treatment was administered with either dendrimer-Cy5 (D-Cy5; to assess corneal macrophage uptake), or D-Dex (therapy). The rats were assessed for corneal edema, corneal opacity and development of corneal neovascularization (NV). Central corneal thickness (CCT) was assessed with anterior segment optical coherence tomography (OCT). The rats were sacrificed 7 days after the alkali burn and the corneas were cross sectioned and then prepared for histological assessment and confocal microscopy.
Results: Alkali burn led to increased macrophage accumulation, corneal thickness, opacity and edema in the central cornea. D-Cy5 was selectively co-localized with the corneal macrophages in the central cornea. D-Dex treatment resulted in favorable outcomes with reduced CCT and edema, fewer central corneal macrophages and improved corneal clarity compared to untreated controls. Corneal NV was assessed one week following alkali burn: the D-Dex treated eyes had reduced corneal NV (1.5±1.2 quadrants) as opposed to untreated eyes (4±0 quadrants) (p=0.02). Eyes treated with D-Dex did not have elevated intraocular pressures compared with controls.
Conclusions: This short-term pilot study conducted in rats demonstrates that dendrimers can target macrophages and be retained in them for up to one week following alkali burn to the central cornea. It also showed that when treated with D-Dex, the CCT was lower than in untreated control eyes. Additionally, the amount of corneal neovascularization was lower in D-Dex treated eyes. These findings suggest that dendrimers may be a potential drug delivery platform in inflammatory ocular surface disorders especially because they target immune effector cells.

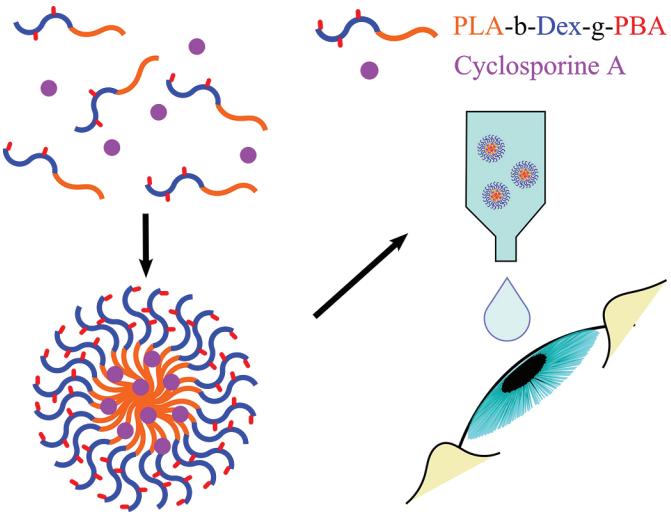


Numerous large mononuclear inflammatory cells are dispersed throughout the central cornea. The majority of these cells are stained by anti-IBA-1 and lectin which suggests that they are indeed macrophages.



Changes in central corneal thickness following treatment (microns).
Commercial Relationships: Uri Soiberman, None; **Siva Pramodh Kambhampati**, None; **Manoj K. Mishra**, None; **Samuel C. Yiu**, None; **Abdul Elah Al Towerki**, None; **Walter J. Stark**, None; **Rangaramanujam M. Kannan**, None
Support: KKESH grant

Program Number: 5036 **Poster Board Number:** C0013
Presentation Time: 3:45 PM–5:30 PM
Weekly dosing regimen of eye drop formulations delivered through mucoadhesive nanoparticles enhances treatment of experimental dry eye
Shengyan Liu[1], Matthew D. Dozois[1], Deborah L. Ng[1], Chuning Chang[1], Denise Hileeto[2], Lyndon W. Jones[2], Frank Gu[1]. [1]Chemical Engineering - Nanotechnology, University of Waterloo, Waterloo, ON, Canada; [2]Optometry & Vision Science, University of Waterloo, Waterloo, ON, Canada.
Purpose: To evaluate the long term efficacy of using once a week dosing of Cyclosporine A (CsA) loaded mucoadhesive nanoparticle eye drop formulation in treating dry eye syndrome (DES).
Methods: Experimental DES in mice was induced by applying scopolamine patches to their midtails and exposing them to desiccating environments. These mice were treated with various formulations including Restasis (thrice daily), nanoparticles without CsA (Blank NPs, once a week), CsA-loaded nanoparticles with a CsA dosage 1-2% that of Restasis (CsA-NP 1-2%, once a week) or CsA-NP 5% (once a week) for a month. Dry eye status was evaluated using tear production measurement, corneal fluorescein clearance, and histopathology analysis.
Results: After 4 weeks of treatment period, the once a week administration of CsA-NP 5% and thrice daily administration of Restasis both showed elimination of inflammatory signs, but did not recovery the integrity of the ocular surface tissues. The once a week administration of CsA-NP 1-2% showed both the elimination of the inflammation and full recovery of the ocular surface.
Conclusions: Weekly dosing regimen of CsA loaded mucoadhesive nanoparticle eye drop platform demonstrated effective treatment of experimental dry eye in mice with up to 50 to 100 fold reduction in overall dosage compared to Restasis, which may significantly reduce side effects and improve patient compliance.



Commercial Relationships: Shengyan Liu, WO2013188979 A1 (P); **Matthew D. Dozois**, None; **Deborah L. Ng**, None; **Chuning Chang**, None; **Denise Hileeto**, None; **Lyndon W. Jones**, WO2013188979 A1 (P); **Frank Gu**, WO2013188979 A1 (P)

Program Number: 5037 **Poster Board Number:** C0014
Presentation Time: 3:45 PM–5:30 PM
Potential therapeutic use of nanoparticle-loaded cells to repair damaged corneal endothelium
Lauren E. Cornell[1], Mauris Desilva[2], Anthony J. Johnson[1], David O. Zamora[1]. [1]Ocular Trauma and Vision Restoration, United States Army Institute of Surgical Research, Fort Sam Houston, TX; [2]Maxillofacial Injury & Disease Department, Naval Medical Research Unit San Antonio-JBSA, Fort Sam Houston, TX.
Purpose: Current methods to replace traumatized corneal endothelium involve the placement of partial or full thickness grafts. However, in the presence of inflammation, these grafts perform poorly and are not an optimal long term solution. An alternative, non-graft treatment for corneal endothelium repair is needed. The overall hypothesis is that corneal endothelial cells (CEC), loaded with superparamagnetic iron oxide nanoparticles (SPIONPs), can be injected into the anterior chamber of the eye while being exposed to a constant external magnetic field and be strategically targeted to damaged areas of the inner cornea. In this study, we investigate the *in vitro* biological effects of SPIONPs on corneal endothelial cells.
Methods: Bovine corneal endothelial cells (BCECs; Astarte Biologics) were cultured in DMEM and 10% FBS. BCECs were seeded in 48 well plates and maintained in culture for 48 hours. Biotin coated SPIONPs (Micromod, Rostock Germany) were added to the wells at 0, $1x10^6$, $10x10^6$, and $100x10^6$ SPIONPs per cell. SPIONP uptake was confirmed with Prussian blue staining. Cell viability was determined via MTT and Live/Dead staining after being in the presence or absence of a magnetic field. The effect of magnetic force on BCEC cytoskeletal structure was evaluated using rhodamine phalloidin staining. Comparisons between groups were made using either a two tailed t-test or a one-way analysis of variance with values of $p \leq 0.05$ considered statistically significant.
Results: BCEC viability was maintained at all SPIONP concentrations over three days with the exception of $100x10^6$ SPIONPs per cell, where a significant decrease ($p<0.05$) in viability occurred. Furthermore, BCEC viability significantly increased ($p<.05$) upon exposure to magnetic force at all SPIONP concentrations tested. There were no observed differences in the arrangement of actin filaments within the cytoskeletal structure of any conditions evaluated.
Conclusions: These results demonstrate that SPIONP loaded endothelial cells have potential for magnetically controlled cell guidance, as their viability and cytoskeletal structure were unaffected upon SPIONP incorporation and exposure to magnetic force. The information gleaned from this study will help advance cell guided delivery technology of human CECs to the damaged corneal endothelium, thus providing an alternative therapeutic option to corneal grafting.
Commercial Relationships: Lauren E. Cornell, None; **Mauris Desilva**, None; **Anthony J. Johnson**, None; **David O. Zamora**, None

Program Number: 5038 **Poster Board Number:** C0015
Presentation Time: 3:45 PM–5:30 PM
Loteprednol Drug Delivery System for Treatment of Choroidal Neovascularization in Age-Related Macular Degeneration
Anjali Hirani[1, 2], Yong W. Lee[2], Vijaykumar Sutariya[1], Yashwant Pathak[1]. [1]Pharmacy, University of South Florida, Tampa, FL; [2]Biomedical Engineering, Virginia Tech, Blacksburg, VA.
Purpose: Age-related macular degeneration is the leading cause of vision loss in adults over 50. The neovascular form is characterized by choroidal neovascularization (CNV); growth of abnormal blood vessels under the retina which can leak blood and fluid and cause deterioration to the macula over time. Current treatments utilize frequent intravitreal injections which can result in retinal detachment and increased ocular pressure. In this study, we investigated a nano sustained drug delivery system for the treatment of choroidal neovascularization present in wet age-related macular degeneration.
Methods: The drug delivery system (DDS) was comprised of loteprednol etabonate, poly(ethylene glycol)-ylated (PEGylated) poly-(lactide-co-glycolide) (PLGA) nanoparticles (NPs), and a PLGA-PEG-PLGA thermoreversible gel. The loteprednol-loaded NPs were prepared using the nanoprecipitation method. The loteprednol NPs were then incorporated into a 20% (w/v) thermoreversible gel prepared using the cold method. An *in vitro* dialysis method was employed to measure the release of loteprednol from the DDS over time. Cellular uptake imaging nanoparticles in human retinal pigment epithelial cells (ARPE-19) was studied via confocal microscopy. The effect of the DDS on VEGF secretion was quantified by ELISA method.
Results: Nanoparticle characterization data showed an average particle size of 168.60 ± 23.18 nm, polydispersity index of 0.0142 ± 0.0023 nm, and encapsulation efficiency of 82.6%. *In vitro* release results demonstrated free loteprednol was completely released within 48 hours; whereas loteprednol was released from the DDS over 10 days. Cellular uptake imaging demonstrate complete uptake of loaded nanoparticles in ARPE-19 cells within 1 hour. Cells treated with 10 μM loteprednol DDS showed a significant reduction of VEGF secretion of 16% as compared to the equivalent treatment of loteprednol alone.
Conclusions: These results suggest that the proposed delivery system for loteprednol could be an effective sustained release treatment for ocular dysfunction.
Commercial Relationships: Anjali Hirani, None; **Yong W. Lee**, None; **Vijaykumar Sutariya**, None; **Yashwant Pathak**, None

Program Number: 5039 **Poster Board Number:** C0016
Presentation Time: 3:45 PM–5:30 PM
Comparison of second and third generation photoswitches in restoration of vision in blind mice
Kuldeep Kaur[1], Laura Laprell[2], Joseph Nemargut[1], Dirk Trauner[2], Russell Van Gelder[1]. [1]Department of Ophthalmology, University of Washington, Seattle, WA; [2]Department Chemie und Biochemie, Ludwig-Maximilians-Universität München, Butenandtstr. 5-13, Haus F, Germany.
Purpose: Small molecular photoswitches bind to endogenous ion channels in neurons and change their activity in response to light. In this study, we used small molecular photoswitches, phenyl-ethyl aniline azobenzene quaternary ammonium (PhENAQ), a second generation photoswitch and red-DAD, a third generation compound to restore light sensitivity in the retina from the adult blind mice.
Methods: **Multielectrode Array Electrophysiology:** The light responses from adult *Opn4$^{-/-}$;rd/rd* blind mice retinal explants were recorded on multi-electrode array following brief application of PhENAQ and red-DAD. We analyzed the light-triggered firing of ganglion cells in response to different wavelengths and intensities of light.
Intravitreal Injections: PhENAQ and red-DAD were tested *in vivo* by injecting these compounds intravitreally into *Opn4$^{-/-}$;rd/rd* mice. The dark-light shuttle box test was performed on *Opn4$^{-/-}$;rd/rd* mice pre-injection and post-injection. Mice injected with PhENAQ and red-DAD were compared to the wild-type mice.
Results: PhENAQ and red-DAD both bestowed light sensitivity onto blind mice retinal explants, which responded to broad-spectrum white or blue light. Typically, the light increased ganglion cell firing with rapid cessation in the dark. PhENAQ and red-DAD selectively photosensitized RGCs from retina with degenerated photoreceptors but not from wild-type retinas. The photoswitch red-DAD is soluble in FDA approved excipients and diffuses very quickly in the vitreous of macaque in comparison to PhENAQ. Red-DAD showed higher potency compared to PhENAQ. *In vivo* experiments indicate that a single injection of red-DAD is sufficient to restore some light-dependent behavior in the blind mice.
Conclusions: Application of PhENAQ and red-DAD on degenerated retina confers light sensitivity *in vitro* and *in vivo*. In the absence of the classical photoreceptors the photoswitch compounds might have therapeutic applications to treat retinitis pigmentosa or macular degeneration by conferring photosensitivity on retinal ganglion cells.
Commercial Relationships: Kuldeep Kaur, None; **Laura Laprell**, None; **Joseph Nemargut**, None; **Dirk Trauner**, None; **Russell Van Gelder**, None
Support: PN2EY018241, 1R24EY023937, P30EY001730, and an unrestricted grant from Research to Prevent Blindness

Program Number: 5040 **Poster Board Number:** C0017
Presentation Time: 3:45 PM–5:30 PM
TRANSCHOROIDAL SUBRETINAL CHIP IMPLANTATION TO REGAIN VISION IN BLIND RP PATIENTS. MANAGEMENT OF CHOROIDAL PROBLEMS
Helmut G. Sachs[1], Karl Ulrich Bartz-Schmidt[2], Florian Gekeler[2], Katarina Stingl[2], Eberhart Zrenner[2]. [1]Eye Clinic, Klinikum Dresden Friedrichstadt, Dresden, Germany; [2]Institute for Ophthalmic Research, Tuebingen, Germany.
Purpose: Active subretinal visual prostheses require a transchoroidal implantation. The reason fot that is the necessity of an external energy supply. Surgically the choroidal access was assumed to be one of the major problems.
Methods: 26 legally blind RP patients were included in the multicentre study to regain vision with electronic chips. Patients were implanted with a chronic active prosthetic subretinal device via a transchoroidal surgical access. A subgroup of 12 out of these 26 patients were operated by one surgeon who had developed the transchoroidal procedure and had experience with it. Only this subgroup was analyzed to eliminate the learning curve effects of multiple surgeons and to estimate the frequency and the outcome after occurence of choroidal promlems.
Results: All implantations were carried out successfully and led to a stable retinal situation. Feared major bleedings were no observed. Accidental perforation of the choroid occurred (1) in the area of the posterior pole. Problems were observed when advancing the guide foil or the implant subretinally due to adhesions between RPE and retina. Adhesions seem to be common in the area of dense pigmentation or scaring in RP patients. Sub RPE implantation occurred twice but could be corrected within the same surgical session. This never was associated with bleedings or other unexpected effects.
Conclusions: Problems resulting from the choroid occur but are are manageable and seem not to be a limiting factor for this procedure. Perforations of the choroid that occurred did not have a threatening or harmful decisive side effect. Proper guide foil and implant design are relevant. Design of this tools are under improvement to minimize incidents resulting from this challenge. Difficult retinal situations are no contraindication for chip implantation.
Commercial Relationships: Helmut G. Sachs, Retina Implant (C), Retina Implant (F), Retina Implant (R); **Karl Ulrich Bartz-Schmidt**, None; **Florian Gekeler**, Retina Implant (C), Retina Implant (F); **Katarina Stingl**, Retina Implant (F), Retina Implant (R); **Eberhart Zrenner**, Retina Implant (C), Retina Implant (F), Retina Implant (I), Retina Implant (P)
Support: Deutsche Forschungsgemeinschaft (EXC 307, CIN) HOPE2-FKZ 01GQ1002, Retina Implant AG study sponsor support
Clinical Trial: (1) NTC00515814, (2) NTC01024803, (3) NTC01497379