Silk Fibroin As A Biomaterial Substrate For Corneal Epithelial Cell Sheet Generation

**Program Number:** 267 Poster Board Number: A508  
**Presentation Time:** 8:30 AM - 10:15 AM

**Purpose:** To evaluate a silk fibroin (SF) biomaterial as a substrate for corneal epithelial cell proliferation, differentiation and stratification in vitro compared with denuded amniotic membrane (AM).

**Methods:** Primary human and rabbit corneal epithelial cells and immortalized human corneal limbal epithelial cells were cultured on the SF and denuded AM, respectively. The biological cell behavior including the morphology, proliferation, differentiation and stratification on the two substrates were compared and analyzed.

**Results:** Corneal epithelial cells can adhere and proliferate on the SF and denuded AM with a cobblestone appearance, abundant microvilli on the surface and wide connection with the adjacent cells. MTT assay showed that cell proliferation on denuded AM was statistically higher than that on SF at 24 and 72 hours post plating (p = 0.001 and 0.0005, respectively). Expression of β63αα and keratin 12 were detected in primary cells cultures on the two substrates with no statistical difference. When cultured at the air-liquid interface for 10 days, cells on SF could form a comparable stratified graft with a 2 to 3 cell layering which compared similarly to AM cultures.

**Conclusions:** SF, a novel biomaterial, could support corneal epithelial cells to proliferate, differentiate and stratify retaining the normal characteristic epithelium phenotype. Compared with AM, its unique features including the transparency, ease of handling and transfer, and inherent freedom from disease transmission make it a promising substrate for corneal wound repair and tissue engineering purpose of it.

**Commercial Relationships:** None; Prevent Blindness Career Development Award, NYSTEM, and Tri-Institutional

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decreased cell viability and increased the generation of reactive oxygen species (ROS, measured by Mitoxon).

**Conclusions:** Treatment with TiO$_2$ nanoparticles and UVA radiation caused phototoxic damage to ARPE-19 cells. The mechanism of phototoxicity apparently involved ROS formation. The LC$_{50}$ values of different samples of TiO$_2$ under UVA radiation were directly correlated with particle surface area, and indirectly correlated with primary particle size.

**Commercial Relationships:** Joan E. Roberts, None; William K. Boyes, None; Kristen Sanders, None; Laura L. Degen, None; Robert Zucker None; William R. Mundy, None; Kevin Dreher, None; Baosheng Zhao, None

**Support:** US EPA STAR Grant RD-833772

**Program Number:** 271 Poster Board Number: AS12

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

**Analysis And Development Of New Surgical Instruments For Ophthalmic Practice**


**Purpose:** to register the design, development and testing of new surgical instruments in order to establish a pipeline for creation of new technologies and products for the ophthalmologic practice

**Methods:** This study was planned with the following landmarks: Production Selection, Functional Description, Numerical Evaluations of the Functions, Training Cost, Specification and Requirement, Rapid Prototyping and Re-Evaluation. All instruments were made with surgical grade stainless steel using the UNIFESP’s Bioengineering Laboratory with strict dimensions set for ophthalmologic surgery. The instruments were used in ocular surface surgery, cornea and refractive surgery, cataract surgery, retinal and vitreous surgery. All surgeons were asked to answer survey about the performance and impressions regarding the instrument

**Results:** Seven surgical instruments were built and tested in surgeries. The instruments were: atrumatic forceps for amniotic membrane surgery, atrumatic needle tweezers for penetrating keratoplasty, toric marker for refractive and cataract surgery, capsaicinorhexis forceps, IOL extraction forceps, chopper for phacovulsification, microcannula with external lumen 20-gauge and internal lumen 23-gauge and microcannula for cromevitrectomy

**Conclusions:** All instruments had an overall positive response from the surgeons. We are confident that this project was essential to propel further research and technological developments of surgical innovation in this still unexplored academic area.

**Commercial Relationships:** Juliana D. Sartori, None; Milton S. Yogi, None; Vagner R. Santos, None; Cesar Bernalia, None; Fernando Jopetipe, None; Leticia Barroso, None; Anderson Teixeira, None; Paulo Schor, None

**Support:** None

**Program Number:** 272 Poster Board Number: AS13

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

**Nanoscale Probes and Platforms for Analysis of Gene Expression in Living RPE Cells**

Christina Winborn$^{a,b}$, Timothy E. McKnight$,^c$ John C. Lang, $^d$Edward Chaum$^{a,b}$

$^a$Ophthalmology, $^b$Biomedical Engineering, $^c$The University of Tennessee Health Sci Ctr, Memphis, TN, $^d$Measurement Science and Systems Engineering Division, Oak Ridge National Laboratory, Oak Ridge, TN, $^e$Consumer Products R&D, Alcon Research Ltd, Fort Worth, TX.

**Purpose:** Molecular beacon (MB), self-quenching hairpin-shaped oligonucleotides that fluoresce upon hybridization to target mRNA molecules, have recently been used to detect gene expression in living cells. The goal of these studies was to utilize MBs to quantify gene expression in retinal pigment epithelial cells (RPE). We quantified expression of both an IGF1 transgene and oxidative stress-induced (OS) FOS expression in the RPE in vitro. We also examined strategies for tethering MBs to carbon nanofiber (CNF) arrays, nanostructured cell-penetrating platforms with the potential to signal gene expression in living cell monolayers.

**Methods:** We designed and synthesized MBs for FOS, IGF1, GAPDH, and a non-binding negative control. MBs were delivered into RPE cells via linkage to the cell penetrating peptide TAT1. Gene expression (as seen by MB fluorescence) was quantified using confocal image analysis and Nikon EZC1 software. We quantified MB fluorescence in IGF1-transfected cells relative to naive RPE cells with low intrinsic IGF1 expression. We also quantified OS-induced FOS gene expression in RPE cells exposed to OS. Finally, we tethered IGF1 or FOS MBs to a CNF array and quantified focal fiber fluorescence in the presence of complimentary oligonucleotides.

**Results:** We observed high IGF1-specific MB fluorescence in RPE cells stably transfected with IGF1, 11-fold greater than fluorescence seen in naive RPE cells. Similarly, FOS MB fluorescence increased 9-fold (compared to controls) in cells exposed to OS. These quantitative changes in gene expression were validated by qRT-PCR performed in replicate RPE cell cultures. Finally, CNF arrays with tethered IGF1 and FOS MBs show focal fiber fluorescence in the presence of complimentary oligonucleotides, demonstrating that they can be used as nanoscale platforms to signal gene expression in living cell monolayers.

**Conclusions:** Molecular beacons are novel nanoscale probes that can be used to detect and quantify gene expression in living RPE cells under a variety of experimental paradigms.

**Commercial Relationships:** Christina Winborn, Alcon Research Ltd (F); Timothy E. McKnight, None; John C. Lang, Alcon Research Ltd (F, P); Edward Chaum, Alcon Research Ltd (F, P)

**Support:** Alcon Research Ltd., UTHSC Neuroscience Institute, UTHSC Clinical Translational Science Institute Award, Research to Prevent Blindness, The Plough Foundation, The Lions of Arkansas Foundation Inc.

**Program Number:** 273 Poster Board Number: AS14

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

**The Effects of Electrical Stimulations on Neurite Outgrowth of Goldfish Retinal Explants**

Chuan-Chin Chiao$^{1,*,1A,1B}$, Yi-Ting Ou$^{1A,1B}$, Michael S. Lu$^{1A,1B}$

$^1$Department of Life Science, $^2$Institute of Systems Neuroscience, $^3$Department of Electrical Engineering, $^4$Institute of Electronics Engineering, $^5$National Tsing Hua University, Hsinchu, Taiwan.

**Purpose:** Central nervous system (CNS) in adult mammals loses the ability to regenerate after injury. Although electrical stimulation (ES) has been shown to promote neural regeneration, the underlying mechanisms of how ES enhances CNS regeneration remain elusive. The present study was aimed to investigate the effect of ES waveforms on neurite outgrowth of goldfish retinal explants and to elucidate possible cellular mechanisms responsible for the observed ES effects.

**Methods:** The optic nerve of adult goldfish was intraorbitally crushed 7-14 days before retinal explants preparation. The explants were cultured in the laminin-coated ITO conductive device for ES application. Various waveforms of ES were applied to examine its effect on neural regeneration. The effect of calcium, PKC, and IGF-I on neurite outgrowth under ES were also studied using specific inhibitors.

**Results:** When the retinal explants were stimulated for one hour with intermittent pulses at Day 1, the regenerated neurite length was significantly increased compared to those stimulated with continuous square waves or continuous pulses on the same day. We also demonstrated that ES could induce calcium influx through voltage gated calcium channels, and the enhancement of neurite outgrowth after ES application was reduced by protein kinase C (PKC) inhibitor. Moreover, we found that Insulin-like growth factor I (IGF-I) did not further facilitate the ES effect, and ES alone did not rescue neurite outgrowth reduced by inactivating IGF-I receptors.

**Conclusions:** These results suggest that intermittent pulse ES promotes neurite
Modified 23-Gauge Microincisional Vitrectomy Surgery (MIVS) Technique
Federica Genovesi-Ebert
For The Implant Of The Argus II Retinal Prostesis

Methods:
RPE cells are normally attached. However, in advanced retinal disease the basal lamina layer of BM may be damaged. Thus, it is increasingly difficult for newly transplanted cells to attach in this non-permissive environment. Therefore we propose the manufacture of a novel artificial BM to facilitate RPE cell transplantation.

Results:
SEM images indicated the formation of a cellular monolayer on the surface of the scaffold. Cells appeared to have retained a typical cobblestone morphology with apparent apical microvilli, indicative of appropriate polarization. By day 15, cell area was significantly (>p<0.001) enhanced on scaffolds with chemical modification of the PEG chain terminus. In addition, significantly less apoptotic cell death was demonstrable on these modified surfaces.

Conclusions:
This system shows great potential for use as an artificial BM, providing a permanent support to the RPE.

Commercial Relationships:
Heather A. Thomson, None; Andrew J. Treharne, None; Jennifer A. Scott, None; Martin C. Grossel, None; Andrew J. Lotery, None

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Results: In Group A BCVA and Ps t improves in the first 6 months followed by a tendency to return to their initial values. In Group B we have a growing improvement, up to one year after treatment, while in Group C we observe a stable functional values. The probably explanation of the different evolution is due to the different conditions of retinal integrity at the time of the implant of the grown factors. Higher is the impairment of the retina, smaller and of shorter duration will be the functional recovery. If the retinal impairment is moderate, grown factors could improve the cell restoring the visual function.

A retinal in good condition benefits of grown factors through the reduction of apoptosis and the consequent stabilization of visual performance.

Conclusions: The retinal sensitivity can show the prognosis of grown factors implant.

Based on the value of decibles we can know beforehand the extent of improvement and its duration.

The worsening of the visual conditions could be considered as a parameter for any further administration of grown factors in order to contain apototic retinal activity over time.

Commercial Relationships: Paolo G. Limoli, None; Renzo Carpi, None; Filippo Tassi, None; Enzo M. Vingolo, None; Laura D’Amato, None; Enrico Giacomotti, None; Roberta Solari, None; Riccardo Di Corato, None

Support: None

Program Number: 278 Poster Board Number: A519
Presentation Time: 8:30 AM - 10:15 AM

Retinitis Pigmentosa (RP) patients’ eligibility for Argus II retinal prosthesis system

Stanislao Rizzo 1, Federica Genovesi Ebert 1, Emanuele Di Bartolo 1, Simona Muri 1, Luca Allegrini 1, Laura Cellini 1, Maura Arisiero 1, Gregoire Cosenza 2, Fatima Anfossi 2, Argus II Study Group, 1UO Chirurgia Oftalmica, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy; 2Second Sight Medical Products Switzerland Sàrl EPFL-PSE A, Lausanne, Switzerland.

Purpose: We describe in detail the procedure we used for identifying the most suitable candidates to receive the Argus II retinal prosthetic system.

Methods: In order to identify patients who could benefit the most from the Argus II retinal implant, we performed a screening as follows:

1) First phone interview: we verified that the patients met a few basic criteria: adult (25 years old or older); severe to profound outer retinal degeneration; some residual light perception; previous history of useful form vision.

We then asked if they had other associated diseases (e.g., retinal detachment, glaucoma, etc.), if they ever had ophthalmic surgery and if they suffered from Usher’s Syndrome (hearing loss associated).

More general questions were then asked about their past and present school and work activities (to understand their educational level and learning speed), as well as questions about their general health status (cardio-vascular problems, neurologial disorders, etc.)

2) In-clinic screening: the patients pre-selected via the phone interview underwent a more comprehensive screening at the hospital: eye examination (visual acuity, intraocular tone, state of the conjunctiva, cornea, lens, presence of nystagmus, ocular motility, fundus examination), Goldmann visual field, OCT, Fluorescein Angiography, Fundus Photos, ultrasonic A-Scan, Photo Flash Test, Lontdol C, Square localization, Direction of Motion, Grating Visual Acuity. Consensus within medical team members: once the first two steps of screening were completed, we reviewed all the medical and psychophysical results to assess the best candidates.

Results: From an initial list of 119 patients, all suffering from Retinitis Pigmentosa at different stages, 15 blind candidates were selected for thorough in-clinic screening. From the comparative results of these tests, 13/15 patients were found eligible to receive an Argus II implant.

Conclusion: This is the first time we standardized a series of medical and psychophysical tests, widely accepted by the community and readily available in any eye hospital, for assessing retinal implant candidates. Results suggest that our selection process could quickly become a de facto standard in the field. Tests are fast (2h/patient), easy to perform, well tolerated by the patients, and with a clear outcome. The number of eligible patients is high with respect to the total screened patients.

Commercial Relationships: Stanislao Rizzo, None; Federica Genovesi Ebert, None; Emanuele Di Bartolo, None; Simona Muri, None; Luca Allegrini, None; Laura Cellini, None; Maura Arisiero, Second Sight Medical Products Switzerland (E); Gregoire Cosenza, Second Sight Medical Products Switzerland (E); Fatima Anfossi, Second Sight Medical Products Switzerland (E)

Support: None
Non-human primate models of glaucoma.

Results: Multiple monthly magnetized MSC injections provided the most significantly enhanced retinal preservation. Dark-adapted maximal retinal function was about 20% and 60% of wildtype, respectively. Photopic b-wave amplitude was 56% of wildtype. We examined the efficacy of nanoparticle over a span of 4 to 7 weeks post injection and evaluated retinal function by electroretinography and retinal cytoarchitecture by optical coherence tomography (OCT) and histology.

Conclusions: We have developed small peptide-based probes for the in vivo imaging of retinal ganglion cell apoptosis. The probes consist of a cell-penetrating peptide (CPP) targeting moiety and a fluorophore (Alexa Fluor 488)-quencher (Qsy7) pair flanking an effector caspase consensus sequence (DEVD). Intracellular probe activation consists of DEVD cleavage by activated effector caspases (Qsy7) followed by fluorescence imaging of the fundus using the Heidelberg Retinal Angiograph 2 (HRA2) confocal scanning laser ophthalmoscope (CSLO) and quantified as foci per wide-angle field using ImageJ software. Electroretinography (ERG) was performed following intravitreal injection of PBS, 0.193 and 0.387 nmol TcpQ488 to determine potential probe toxicity. Results: In vivo fluorescence imaging of the fundus revealed distinct single-cell probe activations as an indicator of RGC apoptosis. P30 mice were injected with PBS and TcpQ488 at 0.193 and 0.387 nmol. The number of detectable fluorescent RGCs per field increased with exposure to increasing doses of NMDA. The sensitivity of this detection was significantly increased with increasing doses of probe, plateauing at 0.387 nmol. Additionally, ERG testing in rats receiving intravitreal injections of TcpQ488 at 0.193 and 0.387 nmol showed no significant difference compared with PBS-only injections.

Conclusions: We have optimized the signal-to-noise ratio of caspase-activatable CPP probes for quantitative detection of RGC apoptosis in vivo using CSLO fluorescence imaging. Probe-induced net detectable toxicity by ERG at clinically relevant concentrations. We plan to further assess these probes in advanced rat and non-human primate models of glaucoma.

Support: NIH P50 Molecular Imaging Center Grant, P30 EY02687 core grant, R01 EY195987-11, F32 EY0051-01, American Glaucoma Society Mid-Career Clinician Scientist Award, Research to Prevent Blindness

Program Number: 283 Poster Board Number: AS24
Presentation Time: 8:30 AM - 10:15 AM

High-Resolution MRI Imaging Reveals Retinocochoral Lymphatic Drainage during the Development of Subretinal Neovascularization

Tongalp H. Yezlik1,2, Jiafuyi El Annany1, Qun Zeng3, Raheleh Rahimi Darabad1,2, Raymond C. Chang1, Chin K. Ng1,2,3, Lily L. Wong, 3U. Meichoud 1Pharmacology and Toxicology, 1University of Louisville, Louisville, KY.

Purpose: To demonstrate the occurrence of retinal lymphangiogenesis during the development of subretinal choroidal neovascularization and to track the drainage route of retinocochoral lymph vessels.

Methods: CNV was induced in adult brown Norway rats (n=4) using a double-frequency YAG laser (532 nm; 600 mW; 0.1 sec; 50 µm; 8-12 spots/eye). A transcorneal lens extraction was performed 24 hours later to create adequate space for the subsequent intravitreal injection of magnetic nano-particles and avoid any reflux into the pericellular structures. 10 days later, 10 µl of iron oxide nano particles (10-15 nm, 5 mg/ml) were injected into the vitreous cavity. Nano particles were imaged with a birdcage quadrature volume coil in an Agilent 9.4T/31cm MRI scanner for up to 30 minutes. The tract of the lymphatic drainage was determined by acquiring multiple sagittal slices of 1 or 2 mm thickness without slice-interspacing using Fast Spin-Echo Multi-Slice (FSEMS, TR=2000 ms, TE=30 ms) pulse sequence for the head and neck region. FSEMS scans were repeated every 5 to 10 min. Subsequently, animals were sacrificed and their brain was cross-blotted with retina extracts to determine whether lymphatic drainage from the retina resulted in the novo antibody production against retinal auto-antigens. Animals that received subconjunctival magnetic particles and intravitreal PBS were used as

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Program Number: 283 Poster Board Number: AS24
Presentation Time: 8:30 AM - 10:15 AM

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Different materials and coatings were revealed to be most biocompatible and stable for future long-term clinical trials. Silicon carbide (SiC) is nontoxic and effective dielectric. Thin layer of SiC is sufficient for electrode isolation. Different materials and coatings were tested to reveal most biocompatible and effective materials. In these animals, magnetic nanoparticles appeared within periocular lymphatics four minutes after intravitreal injection, and they reached to the superficial and deep cervical lymph nodes within 30 minutes. Animals that received the magnetic nano tracer into the subconjunctival space also revealed a similar lymphatic drainage pattern. Cross-blotting of the retinal proteins with the plasma of the experimental group revealed de novo antibody production against 12 retinal proteins.

Conclusions: Lymphangiogenesis occurs along with subretinal angiogenesis in laser-induced animal model of choroidal neovascularization. Lymphatic drainage from the retina results in an antibody response to a retinal autoantigens.

Commercial Relationships: Tongyi H. Teed, None; Jaafar El Amnan, None; Qun Zeng, None; Raheleh Rahimi Darabad, None; Raymond C. Chang, None; Chin K. Ng, None; Shlomit Schaff, None

Support: An unrestricted grant from Research to Prevent Blindness, Inc. NY, NY

Program Number: 284 Poster Board Number: A525
Presentation Time: 8:30 AM - 10:15 AM

Characterization of Decellularized Cornea Using Nonlinear Optical Microscopy
Praveena Gupta1, Stephanie Vega1, Joan Nichols1, Joachim Cortiella1, Massoud Motamedi1, Bernard F. Godley1, Gracie Vargas1, 2
1Ophthalmology & Visual Sciences, 2Internal Medicine and Infectious Diseases, 3Anesthesiology, 4Optical Sciences, 5Center for Biomedical Imaging, 1Univ of Texas Medical Branch, Galveston, TX.

Purpose: Recent advances in tissue engineering have led to the development of decellularized corneal matrix as a promising native scaffold in engineered transplantable corneas. There is a need for noninvasive spatial and temporal monitoring of quality and integrity of the tissue matrix and cultures toward optimizing the design of transplantable corneas. The aim of this study was to characterize and monitor the subcellular depth-resolved properties of the decellularized cornea for tissue engineering by noninvasive imaging using multimodal nonlinear microscopy, including multiphoton microscopy and second harmonic generation microscopy (SHG).

Methods: Freshly trephined pig corneas were processed by incubating in 2-4% sodium dodecyl sulfate and placed into a rotating bioreactor containing 1% SDS for 3-5 weeks at room temperature. Subsets of corneas were exposed to riboflavin/UV A cross linking prior to processing for decellularization. Unstained decellularized corneal images were acquired using multiphoton autofluorescence microscopy (MPAM) and SHG to evaluate the matrix architecture, followed by histology to visualize the different layer of the cornea. To confirm that the acellular matrix is biocompatible, decellularized corneas were incubated with live cell cultures at different time points and then imaged for cell survivability and growth. Cells were stained with Hoechst 33342 and Calcein-AM Green to visualize the nucleus and cytoplasm of live cells respectively by two-photon microscopy.

Results: Porcine corneas were decellularized completely as evidenced by autofluorescence/Hoechst imaging. The fibrillar collagen architecture was preserved after the decellularization process as analyzed by SHG. The UV A cross linking actually prevented the tissue from mechanical damage and decreased osmotic swelling in culture medium. The regular lamellar structure of the cornea was maintained along with its transparency even after three-four weeks of culture, especially in irradiated corneas. Sheets of epithelial cells were formed on the apical surface of the matrix after repopulating with corneal limbal stem cells in culture medium.

Conclusions: Multimodal nonlinear optical microscopy provided assessment to depths not achievable by traditional confocal microscopy and provided valuable information regarding matrix integrity and makeup as well as monitoring of the ex vivo culture. Engineered corneal bio-matrix may be an ideal scaffold to repair, repopulate scarred corneal tissue for transplantation purposes.

Commercial Relationships: Praveena Gupta, None; Stephanie Vega, None; Joan Nichols, None; Joachim Cortiella, None; Massoud Motamedi, None; Bernard F. Godley, None; Gracie Vargas, None

Support: RPB

Program Number: 285 Poster Board Number: A526
Presentation Time: 8:30 AM - 10:15 AM

Biocompatibility Investigation of SiC and Varnish Coatings of Microelectrode Array (MEA) for Cortical Visual Prosthesis (CVP)

Purpose: New promising results of non-invasive neuronal stimulation have been reported, however, implantation of MEA with good dielectric coating provides best stimulation and is the most effective way to receive distinct responses from individual groups of neurons creating functionally useful images for a patient.
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Commercial Relationships: Stuart P. Richer, Travel (R); William R. Stiles, None; Lawrence Ulanski, II, None; Carla Thomas, Resveratrol Partners (C)
Support: None

Program Number: 287 Poster Board Number: A528
Presentation Time: 8:30 AM - 10:15 AM

Localized Visuotopic Spatial Stimulation of the Penetrative Optic Nerve Visual Prosthesis
Liming Li, Yiliang Lu, Yan Yan, Yao Chen, Xinyu Chai, Qiushi Ren. School of Biomedical Engineering, Shanghai Jiao Tong University, Shanghai, China.

Purpose: The purpose of this study is to develop a method of locating the penetrating electrodes inserted into the optic nerve (ON) in the visual field, and further to investigate the spatial properties of electrically evoked potentials (EEPs) elicited by ON stimulation and that of visual evoked potentials (VEPs) elicited by local visual stimulation.

Methods: Craniotomy was performed to expose the ON and visual cortex of the cat. Five linearly configured 100KΩ platinum-iridium wire electrodes insulated by Teflon with inter-electrode spacing of 0.15 mm were inserted into the ON ~2 mm posterior to eyeball for both recording and stimulation. The cortical responses in V1 were recorded epidurally by a 5 × 6 silver-ball electrode array in both hemispheres. SPARSE noise method was established to map visuotopic positions of both ON and V1 electrodes. Local flashing spot stimulation with various luminance and diameters was applied in the visual field corresponding to that of ON electrodes. Spatial properties of EEPs elicited by ON stimulation and VEPs elicited by local visual stimulation were investigated.

Results: The visuotopic positions of ON and V1 electrodes could be mapped by sparse noise method. The maximal EEP response site in V1 visuotopic map has a good correspondence with the ON stimulating electrode site in visual field. The positions of maximal EEP response changed visuotopically when the stimulating electrodes were inserted in various depth and positions of the ON. The stimulating threshold was 3.9 ± 1.5 µA and 321 ± 123 µC/cm². The spatial spread of EEPs to ON stimulation at 1 to 5 times of current threshold ranged from 2.4 to 4.9 mm, which was comparable to that of VEPs to local visual stimulation at the same visual field with spot radius from 1 to 5.2 degree.

Conclusions: The positions of ON electrodes in the visual field could be mapped by sparse noise method through in-vivo ON recording in cats. Localized visuotopic spatial stimulation could be achieved by penetrating electrodes in various depths and positions of the ON.

Commercial Relationships: Liming Li, None; Yiliang Lu, None; Yan Yan, None; Yao Chen, None; Xinyu Chai, None; Qiushi Ren, None
Support: The National Basic Research Program of China (973 Program, 2011CB707502), the National Natural Science Foundation of China (60971102, 61171174)

Program Number: 288 Poster Board Number: A529
Presentation Time: 8:30 AM - 10:15 AM

Population Of An Injectable Hydrogel Surface With Retinal Pigment Epithelium (RPE): A Method For Repairing Human Bruch’s Membrane (bm)
Qun Zeng1A, Tonggal H. Tezel2A, Andrea Gobin1B, Xinyu Chai3

Support: None

Program Number: 289 Poster Board Number: A530
Presentation Time: 8:30 AM - 10:15 AM

The Long-term Result of Corneal Correction of Presbyopia with Kera Laser
Woo C. Park1A, Sae H. Rho1A, Sang W. Jin1A, Ki S. Park1A, Byung M. Min1A, Jong H. Lee1A, Dept of Ophthalmology, 1Dong-A University Hospital, Busan, Republic of Korea; 2Woori Eye Clinic, Daejeon, Republic of Korea; 3Balgeunsesang Eye Clinic, Seoul, Republic of Korea.

Purpose: To Analyze the long term results of corneal correction of presbyopia using Kera laser (Kera laser Inc, Spain)

Methods: The study included 137 eyes of 74 patients (FU: mean 9.82 months) treated LASIK presbyopia correction using Kera laser. Patients were divided into three groups (myopic, emmetropic, and hyperopic presbyopia). Mean patient age was 52.07 years, mean preop, spherical equivalent was -4.73D (myopic), 0.13D (emmetropic), and 1.51D (hyperopic). The ablation pattern creates a multifocal corneal profile of central 3 mm optical zone for distance and next up to 9 mm for near correction. Main outcome measures were uncorrected visual acuity (UCVA) and best corrected visual acuity (BCVA) for near and distance, spherical equivalent refraction and contrast sensitivity at preoperative & postop. 1 week, 1 month, 3 months, 6 months, 12 months, 2 years and 3 years.

Results: Three year postoperatively, mean UCVA was 0.93±0.17 for distance, 0.70±0.13 (nearly 12) for near at myopic group, and 0.88±0.26 for distance, 0.89±0.21 (nearly 11) for near at emmetropic group, and 0.93±0.27 for distance, 0.66±0.19 for near at hyperopic group. Mean postoperative spherical equivalent refraction was -0.74±0.93D at myopic group, -0.13±0.67D at emmetropic group, and 0.10±0.65D at hyperopic group. Three eyes lost more 1 line of BCVA for distance, and 14 eyes gained 1 line or more of distance BCVA. Contrast sensitivity decreased at postoperative 1 month but, recovered after postop. 3 month. Tear break up time and corneal sense was decreased at postoperatology but, tear break up time was recovered after postop. 12month and corneal sense was not recovered at postoperative 3 years.

Conclusions: The Kera laser presbyopia surgery(Cnear) is a safe and efficient treatment that may improve functional near and far vision in myopic, emmetropic and hyperopic presbyopia.

Commercial Relationships: Woo C. Park, None; Sae H. Rho, None; Sang W. Jin, None; Ki S. Park, None; Byung M. Min, None; Jong H. Lee, None
Support: None

Program Number: 290 Poster Board Number: A531
Presentation Time: 8:30 AM - 10:15 AM

Set-β Subcellular Localisation-dependent Regulation Of Retinal Ganglion Cell Neurite Growth
Ephraim F. Trakhtenberg1A,2,2A, Yan Wang1B,2, Stephanie Fernandez1A, Allison Lapins1A, Richa Panara1A,2, Jesse Shechter1A, Raquel Rotmann1A, James Farmer1A, Steven Yang1,2A

Support: None

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A Self-adaptive Wireless Transmission System Based on Visual Image Processing

**Kaijie Wu, Xinyu Chai, Qushi Ren, Yun Gu, Xuping Lei, School of Biomedical Engineering, Shanghai Jiao-Tong University, Shanghai, China.**

**Purpose:** The purpose of this study was to develop a self-adaptive wireless transmission system based on visual image processing. This system could be used for neural stimulation and could help in calculating the power requirement of neural stimulators.

**Methods:** This system consists of a Class E power amplifier, power-controlling circuits, and a visual image processing circuit. The power dissipation of a neural stimulator could be evaluated using this system. The system can change the transmission power and maintain high efficiency.

**Conclusions:** This system was developed to calculate the power requirement of neural stimulators and could be used for neural stimulation.

**Results:** The system was developed and tested with visual images. The system was able to adjust the transmission power and maintain high efficiency.

**Support:** This work was supported by National Natural Science Foundation of China (60901026).

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**Program Number:** 291 Poster Board Number: A532
**Presentation Time:** 8:30 AM - 10:15 AM

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Transfer Prevents Corneal Fibrosis

**Jeffrey Goldberg, 1,2.  1Ophthalm, Univ of Miami Bascom Palmer Eye Inst, Miami, FL; 2Neuroscience Program, Univ. of Miami Miller Sch. of Med, Miami, FL.**

**Purpose:** Adult mammalian central nervous system (CNS) axons are unable to regrow their axons after injury, but immature CNS axons grow robustly. Manipulation of various cell-autonomous factors along with overcoming the inhibitory adult CNS environment only partially restores regeneration. Recent evidence suggests that postnatally, CNS neurons themselves decrease their intrinsic regenerative capacity. We investigated whether Set-β oncprotein is developmentally regulated, whether it affects axon growth, and whether subcellular localization and posttranslational modifications modulate its effect on axon growth.

**Methods:** Embryonic and postnatal rat retinal sections were immunostained against retinal ganglion cell (RGC) marker Brn3b and Set-β. Immunofluorescence intensity was analyzed with AxioVision (Zeiss). Set-β, myristoylated (myr)-Set-β, Set-β phospho-mutants, and mCherry were overexpressed in purified P3 RGCs, incubated for 24 hours, immunostained against transfection and neurite markers, and imaged. Neurite length was quantified using ImageJ Plugin Neurite Tracer. Data was analyzed with SPSS, by ANOVA with post hoc test.

**Results:** We found that Set-β is developmentally upregulated in RGCs' nuclei. Set-β overexpressed in RGCs in vivo localized to the nucleus and suppressed neurite growth. In contrast, experimentally increased myr-Set-β cytoplasmic localization enhanced axon growth. We also found that N-terminal phosphorylation of Set-β blocks nuclear Set-β's ability to suppress neurite growth.

**Conclusions:** Set-β inhibits or promotes axon growth in RGCs depending on its subcellular localization and N-terminal phosphorylation. Further examination of whether Set-β regulates RGC axon regeneration in vivo may be warranted.

**Commercial Relationships:** Ehraim F, Trakhtenberg, None; Yan Wang, None; Stephanie Fernandez, None; Allison Lapius, None; Richa Panara, None; Jesse Shechter, None; Raquel Rottmann, None; James Farmer, None; Steven Yang, None; Jeffrey Goldberg, None.

**Support:** AHA grant 11PRE7310069, EFT; NEI grant EY020913, JLG; NINDS grant NS061548, JLG; Center grant P30 EY014801; an unrestricted grant to the University of Miami from Research to Prevent Blindness, Inc.

**Program Number:** 292 Poster Board Number: A533
**Presentation Time:** 8:30 AM - 10:15 AM

Evaluation of Decellularized Human Cornea in a Mouse Model


**Purpose:** To evaluate the survival of decellularized human corneal graft in a mouse model.

**Methods:** Human cornea is decellularized by treating it with sodium chloride solution, followed by DNase and RNase solution. To test the efficacy of decellularized human cornea in vivo, graft re-cellularized with corneal cells or without cells is transplanted into the C57BL6 mouse eye. Central cornea of 1.5 mm diameter and 70 µm depth is ablated with eximer laser from mouse eye and a thin section of decellularized cornea is grafted onto it. Mice are examined every day or every other day during the course of the study and are graded for neovascularization, opacity, inflammation, and edema with slit lamp microscope. At one week time point, eyes are enucleated and sections are stained with Hematoxylin and Eosin staining to assess the integration of donor tissue with the host tissue, and the fate of donor cells.

**Results:** The preliminary study is conducted for one week and the data suggested that the transplanted decellularized human cornea survived in the mouse eye during this time. In case of decellularized human cornea without the cells, host cells infiltrated the graft. While, in re-cellularized cornea, both host and donor cells migrated into and out of the graft assessed by staining cells with cells’ specific marker. However, the graft over time became slightly opaque but the transparency of the graft is not significantly affected.

**Conclusions:** This study evaluated the survival of decellularized human corneal graft with or without the corneal cells in a mouse model. Transplanted decellularized cornea survived in vivo for a period of one week with little or no graft rejection. The study will be extended to evaluate the long-term survival of decellularized graft in vivo.

**Commercial Relationships:** Maryam A. Shafiq, None; Behrad Y. Milani, None; Mercedes Majdinasab, None; Beatrice Y. Yue, None; Ali R. Djallilian, None.

**Support:** K08EY107561-A1

**Program Number:** 293 Poster Board Number: A534
**Presentation Time:** 8:30 AM - 10:15 AM

Impediment to Whole Eye Transplantation: An Animal Model and Surgical Techniques


**Purpose:** We have previously described the ocular viability impediment to whole eye transplantation. In this report, we describe and attempt to overcome the impediment relating to an animal model. Specifically, we describe the post mortem surgical exposure and feasibility of retro-orbital optic nerve and vascular anastomosis in a porcine model.

**Methods:** Fifty (50) pigheads from the slaughter house were dissected to elucidate the orbital and optic nerve anatomy and enumerate the steps for total eye transplantation. Mercox dye was injected into main arteries and veins to trace the vascular anatomy of the orbit. The external ophthalmal artery (EOA) was identified and cannulated. Fluorescein sodium or Mercox dye was injected into the cannulated EOA which was then anastomosed to the external carotid artery. Fundus photos and fluorescein angiograms were taken. RGCs were mounted and imaged.

**Results:** The EOA was successfully cannulated and anastomosed to the external carotid artery using a specially-designed cannula for a long extraorbital course. EOA anatomy was elucidated and its course around the ON head before entering the globe was noted. Dye was recovered in the retina: the vascular tree is similar to that of human eyes. Two additional surgeries were performed and the meninges were removed and imaged.

**Conclusions:** Adequate surgical exposure for visualization and retro-orbital optic nerve and vascular anastomosis is feasible in the porcine model. Future work is needed to prove its feasibility in living animals.

**Commercial Relationships:** Norma Allemann, Daoud Fahl, Wallace Chamom, Jin-Hong Chang, Sandeep Jain, Dimitri T. Azar, None

**Support:** NIH Grant EY01792 (DFA) and an unrestricted grant from Research to Prevent Blindness, New York, NY.

**Program Number:** 294 Poster Board Number: A535
**Presentation Time:** 8:30 AM - 10:15 AM

Polyethyleneimine (PEI) Nanoparticle-mediated Soluble TGFβRII Gene Transfer Prevents Corneal Fibrosis

Jeffrey Goldberg, 1,2.  1Ophthalm, Univ of Miami Bascom Palmer Eye Inst, Miami, FL; 2Neuroscience Program, Univ. of Miami Miller Sch. of Med, Miami, FL.

**Purpose:** Adult mammalian central nervous system (CNS) axons are unable to regrow their axons after injury, but immature CNS axons grow robustly. Manipulation of various cell-autonomous factors along with overcoming the inhibitory adult CNS environment only partially restores regeneration. Recent evidence suggests that postnatally, CNS neurons themselves decrease their intrinsic regenerative capacity. We investigated whether Set-β oncprotein is developmentally regulated, whether it affects axon growth, and whether subcellular localization and posttranslational modifications modulate its effect on axon growth.

**Methods:** Embryonic and postnatal rat retinal sections were immunostained against retinal ganglion cell (RGC) marker Brn3b and Set-β. Immunofluorescence intensity was analyzed with AxioVision (Zeiss). Set-β, myristoylated (myr)-Set-β, Set-β phospho-mutants, and mCherry were overexpressed in purified P3 RGCs, incubated for 24 hours, immunostained against transfection and neurite markers, and imaged. Neurite length was quantified using ImageJ Plugin Neurite Tracer. Data was analyzed with SPSS, by ANOVA with post hoc test.

**Results:** We found that Set-β is developmentally upregulated in RGCs' nuclei. Set-β overexpressed in RGCs in vivo localized to the nucleus and suppressed neurite growth. In contrast, experimentally increased myr-Set-β cytoplasmic localization enhanced axon growth. We also found that N-terminal phosphorylation of Set-β blocks nuclear Set-β's ability to suppress neurite growth.

**Conclusions:** Set-β inhibits or promotes axon growth in RGCs depending on its subcellular localization and N-terminal phosphorylation. Further examination of whether Set-β regulates RGC axon regeneration in vivo may be warranted.

**Commercial Relationships:** Ehraim F, Trakhtenberg, None; Yan Wang, None; Stephanie Fernandez, None; Allison Lapius, None; Richa Panara, None; Jesse Shechter, None; Raquel Rottmann, None; James Farmer, None; Steven Yang, None; Jeffrey Goldberg, None.

**Support:** AHA grant 11PRE7310069, EFT; NEI grant EY020913, JLG; NINDS grant NS061548, JLG; Center grant P30 EY014801; an unrestricted grant to the University of Miami from Research to Prevent Blindness, Inc.
Multipolar Return Configurations In Microelectrode Arrays Designed For Purpose:

Multipolar return configurations have been used to inhibit fibrosis in human corneal fibroblasts (HCF) with linearized PEI nanoparticles (LEPI) could inhibit fibrosis in the cornea using an in vitro model.

Methods: The TGFβRII containing TGFβRII extracellular domain bound to human immunoglobulin Fc chain was cloned into mammalian pcDNA3.1 expression vector (pcDNA3.1-TGFβRII). Restriction enzyme and DNA sequencing confirmed nucleotide sequence of generated construct. A pcDNA3.1-gfp construct was used for optimization. HCF were used for elaFusion to quantify TGFβRII. Cells were used to analyze fibrosis markers (α-smooth muscle actin (SMA), collagen, β-actin) with real-time PCR, western blotting and immunocytochemistry. Toxicity of LPEI was assessed with phase-contrast microscopy and MTT assay.

Results: Gene delivery efficacy and toxicity of LPEI depended on N/P ratio. N/P ratio of 3 and 8 showed lowest toxicity and highest gene delivery compared to Lipofectamine-2000, a high-efficiency commercial vector, among tested N/P ratios. ELISA quantification of lipofectamine or nanoparticle delivered TGFβRII in HCF ranged from 1620-1636 pg/mL suggesting high transgene delivery by either vehicle. Immunocytochemistry quantification of SMA in TGFβRII-treated non-transfected HCF showed 17±5 SMA+ cells/40x field (90%) whereas TGFβRII delivered with Lipofectamine or LPEI nanoparticles reduced SMA+ cells by 65% (6±4 cells/40x field; p<0.01). Similar level of SMA inhibition was detected with qRT-PCR (65%, p<0.05) and western blotting (65%; p<0.001).

Conclusions: LPEI nanoparticles (up to N/P 30) are an efficient, low toxicity vector for corneal gene therapy. LPEI-nanoparticle-mediated stTGFβRII gene therapy could be used to inhibit fibrotic response in the cornea.

Commercial Relationships: Jason T. Rodier, None; Ajay Sharma, None; Ashish K. Mihlanov, None; Rajiv R. Mohan, None

Support: R01EY17294 National Eye Institute, NIH (RRM), 1I01BX000357-01 Veteran Health Affairs Merit (RRM), and Research to Prevent Blindness (RPB) grants

Program Number: 295 Poster Board Number: A536

Presentation Time: 8:30 AM - 10:15 AM

Multiple Return Configurations In Microelectrode Arrays Designed For Retinal Implants: Modeling Effects On Threshold Levels And Dynamic Range Robert G. Wilke1,2, Gita Khallili Moghadam1, Nigel Lovell1, Socrates Dokos3, Gregg Snauin1,3,1 Biomedical Engineering, University of New South Wales, Randwick, Australia; 2Institute for Ophthalmic Research, University of Tuebingen, Tuebingen, Germany.

Purpose: Estimating the effect of current focusing in the retina using local guard electrodes in conjunction with a distant return electrode. Current thresholds to elicit ganglion cell activation and respective dynamic range are estimated using a finite-element modeling approach.

Methods: A microelectrode array in a hexagonal configuration was modeled solving for required current at the stimulating electrode yielding an electric field magnitude at the ganglion cell layer previously reported to activate ganglion cells. The following parameters has been investigated:

- Ratio of h/D (distance from electrode to ganglion cells/ electrode diameter): 0.5, 1, and 2
- Return electrode configuration: monopolar (distant active return electrode), hexagonal (6 local active electrodes), and quasi-monopolar (QMP, a mix of hexagonal and distant return configuration)

All return electrodes are designed to actull pull current. In the QMP configuration 50% of injected current is recovered through distant and local return electrodes, respectively. Electrodes were chosen to be of 100µm diameter and 110µm pitch.

Dynamic range was defined as the difference between threshold current and the safe current injection limit of the IORF electrodes.

Results: The dynamic range systematically decreased as the electrode configuration varied from MP to QMP to hexagonal. This effect is more significant for larger electrode/target cell distances (h/D of 2), and hardly noticeable for smaller distances. This is due to elevated threshold levels for QMP and hexagonal configuration due to partial shunting of stimulating current directly to return electrodes.

Conclusions: Current focusing using multipolar configuration like the hex-return has the benefit of more localized stimulation that is less prone to electrocrosstalk. However, depending on electrode geometry and distance from target cells it also has higher threshold levels. We investigated if a mix of return configuration using distant return and local hex return can compensate for this limitation. QMP configuration with 50% distant return was found to lower thresholds and yield higher dynamic ranges as compared to pure hex-configuration. If target cells are very close to stimulating electrodes as could be achieved with penetrating electrodes, return configuration can influence on threshold levels.

Commercial Relationships: Robert G. Wilke, None; Gita Khallili Moghadam, None; Nigel Lovell, None; Socrates Dokos, None; Gregg Snauin, Inventor pat. application (P)

Support: ARC Special Initiative Bionic Vision Australia

Program Number: 296 Poster Board Number: A537

Presentation Time: 8:30 AM - 10:15 AM

Enhanced Production of Photoreceptor Precursor Cells (PPCs) from Human Embryonic Stem Cells (hESCs) using Size-Controlled Embryoid Bodies (EBs) and mRNA Induction Christopher R. Laver, Anat Yanai, Kevin Gregory-Evans. Ophthalmology and Visual Sciences, University of British Columbia, Vancouver, BC, Canada.

Purpose: Efficient in vitro production is a critical hurdle in the large-scale manufacture of PPCs for future pre-clinical and clinical stem cell trials in retinal degeneration. We proposed to enhance published protocols by reducing the time taken for differentiation whilst also increasing yield of PPCs for a given number of hESCs. Gene-induction protocols have previously focused on inhibiting pro-growth pathways (e.g., Wnt, BMP, and Nodal), and small-molecule targeted induction agents (e.g., thyroid hormone, retinoic acid, FGF, and IGF). We hypothesize that driving expression of CRX (a transcription factor in photoreceptor differentiation) during directed hESC differentiation will enhance production of PPCs. We propose to achieve this through synthetic mRNA induction.

Methods: We adapted established PPC-production methods by, firstly, using size-controlled embryoid bodies (EBs) to synchronize hESC differentiation and, secondly, by using TEsR2 xeno-free media in our feeder-free differentiation protocol. We have developed a CRX-encoding expression vector for in vitro transcription, with CRX transcripts ‘coupled’ with 7-methylguanosine and treated with phosphatases. Lipoplexes were used to transfect cells with this CRX mRNA. Following this, immunocytochemistry and RT-PCR was used to assess gene expression in differentiating hESCs.

Results: Size-controlled EBs significantly improved PPC yield, as 10,000-cell EBs exhibited a more complete reduction of residual-hESC colony formation relative to 1,000-cell and random-size EBs. We have also calculated the benefit of introducing CRX-mRNA into differentiation media at stage when cells express PAX6 and MITF through NNL and Chx10 expression analysis.

Conclusions: Our data indicates that hESCs can undergo more efficient differentiation with modifications to previous protocols. Such improved cost efficiency in PPC manufacture will enhance productivity in future pre-clinical trials in retinal degeneration.

Commercial Relationships: Christopher R. Laver, None; Anat Yanai, None; Kevin Gregory-Evans, None

Support: None

Program Number: 297 Poster Board Number: A538

Presentation Time: 8:30 AM - 10:15 AM

A Novel Approach for Quantitative Analysis of 3D Phosphenes Eduardo Fernandez1,2, Carlos Agullo1, Andres Omeño1, Aravanta Alfaro1,2, Antonio Alarcon1, Cristina Soto1,2, Instituto de Bioingenieria, Univ of Miguel Hernandez, Elche, Spain; 1CIBER-BBN, Zaragoza, Spain.

Purpose: Appropriate delivery of electrical stimulation to visual structures can evoke patterned sensations of light, called phosphenes, in individuals who have been blind for many years. This pivotal finding settled the physiological basis for present efforts to develop a visual prosthesis for the blind. Various methods have been used to document phosphenes, but a standardized methodology is lacking. Here we aim to introduce a novel wireless system and procedure for the documentation and 3D analysis of phosphenes.

Methods: Phosphenes were induced by non-invasive Transcranial Magnetic Stimulation (TMS) of occipital cortex. The protocol was applied on a group of 20 sighted and 15 legally blind volunteers. For mapping the visual perceptions we used a wireless system including an autofocus infrared (IR) camera and one IR projector. After each TMS pulse, subjects were asked to make drawings of the perceptions with their own hands (the sensors transmit beams of infrared light allowing 3D hand tracking), with particular emphasis on their localization within the 3D visual field. A customized program allowed easy registration and analysis of collected data.

Results: All study subjects (both healthy and vision impaired) perceived phosphenes and tolerated the procedure without complication. The new mapping technique allows to locate phosphenes in real 3D visual space. Furthermore our
procedure allows an easy calculation of the position, volume and area of the subjective perceptions from the coordinates of the drawings.

**Results:** Binding efficiency of Ranibizumab was highly dependent on pH and molar ratio of Ranibizumab to dextran sulfate. The entrapment efficiency of nanoparticles was highly dependent on the nature of PLGA and drug to polymer ratio. As hydrophobicity of PLGA was increased, entrapment efficiency was enhanced and approximately 70%, 75% and 85% entrapment was obtained with PLGA(50:50), PLGA(65:35) and PLGA (75:25) respectively. When PLGA 65:35 was employed for nanoparticles preparation, entrapment efficiency of approximately 75% and 85% were obtained for drug to polymer ratio of 1:10 and 1:1 respectively. The size of the nanoparticles was around 150 nm in all the cases.

**Conclusions:** Development of a nanoparticulate formulation of macromolecule is a significant challenge. Different process variables have shown impact on the entrapment of HIP complex inside the nanoparticles. Nanoparticle preparation by nanoprecipitation method utilizing HIP complex has shown promising result with maximum entrapment of approximately 85%.

**Commercial Relationships:** ASHA Patel, None; Ripal Gaudana, None; Yi Hao, None; Nelson Sabates, None; Ashim Mitra, None

**Support:** St. Luke’s Hospital Foundation.

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**Program Number:** 301 Poster Board Number: A542 Presentation Time: 8:30 AM - 10:15 AM

Development Of Functionalized Gold Nanoparticles As Diagnostic And Therapeutic Agents ForOptical Tomography

Richard M. Awdeh1, Shradha Prabhulkar1, 2, Sanjiv Gambhir1, 2, 3

1Optical Tomography, Bascom Palmer Eye Institute, Miami, FL; 2Electrical Engineering and Radiology, 3Bioengineering & Materials Science and Engineering, 1Stanford University, Palo Alto, CA.

**Purpose:** Molecular in vivo imaging allows for diagnostic imaging, target screening, and therapeutic monitoring of disease on a molecular level, prior to evidence of pathologic changes that can be detected with conventional imaging modalities. Optical Coherence Tomography (OCT) is an imaging modality used to visualize ocular structures with high spatial resolution. We have created optimized gold nanoparticles (GNPs) which produce a strong OCT signal, beyond the background tissue OCT signal, and can fulfill the role of an exogenous OCT contrast agent. In order to achieve molecular diagnostic and therapeutic capabilities, we functionalized these GNPs with anti-VEGF antibodies, allowing...
for the dual purpose of molecular diagnostic imaging and therapy towards age-related macular degeneration (AMD).

**Methods:** GNPs with a longitudinal plasmon resonance at 840nm were synthesized using seed-mediated growth mechanism. The GNPs were covalently attached to anti-VEGF antibodies via carbodiimide chemistry. The anti-VEGF functionalized GNPs in vitro were tested in a laser induced mouse model of choroidal neovascularization. Tail-vein, venous sinus, intravital, and sub-retinal administration routes were tested to determine the optimal delivery technique. OCT images of the control (CNV induced mice with saline injections) and test groups (CNV induced mice with anti-VEGF functionalized GNPs) were acquired following injection to determine the localization dynamics of the GNP construct in this CNV mouse model.

**Results:** OCT imaging reveals that there is a marked increase in the scattering intensity of areas around the laser induced lesions in anti-VEGF functionalized GNP injected mice versus the control group using tail vein injections. The minimal detectable concentration the systemic administration of these functionalized GNPs was 87nM in this set of experiments. These detection limits are in line with our published minimal detectable threshold of GNPs as low as 370pM for nonfunctionalized, locally administered GNPs in the eye.

**Conclusions:** Based on this data, we conclude that our antibody functionalized GNPs can be used with high-resolution, spectral-domain OCT for the molecular in vivo detection of AMD. Our future efforts will focus on the quantitative analysis of disease burden based on in vivo molecular profiling.

**Commercial Relationships:** Richard M. Awdeh, Nanophthalmos, LLC (I, C, P); Shradha Prabhulkar, None; Adam de la Zerda, Nanophthalmos, LLC (P); Sanjiv Gambhir, Nanophthalmos, LLC (P)

**Support:** NIH/ NEI R21 – EY020940

**Program Number:** 302 Poster Board Number: A543

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

**Ultrahigh Photosensitivity Silicon Nanophotonics For Retinal Prosthesis**

**Massoud L. Khraiche**1, Deli Wang2, Yuhua Lo2, Geet Cauwenberghs1, Igor Kazak2, William R. Freeman2, Gabriel Silva3, Bioengineering, UCSD, La Jolla, CA; **Ophthalmology, University of California San Diego, La Jolla, CA;**

**Purpose:** Age related macular degeneration (AMD) and retinitis pigmentosa (RP), lead to the loss of the photoreceptor cells rendering the retina incapable of detecting light. There have been several engineering approaches aimed at replacing the function of the photoreceptors (PRs) by detecting light via an external camera or photodiodes and electrically stimulating the remaining retinal tissue to restore vision. These efforts face several challenges including low resolution, high power consumption and poor tissue integration. In this work, we will introduce a nanophotonic technology that has the potential to be at the heart of nanoengineered retinal prosthesis.

**Methods:** Large arrays of Icm X Icm Nanowires were fabricated via nanoinprint lithography. The Nanowire array was used to stimulate retinal explants from the subretinal stimulation site of a ganglion cell epi-dermis was tested via a Microelectrode array placed at the epiretina.

**Results:** Our results showed the Nanowires possess characteristics that make them ideal replacements for the photoreceptors; Topography and spatial control: The nanowires can be fabricated to match the functional organization of the photoreceptors in the retina. Light adaptation and amplification: The rods and cones can operate on an extremely large range of illumination. The Nanowires can be made to mimic this control via feedback control of bias voltage. Neural stimulation: The PRs stimulate neural tissue via the release of neurotransmitters. Driving a current through neural tissue can also excite neurons. Our data shows the NW platform capable of producing current levels and waveforms sufficient for neural stimulation in response to light. The Nanowires were stimulated with a laser at 635nm, a wavelength we confirmed is not able to produce an electrophysiological response on its own the rat retina.

**Conclusions:** The presented body of work shows our Nanowires technology has a lot of promise for application in a prosthetic platform to replace the function of the PRs. The long term aims of our group is to produce a nanoengineered retinal prosthesis capable of tissue integration on a scale comparable to those of proteins and lipids, with light sensing and stimulation elements near rod and cones light sensitivity and spatial distribution.

**Commercial Relationships:** Massoud L. Khraiche, SD2010-348 (P); Deli Wang, SD2010-348 (P); Yuhua Lo, SD2010-348 (P); Geet Cauwenberghs, SD2010-348 (P); Igor Kazak, None; William R. Freeman, SD2010-348 (P); Gabriel Silva, SD2010-348 (P)

**Support:** Qualcomm Health & Life Sciences Wireless Health Innovation Challenge, Von Liebig Center UCSD

**Program Number:** 303 Poster Board Number: A544

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

**Functionalized Nanoparticles To Enhance Regenerative Axon Growth**

**Daniel W. Pita-Thomas, Michael Steketee, Jeffrey L. Goldberg,** Karl Kador. **Ophthalmology, University of Miami, Miami, FL**

**Purpose:** Neurons in the central nervous system fail to regenerate their axons after injury. Although there are approaches to neutralize inhibitory signals and add growth-promoting signals, delivery therapeutics to the lesion site is problematic, limiting efficacy. In the present study, we propose use functionalized magnetic nanoparticles (fMNPs) as a treatment delivery system to enhance regenerative axon growth.

**Methods:** We used chemical coupling of fluorescent nanoparticles to agonist anti-CD40 antibodies, cholera toxin B and peptidase; coculture of retinal ganglion cells (RGCs) with fMNPs to promote endocytosis; and manipulation of the fMNPs using magnetic forces. Intravitreal injection of fMNPs in rats was followed by immunohistochemistry to assess fMNPs' location.

**Results:** fMNPs functionalized with different molecules were endocytosed into long-lived signaling endosomes by RGCs and transported along the axon. Application of magnetic forces to cultured RGCs loaded with fMNPs induced changes in axon growth rates. fMNPs injected intravitreally were internalized by RGCs and trafficked to axons in the optic nerve and dendrites in the inner plexiform layer.

**Conclusions:** fMNPs provide a flexible platform for targeting nano-therapeutics to previously inaccessible regions of the central nervous system and advance our understanding of fundamental mechanisms regulating axon growth.

**Commercial Relationships:** Daniel W. Pita-Thomas, None; Michael Steketee, None; Jeffrey L. Goldberg, None; Karl Kador, None

**Support:** Technology Transfer Feasibility Grant ID 2KF03, EY017971 (JLG), P30-EY014801 (University of Miami), and NRSA T32NS007044 (MBS), as well as an unrestricted grant from Research to Prevent Blindness

**Program Number:** 304 Poster Board Number: A545

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

**Towards Photochemical Measurement of Nano-Emulsion Lubricant Eye Drop Coalescence in the Tear Film**

**Howard A. Ketelson**1, James W. Davis2, Minrui Chen1, Yuguo Cui2, Robert Pelton2,1Alcon Research Ltd, Fort Worth, TX; 2McMaster University, Hamilton, ON, Canada.

**Purpose:** A challenge in the formulation of emulsion eye drops containing polymer, lipids and oil ingredients is to maintain colloidal stability without impacting performance in the eye. Ideally, emulsion droplets are indefinitely stable towards creaming and coalescence until they are dropped onto the eye whereupon there is breakdown, release and retention of the critical chemical components in the eye. We have developed photochemical approaches to tracking nano-emulsion colloidal stability. The method has been demonstrated in vitro with the potential to perform real-time emulsion stability measurements in the tear film.

**Methods:** Two samples of negatively charged oil-in-water nano-emulsions stabilized with phosphatidylcholine (PC) were prepared. The average diameters were controlled by the processing conditions and could be varied between 100 and 250 nm. One emulsion sample was doped with nitrobenzylidazolabeled PC, the donor, and the other with rhodamine B- labeled PC, the acceptor. The two types of labeled emulsions were mixed and emulsion aggregation was measured by Fluorescence Resonance Energy Transfer (FRET) that reports a change in the fluorescent spectrum when the donor and acceptor come in contact. The FRET-based approach for monitoring nano-emulsion stability was validated by traditional dynamic light scattering methods.

**Results:** Hexadecane nano-emulsions were mixed with three guar derivatives to induce either: 1) no effect in the presence of low molecular weight partially hydrolyzed guar; 2) depletion flocculation and coalescence, induced by high molecular hydroxypropyl guar; or, 3) bridging flocculation (cationic guar). FRET was a sensitive probe for depletion induced coalescence but was not a sensitive measure of bridging flocculation where the emulsion droplets have little opportunity to coalesce.

**Conclusions:** We have demonstrated that FRET is a sensitive measure of nano-emulsion stability and that it may be useful for tracking emulsion distribution and delivery mechanisms in the tear film.

**Commercial Relationships:** Howard A. Ketelson, Alcon Research Ltd (E); James W. Davis, Alcon Research Ltd (E); Minrui Chen, None, Yuguo Cui, None, Robert Pelton, None

**Support:** None

**Program Number:** 305 Poster Board Number: A546

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A Biodegradable Thin-film Polycaprolactone Scaffold As A New Tool To Enhance Differentiation Of Mouse Retinal Progenitor Cells

Methods:

Presentation Time: 8:30 AM - 10:15 AM

The results of this study indicated the potential of using ASCs in the treatment of diabetic retinopathy. The study showed that ASCs could be used to regenerate retinal tissue and improve vision in diabetic patients.

Purpose: The purpose of this study was to evaluate the potential of using ASCs in the treatment of diabetic retinopathy.

A 75-year-old diabetic patient with a history of proliferative diabetic retinopathy was enrolled in this study. The patient was treated with ASCs injected into the subretinal space of the affected eye. The patient's vision improved from 20/400 to 20/200 after 3 months of treatment.

Results:

The study observed that ASCs were able to migrate into the retina and differentiate into various retinal cell types. The ASCs were integrated into the retinal ganglion cell layer and improved photoreceptor function.

Conclusion: The study demonstrated the potential of using ASCs in the treatment of diabetic retinopathy. Further studies are needed to evaluate the long-term effects of ASC therapy in diabetic retinopathy.

Commercial Relationships: Nothing to disclose.

Support: None

ARVO 2012 Annual Meeting Abstracts by Scientific Section/Group – Nanotechnology and Regenerative Medicine Group (NT)

Program Number: 306 Poster Board Number: A547

Presentation Time: 8:30 AM - 10:15 AM

Regenerative Therapeutic Potential of Adipose Stromal Cells in Early Stage Diabetic Retinopathy

Gangaraaju Rajashkekar1, Rachel Richardson18, William Roell18, Timothy S. Kern1, Alan Harris19, Keith March30

1Department of Ophthalmology and Cellular & Integrative Physiology, 2Department of Ophthalmology, 3Department of Cellular & Integrative Physiology, 4Department of Medicine and Cellular & Integrative Physiology, 5Indian Univ Sch of Medicine, Indianapolis, IN; 6Department of Medicine, Case Western Reserve Univ, Cleveland, OH.

Purpose: Early stage diabetic retinopathy (DR) is the most common vascular complication in patients with long-standing diabetes, and is the leading cause of blindness in working-age adults. Early stage DR has been shown to involve inflammation, vascular leakage and apoptosis of vascular cells. In this study, we hypothesized that cells derived from the stromal fraction of adipose tissue (ASC), which have been shown to improve ischemia reperfusion, limit myocardial infarction and possess neuroprotective function, might have potential to enhance early stage DR features. Furthermore, we hypothesized that ASC therapy might be beneficial for treatment studies.

Methods: Streptozotocin (STZ) induced diabetic athymic nude rats were intravitreally injected 150,000 human ASC cells into the right eye, and the ipsilateral eye served as control with equal volume of saline. After 7 days, rats were sacrificed, retinal vascular leakage was analyzed by FITC-albumin extravasation assay, apoptosis was measured by TUNEL assay, and inflammatory genes were assessed by real-time RT-PCR method. In vitro, ASC were subjected to varying concentrations of glucose and proliferation assessed by MTT assay, cell death by Caspase-3 assay, and oxidative stress by DCF assay.

Results: In vivo, ASCs subjected to elevated glucose concentrations of 250mg/dL and showed impaired glucose tolerance. Diabetic rats that received saline injection after day 7 demonstrated significantly (p<0.01) increased vascular leakage, TUNEL-positive cells in the retinal ganglion cell layer (as well as around vessels), and upregulation of inflammatory genes such as ICAM-1, Eselectin, ICAM-2, Timp1, TGFβ, TNFα and Stat3 (>2 fold) compared to non-diabetic rats. On the other hand, diabetic rats that received ASC injection were as hyperglycemic as control diabetic, but nevertheless demonstrated significantly less of all the retinal parameters above. In vitro, ASC subjected to elevated glucose concentration displayed sustained proliferation, and decreased apoptosis and oxidative stress.

Conclusion: This is the first demonstration of the use of ASC in the treatment of early lesions of DR. Future studies in long-term diabetes models will address if ASC repair and stabilize vasculature in the treatment of DR. By evaluating this approach in the rodent model, we will be in a better position to determine whether such an approach should be tested in humans. The diabetic rat studies will provide valuable insights to guide the design of our future clinical studies in human patients.

Commercial Relationships: Gangaraaju Rajashkekar, None; Rachel Richardson, None; William Roell, None; Timothy S. Kern, None; Alan Harris, None; Keith March, None.

Support: Janssen Research Trust Fund & Signature Center/CBIVM.

Program Number: 307 Poster Board Number: A548

Presentation Time: 8:30 AM - 10:15 AM

Human Embryonic Stem Cell-derived Hemangioblasts Can Repair Acute Retinal Vascular Damage

Wei Zhang1, Jin-Du Wang1, Ying Yu1, Jing-Shang Zhang1, Wei Wang1, Shi-Jiang Lu1, Robert Lanza2, Jing Xu1

1Department of Biochemistry, 2Department of Physiology, 3Beijing Institute of Ophthalmology, Beijing, China; 4Advanced Cell Technology, Inc, Boston, MA.

Purpose: It was shown that transplanted human embryonic stem cell-derived hemangioblasts localized to injured retinal vasculature in diabetic retinas and retinal ischemia-reperfusion injured mice. The study was to verify that the hemangioblasts-derived neovasculature was healthy but not leaky in diabetic rats. Using mice model of oxygen-induced retinopathy (OIR), this study was to investigate the potential of hES-hemangioblasts to rebuild functional vasculature on retina obliteration region and to suppress pre-retinal neovascular tufts.

Methods: OIR model was induced using C57Bl/6 neonatal mice as described. GFP-labeled hES-derived hemangioblasts were injected intravitreally. At P17, retinas were whole mounted and vascular architectures were visualized by GS-lectin staining or FITC-dextran cardiac perfusion. The area of central vascular obliteration and pre-laminar neovascular tufts were quantified. STZ-induced diabetic rats were given hES-derived hemangioblasts intravitreally 6 weeks after induction. Blood-retina-barrier (BRB) function was evaluated by measuring the concentration of FITC-dextran in retina homogenate. Human CD31 protein expression in retinas was detected by immunofluorescence staining.

Results: In diabetic rats, GFP-positive hemangioblasts integrated into retinal vessels two days after cell transplantation. The injection of hES-derived hemangioblasts prevented BRB breakdown in diabetic rats. In OIR mice, only hES-derived hemangioblasts but not endothelial cells integrated into retinal vasculature, promoting the process of vascular re-growth and hindering the abnormal formation of neovascular tufts. The areas of both vascular obliteration and neovascular tufts in hemangioblasts-treated eyes were significantly lower than those in control eyes. In both OIR and diabetic models, integrated GFP-positive cells expressed human CD31, indicating the differentiation of hemangioblasts to endothelial cells.

Conclusion: Our study showed that hES-derived hemangioblasts had the ability to form new, functional vasculature network; and repaired vascular inhibited the growth of pathological neovascularization.

Commercial Relationships: Wei Zhang, Advanced Cell Technology, Inc (F); Jin-Du Wang, Advanced Cell Technology, Inc (F); Ying Yu, Advanced Cell Technology, Inc (F); Jing-Shang Zhang, Advanced Cell Technology, Inc (F); Wei Wang, Advanced Cell Technology, Inc (E); Shi-Jiang Lu, Advanced Cell Technology, Inc (E); Robert Lanza, Advanced Cell Technology, Inc (E); Jing Xu, Advanced Cell Technology (F)

Support: None

Program Number: 308 Poster Board Number: A549

Presentation Time: 8:30 AM - 10:15 AM

Absence Of Ctrp5/c1qtlf5 Leads To RPE Degeneration In Ctrp5 Gene Knock-out Mice

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Venkata R. Chavali, Bhubanananda Sahu, Dirk-Uwe Bartsch, Tarun Bansal, Christine A. Curcio, Sheldon S. Miller, Monica M. Jablonski, Radha Ayyagari.

Shiley Eye Center, University of California San Diego, La Jolla, CA; National Eye Institute, NIH, Bethesda, MD; Ophthalmology, Univ of Alabama at Birmingham, Birmingham, Birmingham, AL; Hamilton Eye Institute, Univ Tennessee Health Sci Ctr, Memphis, TN.

**Purpose:** Autosomal dominant late-onset retinal degeneration (L-ORD) in humans is caused by a S163R mutation in the CTRP5 gene. To study the function of this gene and to understand the molecular pathology of L-ORD, a Ctrp5 gene knock-out mouse model (Ctrp5+/−) was generated and its retinal phenotype was characterized.

**Methods:** The Ctrp5+/− mice were generated on a C57BL/6 background. The homozygous knock-out mice were characterized by examining the Ctrp5+/− transcript and protein by western blot analysis. Retinal pathology was evaluated by fundus photography, fundus autofluorescence (FAF) imaging, fluorescein angiography (FA), optical coherence tomography (OCT), dc-electrotetrogrammetry (dc-ERG). Retinal morphology was evaluated by light and electron microscopy; immunohistochemistry with retinal and RPE marker antibodies and staining for lipids. Expression levels of retinal and RPE cell marker genes were determined by qRT-PCR. All assays were done through age 8 months and some assays up to 18 mo.

**Results:** The Ctrp5 transcript and protein were not detected in the homozygous knock-out mice. Fundus examination revealed increased accumulation of hyperfluorescent spots in Ctrp5+/− mice from age 5 months compared to littermate controls. Morphological analysis revealed abnormalities in the RPE and in the outer segments (OS). The RPE developed many vacuoles and appeared to be necrotic. The underlying photoreceptor OS below the necrotic RPE appeared shorter and disorganized along with presence of swollen inner segments (IS). The sub-retinal space was occupied with debris that appeared to contain undigested packets of OS, melanin granules and other material of unknown origin. These morphological changes were found to be progressive with age. The Ctrp5+/− mice had little or no basal laminar deposits, compared to what we previously observed in Ctrp5 S163R knock-in mice. In addition, expression levels of photoreceptor marker genes were significantly reduced in the age in the Ctrp5+/− mice, compared to the wild type littermate controls. The de-ERG response was significantly reduced in the Ctrp5+/− mice by 8 months.

**Conclusions:** The homozygous Ctrp5 gene knock-out mice developed RPE and photoreceptor degeneration. This model is complementary to the Ctrp5 S163R knock-in model previously developed in the lab and will also help elucidate the molecular pathology of L-ORD.

**Commercial Relationships:** Venkata R. Chavali, None; Bhubanananda Sahu, None; Dirk-Uwe Bartsch, None; Tarun Bansal, None; Christine A. Curcio, None; Sheldon S. Miller, None; Monica M. Jablonski, None; Radha Ayyagari, None.

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**Program Number:** 309 Poster Board Number: A550

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

**Development Of Multifunctional Collagen Constructs For Retinal Cell Transplantation Of miLier Stem Cell Derived RPE**

lauren M. James, Harris K. Check, Philip Levenson, Karen Eastlake, Peng T. Khaw, Robert A. Brown, Karl Matter, G Astrid Limb.

Orbit, Institute of Ophthalmology UCL, London, United Kingdom; NIH Biomedical Research Centre, London, United Kingdom; UCL Institute of Orthopaedics and Musculoskeletal Science, London, United Kingdom.

**Purpose:** We aimed to design multifunctional collagen constructs containing therapeutic agents to facilitate the migration, integration and survival of transplanted retinal ganglion cells (RGC) derived from human Müller stem cells (hMSC).

**Methods:** Triaminocoline and Chondroitinase ABC (ChABC) were incorporated into type I collagen constructs; following polymerisation and subsequent vacuum plastic compressed to form cellular scaffolds. Following pH neutralisation of collagen solutions, a suspension of triaminocoline containing 4mg of the drug was added to the collagen, as well as the addition of alginate microparticles containing ChABC. Inclusion of ChABC into alginate particles was achieved using an encapsulator, at a voltage of 9kV and an air gap distance of 7cm. Following polymerisation at 37°C, collagen was then compressed under 150g for 5 minutes to form ultrathin scaffolds to which RGC derived from hMSC could adhere. Electron microscopy was used to examine the microstructure of the collagen matrices containing the agents to assess any morphological alterations to the fibrillar architecture of the scaffolds. Cellular viability of hMSC grown on the collagen scaffolds was examined using a hexosaminidase assay. Optical density of the various scaffolds was also determined over a range of 350nm-800nm using a spectrophotometer. Cellular with RGC derived from hMSC were transplanted onto explants of dermal human retina.

**Results:** Scanning electron microscopy showed that cells seeded onto collagen scaffolds were able to develop firm attachments to the collagen matrices. Cellular viability was maintained throughout 7 days in culture; where cells were unaffected under the varying culturing microenvironments when compared to MatrigelTM alone. Scaffolds containing therapeutic doses of both triamcinolone and ChABC did not induce any light within the visual spectrum. Transplantation of cellular scaffolds onto the inner surface of human retinal explants ex vivo showed that cells were capable of migrating into the RGC layer, as determined by immunostaining of retinal sections prepared from the in vitro transplanted retina.

**Conclusions:** The results suggest that plastic compressed collagen constructs provide suitable vehicles for drug and cell delivery into the eye. These composite multifunctional constructs can be produced consistently under sterile conditions, providing a viable method of supplying transplanted cells with pro-survival factors when transplanted into a host environment. These therefore constitute a viable strategy for transplantation of Müller stem cell derived RGC into the retina. 

**Commercial Relationships:** laureen M. James, None; Hari Jayaram, None; Philippa Cottrill, None; Karen Eastlake, None; Peng T. Khaw, None; Robert A. Brown, Plastic compression of collagen (P); Karl Matter, None; G Astrid Limb, None.

**Support:** BMRC 044

**Program Number:** 310 Poster Board Number: A551

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

**Development of a Clinical Vision Research Training and Mentoring Program for minority undergraduate and graduate students**

Lisa A. Hark,1 Bianca Collymore,2 Krystal Caraballo,2 Deiana Johnson,3 Shayla Stratford,4 Joseph Malunda,1 David Weiss,1 Jacquelyn Thomas.1

Research, Wills Eye Institute, Philadelphia, PA; 2Research, Temple University, Philadelphia, PA; 3University of Pittsburgh, Pittsburgh, PA; 4University of Southern California, Los Angeles, CA.

**Purpose:** To develop, implement, and evaluate a Research Training and Mentoring Program for undergraduate and graduate minority health science, medical, and nursing students to enhance research skills by engaging in clinical vision research.

**Methods:** The Department of Research at the Wills Eye Institute in Philadelphia, Pa, developed and implemented and evaluated a full-time, 9-week, paid summer research training and mentoring program, funded by the Pennsylvania Department of Health. Students were recruited from Jefferson Medical College, Thomas Jefferson University School of Nursing, Philadelphia College of Osteopathic Medicine, University of Pennsylvania, St. Joseph’s University, and Temple University. Students attended research methods lectures, reviewed scientific literature, participated in grant writing, conducted chart reviews, surveyed patients, developed individual research projects, wrote IRB protocols, and met with research mentors on a weekly basis. Students were administered a 65-item pre/post-test to assess diabetes knowledge and research methodology as well as an overall evaluation of all components of the program on a Likert scale ranging from 1-5, where 5 was the highest rating.

**Results:** Two students participated during the summer of 2010 and 5 students participated during the summer of 2011. The group consisted of 6 African Americans students and 1 Hispanic student enrolled in nursing, medical, and public health undergraduate and graduate programs. Pre/post-test scores showed improvement with mean pre-test score=62% and post-test score=95%. Students evaluated their overall experience in the Minority Research Training and Mentoring Program as 4.5 (scale 1-5). They learned a significant amount about ophthalmology by working on research projects (4.67) and evaluated that working on their own vision research project was a worthwhile experience (4.8).

**Conclusions:** This Research Training and Mentoring Program for undergraduate and graduate minority health science, medical, and nursing students has the potential to increase students’ interest in careers in clinical vision research. Currently we are tracking the future career paths of these students and will continue to offer this full-time, 9-week summer program.

**Commercial Relationships:** Lisa A. Hark, None; Bianca Collymore, None; Krystal Caraballo, None; Deiana Johnson, None; Shayla Stratford, None; Joseph Malunda, None; David Weiss, None; Jacquelyn Thomas, None.

**Support:** Pennsylvania Department of Health

**Program Number:** 311 Poster Board Number: A552

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

**Morphological and Functional Evaluation of hESC-RPE Cell Suspension Injection in RCS Rats**

Laura Liu,1,2 Yuntuo Hu1,2, Alejandro Gonzalez-Calle,1 Danhong Zhu,1,3 Padmaja B. Thomas1,2, Birja B. Thomas1,2, Gerald J. Chauder,3 Dennis O. Clegg,3 David R. Hinton1,2, Mark S. Humayun1,2,3 Cell and Neurobiology,1,2 Pathology,3 University of Southern California, Los Angeles, CA; 3Ophthalmology, Chang Gung Memorial Hospital, Taoyuan, Taiwan; 4Ophthalmology, Doheny Eye Institute, Los Angeles, CA; 5Ophthalmology, Peking University Third Hospital, Beijing, China; 6Center for Stem Cell Biology and Engineering, University of California-Santa Barbara, Santa Barbara, CA.

**Purpose:** To evaluate whether transplantation of retinal pigment epithelial (RPE) cells derived from H9 human embryonic stem cells (hESC-RPE) delivered as cell suspension can maintain the RPE functionality and prevent photoreceptor degeneration in the dystrophic Royal College of Surgeons (RCS) rat. The study will
also evaluate the immunological reactions of the host retina to non-polarized hESC-RPE.

**Methods:** hESC-RPE cells were cultured for 4 weeks and digested into cell suspensions using collagenase at the cell density of 5.0x10^6/ml. Subretinal injections of hESC-RPE cells (10^5, 2μl) were performed in 28 to 31 day old RCS rats. Pre-injection was administrated to all rats through drinking water (0.002mg/liter) for the entire period of study. Animals were sacrificed and eyes enucleated at 10 days (n=3), and 2 months (n=9) after surgery; tissue sections underwent histological evaluation using hematoxylin and eosin (H&E) staining and immunostaining for RPE markers (RPE65, human marker (TRA-1-85), and immune response markers (CD3, CD68, GFAP)). Visual functional evaluation was performed using optokinetic testing. Aperio Scanscope was used for measurement of outer nuclear layer thickness and performing cell counts.

**Results:** Based on H&E staining, presence of pigmented cells was observed in all transplanted rats. In the 10 days post-transplantation group, hESC-RPE cells showed co-localized expression of TRA-1-85 and RPE-65 indicative of viable donor cells. In this group, transplanted cell clumps were surrounded by CD68 positive cells indicating macrophage activity. In the 2 months post-transplantation group, CD68 expression was observed mostly over the cell clumps, but TRA-1-85 and RPE-65 markers were not identified. Optokinetic testing suggested some improvement in the implanted eye; however the difference with the non-transplanted eye was not significant. Based on histological assays, no appreciable rescue of the outer retinal layer was observed in the transplanted area.

**Conclusion:** Dissociated hESC-RPE cells do not survive long-term and do not function in the rat’s subretinal space. One possible mechanism for the accelerated loss of the transplanted hESC-RPE cells can be due to the increased host immune reaction to non-polarized hESC-RPE.

**Commercial Relationships:** Laura Liu, None; Yuntao Hu, None; Alejandra Gonzalez-Calle, None; Danhong Zhu, None; Padmaja B. Thomas, None; Biju B. Thomas, None; Gerald Chader, None; Dennis O. Clegg, None; David R. Hinton, None; Mark S. Humayun, None

**Support:** California Institute for Regenerative Medicine (CIRM), NEI EY03040

**Program Number:** 312 Poster Board Number: A553

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

**Safety study in Mini Pigs of transplanted Human Embryonic Stem Cell Derived Retinal Pigment Epithelium (hESC-RPE)**

**Methods:** All animal procedures were performed according IACUC and ARVO rules regarding animal studies. Ultrasound films made from porcine were seeded with hESC-RPE and surgically implanted into the subretinal space of eight 2 months old female Yucatan mini Pigs. The surgical procedure comprised a pars plana vitrectomy plus retinal detachment with balanced saline solution and a limited peripheral retinectomy for insertion of the substrate seeded with cells. Silicone oil tamponade plus laser were performed in all subjects. All subjects received oral cyclosporine (100 mg/day for the first month, 200 mg/day for the last two months) during the entire follow up. Three months after implantation, the pigs were sacrificed. Eyes and organs were fixed (Davidson’s solution and formalin, respectively), embedded in paraffin, and subjected to histological analysis based on hematoxylin and eosin (H&E) staining. Adjacent sections were used for immunohistochemical analysis (when used) using the following antibodies: anti-RPE65 (RPE-specific antigen) and TRA-1-85 (human blood group antigen).

**Results:** The cell monolayer over the porcine scaffold was immunopositive for TRA-1-85 and RPE-65 three months after surgical implantation. Human cells did not migrate off the porcine substrate. In one eye there was a mild inflammatory reaction around the implant, but it was negative for human biomarkers. There was no evidence of intraretinal tumor formation. Systemic organs did not show gross evidence of tumor and are now being evaluated microscopically.

**Conclusion:** The hESC-RPE survived for at least three months in this animal model. The surgical procedure and subretinal implantation of the substrate with cells proved feasible and safe without the induction of tumors in the eyes and organs of the immunosuppressed animals.

**Commercial Relationships:** Rodrigo A. Brant fernandes, None; Bruno Diniz, None; Ramiro Ribeiro, None; Yuntao Hu, None; Laura Liu, None; Padmaja Thomas, None; Biju Thomas, None; Ashish Ahuja, None; David R. Hinton, None; Mark S. Humayun, None

**Support:** CIRM

**Program Number:** 313 Poster Board Number: A554

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

**Behavioral and Electrophysiological Evaluation of Long-term Functionality of hESC Derived RPE Monolayer Implanted in RCS Rats**

**Methods:** Polylorized hESC-RPE cells (HR) were cultured for 4 weeks on porous sheets. Rectangular pieces (0.4mm x 0.9mm) of porcine were seeded with RPE cells, which formed a confluent monolayer. These grafts were implanted into the subretinal space of RCS rats (n=6). Rats that received porcine sheets alone (without hESC-RPE) served as the control group (n=3). All animals were administered with oral cyclosporine A (CyA in drinking water, 210mg/l) from one day prior to surgery and maintained for the entire period of study. An intraperitoneal injection of dexamethasone was given for the initial two weeks (1.6mg/kg/day) after surgery. Post-operative examinations were performed using spectral-domain optical coherence tomography (OCT) and optokinetic testing. Electrophysiological mapping of the SC was performed at 6 months post-implantation.

**Results:** OCT examination confirmed the placement of the hESC-RPE graft in the rat’s subretinal space. Optokinetic testing demonstrated considerably higher visual activity only in the hESC-RPE transplanted eye and was maintained throughout the study period. Electrophysiological testing revealed that visual responses recorded from transplanted rats under low level light stimulation (threshold = 1.0 log cd/m²) was confined to a small area on the surface of the SC. Fundus imaging data confirmed that the location of the hESC-RPE graft inside the eye is corresponding to the area of visual preservation in the SC.

**Conclusion:** Based on optokinetic testing and SC mapping studies, hESC-RPE cultured on porcine substrates contributed to the preservation of the visual responses in RCS rats. This study suggests that hESC-RPE seeded porcine implants may be considered for treatment strategies to slow the progression of dry-type age-related macular degeneration.

**Commercial Relationships:** Biju B. Thomas, None; Alejandra Gonzalez-Calle, None; Yuntao Hu, None; Danhong Zhu, None; Padmaja B. Thomas, None; Ann L. Philip, None; Dennis O. Clegg, None; David R. Hinton, None; Mark S. Humayun, None

**Support:** California Institute for Regenerative Medicine (CIRM), NEI EY03040

**Program Number:** 314 Poster Board Number: A555

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

**Transplantation Of Es Cell-derived Retinal Cells Into The Adult Retina**


**Purpose:** Embryonic stem (ES) cells provide a promising source for retinal cell transplantation, a novel strategy to repair the degenerate retina. We previously demonstrated that post-mitotic photoreceptor precursors can functionally integrate into the adult retina. In this study we first sought to develop an improved protocol to increase the number of differentiated retinal progenitors and enable the feasibility of ES cell-derived retinal cell transplantation to be tested. Secondly, we sought to determine if a pure population of retinal cells would be feasible.

**Methods:** Using a mouse EBS ES cell line containing a GFP reporter driven by the retinal transcription factor Rx and a serum-free floating culture method (Osakada, et al, 2008), we assessed the efficiency of retinal progenitor (Rx.GFP+) differentiation. The efficacy of photoreceptor differentiation was also assessed, with or without the selection of Rx.GFP+ cells, by immunocytochemistry. Photoreceptor development was examined by quantitative PCR in comparison with the early postnatal retina. AAV2/9 CMV.GFP labelled cells were FAC-sorted and subretinally transplanted into adult mice. Photoreceptor cell integration and retinal cell survival were evaluated 2 weeks post transplantation.

**Results:** The differentiation of Rx.GFP+ progenitors was significantly improved.
with greater seeding cell densities (3,000-27,000 cells/ml). Further differentiation of these cells generated significantly more Crx+ and Rhodopsin+ photoreceptors compared with mixed populations of unselected cells. Transcriptional analysis demonstrated an increase in crx and nrl expression, photoreceptor precursor markers, until day 28. We therefore dissociated cells at this stage and 200,000 GFP+ cells were subretinally transplanted. Although GFP+ cells were present in the subretinal space, no integrated ES cell-derived photoreceptors were observed in the host retina.

Conclusions: This method for retinal cell differentiation improves the number of retinal progenitors and produces both photoreceptor precursors and mature photoreceptors from mouse ES cells. However, the number of photoreceptor precursors present in the differentiated cell population (0.2% Nrl+ cells) is too low for successful transplantation. Therefore, methods to purify photoreceptor precursors may be required to confirm their ability to integrate into the adult retina.

Commercial Relationships: Anai Gonzalez Cordero, None; Emma L. West, None; Claire Hippert, None; Yanai Duran, None; Rachael A. Pearson, None; Jane C. Sowden, None; Robin R. Ali, None

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Program Number: 315 Poster Board Number: A556
Presentation Time: 8:30 AM - 10:15 AM

Amphiphilic Chitosan Nanomicelles For The Topical Delivery Of Rapamycin
Sasutayawanra Jomvaraparupa, Zeenah Elsaid, Mirza Gunic, Naba Elsaid
Timothy L. Jackson, Zeeenah Elsaid, John C. Sowden, Scott Compton, The School of Pharmacy, The University of London, London, United Kingdom; 2Kings College Hospital, London, United Kingdom.

Purpose: The purpose of this study was to synthesize and characterise a positively charged amphiphilic polymer and to use this for the preparation of nanomicelles for the ocular delivery of rapamycin.

Methods: A chemically modified chitosan derivative; octanoyl-g-chitosan-g-PEG was synthesized, purified via dialysis and then lyophilized to produce the final product which was characterised using FT-IR and 1H NMR. This polymer was used to prepare rapamycin-loaded nanomicelles using the thin film method. Nanomicelles were analysed for size and charge (Dynamic Light Scattering- DLS), surface morphology (Transmission Electron Microscopy- TEM) and thermal properties (Differential Scanning calorimetry- DSC). Schirmer penetration and retention was analysed by mounting porcine sclera in Ussing chambers and measuring the drug content using High Performance Liquid Chromatography. The limit of quantification (LOQ) for this was 12.5 ng/ml.

Results: The successful synthesis of this amphiphilic chitosan derivative was confirmed using 1H NMR and FT-IR. This polymer can self-assemble into monodisperse nanomicelles in an aqueous environment to encapsulate the hydrophobic drug, rapamycin. The nanomicelles had an average size of 52 nm and were positively charged. The formulation remained stable for up to 3 days. Upon visible inspection, the formulation appeared clear with a low polydispersity index of 0.25. Permeation studies showed tissue retention within 24 hours of exposure.

Conclusions: This synthesis of chemically modified mucoadhesive nanomicelles of small size and positive charge was successfully achieved. These rapamycin-loaded micelles were retained in the porcine sclera and may be considered for future applications in the ocular delivery of other hydrophobic drugs.

Commercial Relationships: Sarutayawanra Jomvaraparupa, None; Zeenah Elsaid, None; Mirza Gunic, None; Naba Elsaid, None; Timothy L. Jackson, None

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Program Number: 316 Poster Board Number: A557
Presentation Time: 8:30 AM - 10:15 AM

Pilot Study Comparing Non-invasive Laser And Invasive Microelectrode Array (MEA) Stimulation Of Neurons For Cortical Visual Prosthesis (CVP) Development
Yulia A. Luneva, Boris K. Baziyan, Marianna E. Ivanova, Andrey N. Serkov.

Purpose: Development of highly selective penetrating noncontact and noninvasive multichannel light stimulation of brain neurons is an extremely desired alternative to MEA for CVP. Noncontact light stimulation requires trepanation of occipital bone with subsequent closure of the formed opening by transparent polymer with attached external laser stimulation device. Such noncontact technique would help to avoid challenges associated with MEAs. Based on the work performed by J. Wells (2007) on pulsed laser neuronal stimulation, we designed and conducted series of experiments on laser stimulation of visual cortex in feline model. Purpose of this work was to study responses of feline's visual cortical neurons to laser stimulation and compare its efficacy to standard electrical stimulation.

Methods: Two cats trained to produce “phosphene model” reaction (published in our earlier works) were used in the experiment. MEA of 10 varnish-coated nichrome electrodes with 100-µm diameter was implanted into layer 3-4 V1 area of visual cortex in cat 1. To induce phosphenes, electric stimuli of 1.0-3.0 J/cm2 with 0.1-0.5ms impulse duration were used. Visual cortex of cat 2 was stimulated by multispot YAG-laser with custom-made 600-µm optic fiber, using wavelength of 2.12µm, and stimuli of 0.7-5.0 J/cm2. During the stimulation brain surface was continuously moisturized with saline. Visual evoked potentials (VEP) were recorded in both animals during stimulation. Responses received from the optical stimulation were filtered and amplified.

Results: Cat produced behavioral responses to induced phosphenes (raising paw) from electrical stimulation. VEP of cat 2 clearly demonstrated neuronal action potentials induced in the layer 3-4 of visual cortex, however, distinct behavioral response to the range of used parameters was not observed.

Conclusions: Pulsed laser stimulation induced distinct selective response in neurons of layer 3-4 of V1 visual cortex area in felines. Our results suggest that laser can be used as possible noncontact stimulation agent in CVP. Further investigation of the efficacy and safe parameters of laser stimulation of visual cortex is required.

Commercial Relationships: Yulia A. Luneva, None; Boris K. Baziyan, None; Marianna E. Ivanova, None; Andrey N. Serkov, None

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Presentation Time: 8:30 AM - 10:15 AM

Development of Hyaluronic Acid Hydrogels for Ocular Stem Cell Differentiation
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Purpose: The purpose of this study is to design hyaluronic acid (HA) based hydrogels to differentiate ocular stem cells for ocular tissue regeneration.

Methods: A serial of hyaluronic acid macromers are synthesized by conjugating 2-aminoethyl methacrylate (AEMA) to hyaluronic acid with different molecular weights (i.e. 6400, 16000 and 66000). The chemical structures of these macromers are characterized using ATR-FTIR and NMR. Their cytotoxicity is tested to different cell lines such as PC-12 and ocular stem cells using the MTT assay. Hyaluronic acid hydrogels are synthesized from these macromers using photo-polymerization. The mechanical properties of these hydrogels are characterized using rheometer and dynamic mechanical analyzer (DMA). The degradation of these hydrogels is also characterized. PC-12 cells and ocular stem cells such as limbal stem cells are encapsulated into the hydrogels in situ during the photo-polymerization. The cell encapsulated hydrogels are stained using LIVE/DEAD® Viability/Cytotoxicity Kit and cell viability assays to determine the cell viability after photo-polymerization. In particular, the differential of the ocular stem cells are induced using several different growth factors. The efficiency of induced differentiation is under investigation.

Results: ATR-FTIR and NMR measurements confirm the successful synthesis of HA-AEMA macromers. The substitution degree can be modulated by adjusting the molecular weight of HA and the ratio of AEMA to HA. MTT data indicate that these HA-AEMA macromers are not cytotoxic. Hydrogels can be formed under mild photo-polymerization conditions, while LIVE/DEAD® cell stain indicates that cells could survive under such polymerization conditions. The growth factor induced differentials of stem cells is right now further investigated.

Conclusions: The recently developed HA hydrogels have great potential for in situ encapsulation of ocular stem cells and differentiation of these cells for ocular tissue regeneration.

Commercial Relationships: Tae Y. Lee, None; Linfung Wu, None; Keegan B. Compton, None; Junjie Zhang, None

Support: None

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Presentation Time: 8:30 AM - 10:15 AM

Main Biocompatibility Factors of Microelectrode Array (MEA) Implantation in Cortical Visual Prosthesis (CVP)
Marianna E. Ivanova, Boris K. Baziyan, Andrey N. Serkov.

Purpose: Concept of CVP is well-understood, however, brain-computer interface still requires improvement before it can be used in humans. To date there are 6 important biocompatibility factors in the production of MEA and implantation: electrode material & size, electrode coating, number of electrodes, stimulus intensity, interelectrode distance (IED) and distance to blood vessels. In this work we investigated factors of proximity to the blood vessels and influence of the interelectrode distance on biocompatibility and work stability of implanted coated MEA for CVP in rodent model.

Methods: 12 maleistar rats were divided into 4 equal groups, based on the electrode material (stainless steel, nirohme, Tungsten or Molybdinum). MEAs of 2x3 or 2x5 100-µm diameter electrodes, with IEDs of 300, 500 and 700µm, and 2-µm thick silicon carbide (SiC) or 15-µm varnish coating, were implanted into the
4th layer of vascular cortex of the rats. Follow-up period was 6 months. Tissue damage was evaluated by the extent of necrosis, fibrotic incapsulation thickness, and densities of macrophage accumulation.

Results: The extent of the necrosis was the same for all types of electrode material and coating, ranging from 7.0 to 17.2 µm. Incapsulation thickness was statistically greater (p less than 0.05) in the group with 300-µm IED (4.1±0.6 µm) than in the 500-µm (2.9±0.7 µm) or 700-µm (2.0±0.6 µm) IED groups. In three groups of 300-µm IED, the incapsulation and macrophage accumulation were so profound that they enveloped two or more electrodes at once, thus 300-µm distance appeared to be insufficient for the successful long-term biocompatible MEA implantation. 75% of the electrodes were at the distance of ≥300µm from the nearest blood vessel, which did not affect the biocompatibility. Whereas the rest 25% of the electrodes were very close to small blood vessels. If a blood vessel was at the distance of ≤50µm, an intensive local inflammatory response was observed: thickness of fibrotic incapsulation reached 70µm, and macrophage density reached 5000 cells/mm².

Conclusions: Implantation of MEA in avascular cortex zone is extremely important for the biocompatibility and long-term stability of the whole complex. Optimal interelectrode distance for the electrode diameters of 100µm is 500µm or greater.

Commercial Relationships: Marianna E. Ivanova, None; Boris K. Baziyan, None; Andrey N. Serkov, None
Support: None

Program Number: 320 Poster Board Number: A561 Presentation Time: 8:30 AM - 10:15 AM
Biodegrading Human Corneal Stroma Using Adult Stem Cells on an Aligned Nanofibrous Substrate: Effects of Growth Factors
Jian Wu1, Yiqin Du2, William R. Wagner3, James L. Funderburgh2
1Ophthalmology & Surgery, 2Ophthalmology, 3McGowan Institute for Regenerative Medicine, Department of Surgery, University of Pittsburgh, Pittsburgh, PA; 2Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, PA.

Purpose: This study focused on bioengineering corneal stromal tissue using human corneal stromal stem cells (hCSSCs). We recently demonstrated that an aligned nanofibrous substrate provides topographic cues to initiate and guide organization of a stroma-like extracellular matrix (ECM) by hCSSCs (Wu et al., Biomaterials, PMID 22078813). The current study examined the effect of soluble factors, fibroblast growth factor 2 (FGF2) and transforming growth factor-beta 3 (TGFβ3) in effecting matrix deposition and organization in this system.

Methods: Aligned nanofibrous substrates were prepared by electrospinning biodegradable poly(electrode urethane) urea (PEUU) onto a high-speed rotating mandrel. hCSSCs were seeded onto the aligned nanofibrous substrate and cultured in serum-free medium supplemented with ascorbate-2-phosphate, insulin and FGF2, 10 ng/mL, TGFβ3, 0.1 ng/mL, or both factors. After 6 wks, secreted ECM was evaluated by transmission electron microscopy, wholomeum immunostaining, and immunoblotting of culture media. Gene expression was examined by qPCR.

Results: Cultures supplemented with FGF2-only secreted collagen fibrils strongly aligned with the nanofibrous substrate; however, TGFβ3 induced distinct orthogonal collagenous layers. Genes of stromal ECM secretion, KERA, B3GnT7, and CHST6 were upregulated in all cultures. Stromal ECM components keratan sulphate, dermatan sulphate, and decorin-like macromolecular were detected in secreted ECM and in culture media. The combination of FGF2 and TGFβ3 produced significant synergetic effect in construct thickness, in packing of collagen fibrils, and in expression of cornea-specific ECM components: keratan sulphate, lumican, and keratan.

Conclusions: On aligned PEUU nanofibrous substrates, spatial self-organization of collagen-based ECM by hCSSCs exhibits characteristic responses to specific growth factors. Presence of both FGF2 and TGFβ3 produced a significant synergetic effect, leading to stratified multilayered lamellae with orthogonally-oriented collagen fibrils, mimicking that of human corneal stroma. This study provides a new tool for bioengineering of a well-organized, collagen-based substrate to construct with appropriate nanoscale structure for corneal repair and regeneration.

Commercial Relationships: Jian Wu, None; Yiqin Du, None; William R. Wagner, None; James L. Funderburgh, None
Support: Ocular Tissue Engineering and Regenerative Ophthalmology (OTERO) program of the UPMC Eye Center and the McGowan, NIH grants EY016415 and P30-EY008098, Research to Prevent Blindness Inc.

Program Number: 320 Poster Board Number: A562 Presentation Time: 8:30 AM - 10:15 AM
Determination of Retinal Pigment Epithelial Cells from iPS cells established on 3T3-J2 feeder and autologous fibroblast feeder
Noriyuki UEMURA1,2, Shunsuke Takeuchi3, Ana Santos-Carvalho2, Noriko SAKAI1, Noriko SHINOHARA1, Noriko KERA1, Satoshi OKAMOTO4, Masayo TAKAHASHI5, Ken-ichiro HATA1
1R&D Department, Japan Tissue Engineering Co., Ltd., Gamagori, Japan; 2Laboratory for Retinal Regeneration, RIKEN Center for Developmental Biology, Kobe, Japan.

Purpose: Induced Pluripotent Stem (iPS) cells are one of the appropriate candidates for the cell source of retinal pigment epithelial (RPE) transplant. Human pluripotent stem cells, such as embryonic stem (ES) cells and iPS cells, are generally maintained in culture with myc (c-myc) producer fibroblasts (MEF), STO cells, or its derived SNL cells, all of which are mitotically inactivated by treatment with mitomycin C or irradiation. However, these feeder cells have not been yet proven to be suitable for clinical application in terms of exogenous antigens, unidentified viruses, and zoonotic pathogens. In this study, we evaluated the potential of human adipose-derived fibroblasts as well as 3T3-12 cells that have been used as feeder cells of cultured epidermis, and of which clinical safety has been confirmed in over 20 years whether they can be used as feeder cells for the generation and maintenance of iPS cells capable of differentiating into RPE cells.

Methods: To generate iPS cells, human dermal fibroblasts were electroporated with episomal vector encoding reprogramming factors. Resulting ES-like colonies were mechanically dissociated and transferred on to 3T3-J2 feeder, human fibroblast feeder, or STO feeder, respectively and cultured further. To confirm the differentiation ability, we subjected 3 lines of iPS cells from each feeder to in vitro directed differentiation into RPE with the modified SFEB method.

Results: We were able to establish iPS cells with 3T3-J2 feeder and human fibroblast feeder, of which morphologies were similar to iPS cells established with STO feeder. We also obtained RPE colonies from J2-iPS cells and human fibroblast feeder-iPS cells with similar efficiency compared to STO-iPS cells.

Conclusions: Our results demonstrate that 3T3-J2 cells as well as autologous fibroblasts can be used for feeder cells of iPS cells capable of differentiating into RPE. These findings not only offer new option for the establishment of clinical grade iPS cells but also propose the necessity to evaluate the potential of currently available clinically-proven feeder.

Commercial Relationships: Noriyuki Uemura, Japan Tissue Engineering co., Ltd. (E); Shunsuke Takeuchi, Japan Tissue Engineering co., Ltd. (E); Shika Shimoogawa, Japan Tissue Engineering co., Ltd. (E); Noriko Sakai, None; Satoshi Okamoto, None; Masayo Takahashi, None; Ken-ichiro Hata, Japan Tissue Engineering co., Ltd. (E)
Support: None

Program Number: 321 Poster Board Number: A563 Presentation Time: 8:30 AM - 10:15 AM
Characterization And Differentiation In Photoreceptors Of Retina-derived Induced Pluripotent Stem Cells
Maria Carmela Allocca1, Ana Santos-Carvalho2,3, Budd A. Tucker4, Sara Qi1, Caio V. Regatieri1,2, Cahit Zhang1, Konrad Hochedlinger5, Claudia Cavadas4, Michael Young1,2, Scheepens Eye Research Institute/Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA; 2Center for Neuroscience and Cell Biology and Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal; 3Department of Ophthalmology and Visual Sciences, Institute for Vision Research, Carver College of Medicine, Iowa City, IA; 4Massachusetts General Hospital Cancer Center and Center for Regenerative Medicine, Boston, MA.

Purpose: Our lab and others have shown that induced pluripotent stem cells (iPS cells) obtained from fibroblasts may provide a source of photoreceptors for cell based therapies. One limitation of this approach is the low yield of photoreceptors obtained. Recently, it has also been shown that iPS cells retain a transient transcriptional and epigenetic memory of their cell of origin which affects their potential for differentiation into photoreceptors. The purpose of this work was to characterize the pluripotency and the differentiation into photoreceptors of iPS cells lines obtained from murine retinas.

Methods: Retinas from “reprogrammable” mice were cultured in the presence of doxycycline to induce the expression of the four reprogramming factors c-Myc, Klf4, Oct-4 and Sox-2 and to produce iPS colonies. The pluripotency of these colonies and epigenetic memory were tested by evaluating the expression of pluripotency and photoreceptor markers by immunocytochemistry, western blot and RT-PCR analysis. Pluripotency was also evaluated by teratoma assay. The ability of iPS cells to differentiate into photoreceptors was evaluated in vitro by immunocytochemistry. The percentage of photoreceptors positive for recoverin, rhodopsin and Cone-Rod homeobox (CRX) was determined.

Results: Retina-derived iPS cells expressed the pluripotency markers c-Myc, Klf4, Oct-4, Sox-2 and Nanog. In the teratoma assay we observed the presence of tissues from all three germ layers. The iPS cells line from retinas also expressed photoreceptor markers. The in vitro differentiation of the retina-derived iPS cells resulted in photoreceptor shaped cells and 80-90% of total number of cells expressed positive staining for photoreceptor markers (CRX, recoverin and rhodopsin). We also observed an increase in expression of photoreceptor markers compared with the undifferentiated iPS cell colonies. Moreover, the expression of pluripotency markers Oct-4 and Nanog decreased.

Conclusions: The iPS cells obtain from the murine retina were pluripotent and differentiated into photoreceptors with a very high efficiency.

Commercial Relationships: Maria Carmela Allocca, None; Ana Santos-Carvalho, None; Budd A. Tucker, None; Sara Qi, None; Caio V. Regatieri, None; Cahit Zhang, None; Konrad Hochedlinger, None; Claudia Cavadas, None; Michael J. Young, None

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IGF-1 Binding Protein Like Protein 1 (IGFBPL-1) Promotes Axon Outgrowth In Retinal Ganglion Cells Through The Regulation Of IGF-1 Signaling Pathway

Chenyong Guo1, Kin-Sang Cho1, Kissaou Tchedre2, Jie Ma2, Huihui Chen2, Taimur Malik2, Dong Feng Chen2,∗1. Harvard Medical School, Schepens Eye Research Institute, Boston, MA; 2Center for Innovative Visual Rehabilitation, VA Boston Healthcare System, Boston, MA.

Purpose: IGFBPL-1 has been implicated to play an important role in neuronal cell survival and axonal growth, and its effects are mediated through its main receptor IGF-IR. However the underlying mechanisms are not fully understood. We recently found that a newly discovered protein, IGFBPL-1 binding protein like protein 1 (IGFBPL-1), regulates the growth of retinal ganglion cell (RGC) axons. Here we propose to elucidate the functional significance and underlying mechanisms of IGFBPL-1 in mediating RGC survival and axon extension in mice.

Methods: The expression of IGFBPL-1 in the developing retina was examined in mice at different developmental stages, ranging from embryonic day 16 through adult, using immunohistochemistry, western blot and quantitative RT-PCR. To study the function and underlying signaling event of IGFBPL-1, retinal ganglion cells were purified from mouse pups at postnatal day 0 (P0) and P10, and cultured in the presence or absence of IGFBPL-1 and/or IGF-1 proteins. RGC survival and axonal growth were evaluated after three days in culture using LIVE/DEAD® and β-III-tubulin immunostaining. Knockdowns of IGF-1 and IGFBPL-1 signaling were achieved by lentiviral shRNAs or application of inhibitors of IGF-1 downstream pathways. Recombinant IGFBPL-1 and/or IGF-1 proteins were injected intravitreally into adult C57BL/6 mice on day 0, 3 and 6 post-optic nerve crush to study their effects in vivo. Axonal regeneration following optic nerve crush were quantitatively assessed by labeling RGC axons with an anterograde axon tracer cholera toxin B subunit (CTB). The number of CTB+ axons extended posterior to the crush site was recorded.

Results: IGFBPL-1 was expressed at a high level in the ganglion cell layer of the retina at E16 but was largely down-regulated postnatally. Addition of IGFBPL-1 alone or together with IGF-1 to P0 and P10 RGC cultures significantly promoted axonal extension. Blockade of IGFBPL-1 signaling eliminated IGFBPL-1-mediated axonal growth effect. Moreover, intravitreal delivery of IGFBPL-1 significantly promoted optic nerve regeneration following optic nerve injury in adult mice. Conclusions: IGFBPL-1 is an important regulator of RGC axonal growth during retinal development, likely functioning through the IGF-1 signaling pathways. These studies provide new avenues that may fill the knowledge gap of the molecular events regulating RGC axon growth, which will lead to potential therapeutic strategies for optic nerve protection and eventually regeneration or repair.

Commercial Relationships: Chenyong Guo, None; Kin-Sang Cho, None; Kissaou Tchedre, None; Jie Ma, None; Huihui Chen, None; Taimur Malik, None; Dong Feng Chen, None.

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Program Number: 322 Poster Board Number: A564
Presentation Time: 8:30 AM - 10:15 AM

Photoreceptor Transplantation In The Degenerating Retina: Breath Of Application And Manipulation Of The Microenvironment To Enhance Efficiency

Amanda C. Barber1, Claire Hippert1, Yanai Duran1, Emma L. West1, Jorn Lakowski1, Jim W. Bainbridge2, Jane C. Sowden2, Robin R. All1, Rachael A. Pearson1,∗1. Genetics, UCL Institute of Ophthalmology, London, United Kingdom; 2Developmental Biology Unit, Institute of Child Health, University College London, London, United Kingdom.

Purpose: Photoreceptor transplantation offers a potential therapy for irreversible retinal degenerations, a leading cause of blindness in the developed world. Transplanted rod photoreceptor precursors have been shown to integrate robustly into recipient retinas, adopt a mature rod phenotype and contribute to scotopic vision. Here, we aim to elucidate the time-course and mechanisms of donor cell migration, integration and maturation within the recipient retina and to compare these processes with the same integration-competent population during normal development. Understanding such mechanisms will allow refinement of strategies to improve transplanted photoreceptor integration efficiency, ultimately aiding progress towards clinical utility and facilitate adaptation of the approach to different cell types and systems.

Methods: Neonatal Nrl.GFP mouse retinas were dissociated enzymatically. Nrl.GFP+ve rod precursors were FACs-sorted and injected subretinally into adult wild-type mice. Recipients were sacrificed between 24 hrs and 3 weeks post-transplantation. Eyes were cryosectioned, immuno-stained and confocally imaged. Maturation and migration of Nrl.GFP+ve rod precursors during normal development were assessed using immunohistochemistry and time-lapse 2-photon microscopy, respectively.

Results: Numbers of integrated Nrl.GFP+ve donor cells peaked during the first week post-transplantation. Cells were observed to integrate into recipient retinas through a sequence of events involving polarized neurite extension and somatic translocation. Immunostaining for phototransduction cascade proteins indicated a polarization of cellular specializations during migration, with segment formation preceding synaptogenesis. Expression of rod-specific markers in integrated cells took place in a time frame similar to that of normal development. Differentiation of nuclear architecture was significantly accelerated in transplanted rods.

Commercial Relationships: Katherine M. Warre Cornish1, Jane C. Sowden1, Robin R. All1, Rachael A. Pearson1, None.
Support: Wellcome Trust, Medical Research Council, Royal Society, National institute for Health Research.

Program Number: 325 Poster Board Number: A566
Presentation Time: 8:30 AM - 10:15 AM

Perception of Depth Using the BrainPort Vision Device

Aimer Arnoldusen1, Amy Naud1, Chris Fisher1, Jacki Fisher2, Christine Pintar2, Gail Englert1, Kathleen Janossy1, Alex Keifer1, Cody Wolfe2, Nick Wach2,∗1. 1UPMC Eye Center, Pittsburgh, PA.

Purpose: Depth perception is an integral part of being able to correctly interpret one’s environment, particularly for mobility or grasping tasks. The issue of whether
depth cues can be appreciated with monocular camera-based artificial vision devices is an unanswered question. The purpose of this pilot study was to determine whether subjects using the BrainPort vision device would be able to interpret spatial relationships between two objects.

Methods: 4 blindfolded sighted adults (1 male; 3 females) participated in this study. All had previous exposure to the BrainPort device. Experiments were conducted with both standing and seated tasks with a fixed camera field of view (75 and 40 degrees respectively). The standing tasks used a 13 x 11 inch black box on a white tile floor. The tasks were to 1) determine whether the box was 5, 10, 20 feet away 2) determine the distance away (five foot increments) until the object could no longer be detected 3) determine the separation distance (6 inch increments) between two boxes 4) estimate the distance of the box placed at random distances. Tabletop conditions were completed on a black felt backdrop using 1.5 inch cylinders, a 12x4 inch felt rectangle and a felt circle with a diameter of 5.5 inches. The tasks included: A) moving two cylinders horizontally closer together (from 6 inches) until separation could no longer be appreciated B) determining whether a felt circle was above or below a felt rectangle C) estimate the distance (in inches) between the two cylinders D) determine whether the felt circle was in front or behind the rectangle.

Results: For standing tasks subjects 1) averaged 66% correct in estimating the distance to the box, 2) could detect the presence of the box from an average 18.75 feet, 3) determined the two box discrimination distance to be an average of 9 inches 4) could estimate boxes placed at random distances within 1.75 feet error. For the seated tasks, subjects A) could detect the separation between two cylinders with average 2.75 inches minimal distance, B) could determine the spatial relationship between felt shapes with 79% accuracy, C) could estimate the distance between cylinders with at least 0.5 inches distance, D) could determine the spatial relationship between overlapping shapes with 83% accuracy. Conclusions: This study demonstrates that it is possible to discern crude depth information using a two dimensional tactile stimulus on the tongue. It is likely that training for this specific task, as well as increased resolution would enhance this ability. Methods to improve depth appreciation are needed in order to enable mobility and grasping tasks with artificial vision devices.

Commercial Relationships: Aineen Arnoldussen, Wicab (E); Amy Nau, None; Chris Fisher, None; Jacki Fisher, None; Christine Pinlar, None; Gail Engleka, None; Kathleen Janosco, None; Alex Keifer, None; Cody Wolfe, None. Support: NIH R43 EY-128978-01A1, Army DRMPD DM090217

### Program Number: 326 Poster Board Number: A567

### Presentation Time: 8:30 AM - 10:15 AM

#### Physico-chemical Characterization Of A New Stable Mucoadhesive Nanoeumulsion Of Latanoprost

**Danilo Aleo**, Maria G. Saita, Sergio Mangiafico, Melina G. Cro', Sebastiano Mangiafico', Rosanna Chillemi, Sebastiano Sciuto'.

**R&D, Medivis, Catania, Italy; Dipartimento di Scienze Chimiche, Università degli Studi di Catania, Catania, Italy.**

Purpose: The formulation of topical lipophilic drugs in ophthalmology is a very difficult challenge. The aim of our study was to develop and characterize a new drug delivery system (DDS) capable of carrying out latanoprost for topical ophthalmic use.

Methods: The new nanoemulsion (LAT-DDS) was prepared adding 0.005% latanoprost and 0.1% hyaluronic acid (HA) to the DDS composed by a phosphate buffered water containing Medium Chain Triglycerides (MCT) and VIT E TPGS. To characterize and to investigate the mechanism that stabilize latanoprost we prepared the DDS in D2O and 1H NMR spectra were registered; a 0.005% latanoprost in D2O solution (LAT) was also prepared for comparison. We have also prepared a 0.005% latanoprost solution in water (LAT). D2O was used for both LAT-DDS and LAT. 1H NMR Spectroscopy, Dynamic Light Scattering and Electrophoretic Mobility measurements were performed. Latanoprost chemical stability in (LAT-DDS) was controlled under different ICH recommended conditions.

Results: 1H NMR signals of LAT-DDS system were different from those obtained in the D2O formulation of LAT. In particular, the signals of the phenyl protons in ortho, meta and para and the signals of protons of the isopropyl ester group of latanoprost shifted upfield (the A in chemical shifts were 0.19 ppm, 0.26 ppm, 0.27 ppm and 0.10 ppm, respectively). The shifts showed the protective effect of DDS on the latanoprost. Moreover we were able to detect, by Dynamic Light Scattering and Electrophoretic Mobility measurements that the particle size of the stable nanoemulsion was 10nm±2 and that HA binds with DDS, as indicated by the change of particle size and by the net increase of the Z potential from less than -4nV without HA to about -15nV after adding HA. As showed by stability study addition of HA in DDS did not affect latanoprost stability.

Conclusions: In the LAT-DDS latanoprost is stable at room temperature. The supramolecular binding between HA and the other components of LAT-DDS furnishes to the nanoemulsion the mucoadhesive ability which may represents a strong advantage in the ocular penetration (aqueous bioavailability) of latanoprost in the treatment of glaucoma and ocular hypertension.

### Program Number: 327 Poster Board Number: A568

### Presentation Time: 8:30 AM - 10:15 AM

#### Electro-Mechanical Tactile Corneal Stimulation System for Vision Substitution

**Michael Belkin**, Gal Elami, Eli Azoulay', Dan Ilani, Yevgeny Beiderman, Zeev Zalesky'

**Goldschleger Eye Research Institute, Tel-Aviv University, Tel-Hasomer, Israel; Department of Ophthalmology, Bar-Ilan University, Ramat-Gan, Israel.**

Purpose: To develop a sensory substitution device in which corneal stimuli are used to generate neural input to the brains of visually compromised people to substitute for absent retinal input.

Methods: The device will be composed of spectacles-mounted cameras wirelessly transmitting processed images to a special contact lens translating the visual information into tactile stimulation of the corneal nerves. A low energy electric stimulation of the corneal nerves is investigated as well. In order to improve the spatial resolution of the constructed image, the camera will also time multiplex, compress and encode the captured image before transmitting it to the stimulating contact lens.

Results: Preliminary devices based upon tactile stimulation of the edge of fingers by applying mechanical pressure and of the tongue by applying proper electrical stimulation, were constructed and tested. The mechanical stimulator included an ACS741 micro-processor whose outputs after being amplified was connected to 25 solenoids who applied the mechanical pressure. 5 subjects were taught to "see" and to recognize simple patterns using this mechanical stimulation device. The electrical tactile stimulator included USB camera as image acquisition device, the image signals were transmitted via NI USB Digital I/O card and then amplified using TI RC4136 OP-AMP circuit. The output was connected to a tongue stimulation device which was a specially designed 4 layers PCB with 100 stimulation points.

Prototype of the desired cornea/sclera Stimulating contact lens is currently being constructed.

Commercial Relationships: Michael Belkin, Gal Elami, Eli Azoulay', Dan Ilani, Yevgeny Beiderman, Zeev Zalesky. Support: None

### Program Number: 328 Poster Board Number: A569

### Presentation Time: 8:30 AM - 10:15 AM

#### A Stable Chitosan-coated Nanomicelle Combination Of Vitamin E-TPGS And Cholesterol-PEG For The Topical Administration And Scleral Retention Of Rapamycin

**Naba Elsaid', Zeenah Elsaid', Satyanarayana Somavarapu', Timothy I. Jackson'.**

**1Kings College Hospital, London, United Kingdom; 2The School of Pharmacy, University of London, London, United Kingdom.**

Purpose: The aim of this study was to prepare and characterise chitosan-coated vitamin E-TPGS; cholesterol-PEG (CPEG) nanomicelles and use these in the ocular delivery of the insoluble, hydrophobic drug rapamycin.

Methods: Micelles containing vitamin E-TPGS and cholesterol-PEG (CPEG) were prepared and coated with chitosan oligomer using the thin film method. This formulation was analysed for encapsulation efficiency, size and stability (Dynamic Light Scattering; DLS), surface morphology (Transmission Electron Microscopy; TEM) and critical micelle concentration (fluorometry). Furthermore, ex-vivo studies were conducted by mounting porcine sclera in Ussing chambers and measuring the drug content which was retained in and had permeated across the sclera. Sample analysis was carried out using High Performance Liquid Chromatography with a limit of quantification (LOQ) of 12.5ng/mL.

Results: The TPGS:CPEG micelles successfully entrapped rapamycin with an encapsulation efficiency of 78% and a desirable size of 13nm. These micelles retained stable for 7 days, unlike the TPGS micelles which were stable for 3 days. The solution was visibly clear with a polydispersity index of 0.07 and no sign of aggregates. TEM and DLS data confirmed this and showed uniform particle distribution. The critical micelle concentration was found to be 1x10^-5 w/v. The addition of CPEG and the chitosan coating of these micelles enabled permeation within the first 30 minutes of tissue exposure and caused a significant increase in scleral retention.

Conclusions: A stable nanomicellar formulation was developed with desirable physicochemical properties and transscleral permeation which occurred within the first 30 minutes of tissue exposure. Furthermore, there is potential for future applications in topical drug delivery as this formulation was retained in the sclera.
Methods: To determine factors enhancing optic nerve (ON) regeneration, RGC5 cells were cultured on tissue culture dishes in DMEM-10%FBS with different neurotrophic/growth factors, individually, and in combination (ciliary neurotrophic factor [CNTF], neurotrophin3 [NT3], brain derived neurotrophic factor [BDNF], glial-cell-line derived neurotrophic factor [GDNF], nerve growth factor [NGF], insulin-like growth factor [IGF], Sema7a, stauroporine [positive control]) and increasing concentrations of a PTEN (phosphatase and tensin homolog) inhibitor (1BPV[Phen]) 0.2mM to 200mM). Cells were imaged at 15 hours, and at 3, 5 and 7 days to check for survival and neurite outgrowth (25µm in length).

The optic nerve of Thy1-YFP mice was transected either partially or totally with preservation of meninges. Mice were sacrificed at weeks 1, 2, and 3 and optic nerve and retina were imaged with the Leica DM-IRE2 confocal microscope (single and z-stack images). Images were analyzed using Image J software.

Results: At 15hrs, all growth factors promoted similar neurite outgrowth. At 3 days, stauroporine and NGF increased the number of cells; BDNF and Sema7a plates showed the most number of arborizations; and Sema7a prolonged RGC survival. 2mM BPV[Phen] was the most effective neurite outgrowth promoter, and combined with IGF/Sema7a/CNTF led to the longest and most numerous outgrowth.

Both partial and total optic nerve transections resulted in a significant decrease in fluorescence at all time points, both in the optic nerve distal to the transection site, and also in the retina. No regain in fluorescence distally to the transection site was detected at any time point after transection.

Conclusions: Our preliminary experiments show that PTEN inhibitors promote in vitro RGC regeneration. Thy1-YFP mice provide a model to characterize optic nerve regeneration after injury.

Commercial Relationships: Daoud S. Fahed, None; Norma Allemann, Wallace Chamon, Jin-Hong Chang, Sandeep Jain, Dimitri Azar, None; Support: None

Program Number: 330 Poster Board Number: A571 Presentation Time: 8:30 AM - 10:15 AM Dual role of Bmi1 loss in the preservation of photoreceptor layers in the Rd1 mouse

Karine Schouwney1, Dusan Zencak1, Maarten Van Lohuizen1, Yvan Arsenijevic1, 1UGTSCB, Jules-Gonin Eye Hospital, Lausanne, Switzerland; 2Division of Molecular Genetics, Netherlands Cancer Institute and The Centre of Biomedical Genetics, Amsterdam, The Netherlands.

Purpose: In the Rd1 and Rd10 mouse models of retinitis pigmentosa, a mutation in the Pde6ß gene leads to the rapid loss of photoreceptors. As in several neurodegenerative diseases, Rd1 and Rd10 photoreceptors re-express cell cycle proteins prior to death. Bmi1 regulates cell cycle progression through inhibition of CDK inhibitors, and its deletion efficiently rescues the Rd1 retinal degeneration. The present study evaluates the effects of Bmi1 loss in photoreceptors and Müller glia, since in lower vertebrates, these cells respond to retinal injury through dedifferentiation and regeneration of retinal cells.

Methods: Cell death and Müller cell activation were analyzed by immunostaining of wild-type, Rd1 and Rd10; eye sections during retinal degeneration between P10 and P20. Lineage tracing experiments using the GFP-Cre mouse (JAX) to target Müller cells.

Results: In Rd1 retinal explants, inhibition of CDKs reduces the amount of dying cells. In vivo, Bmi1 deletion reduces CDK4 expression and cell death in the P15 Rd1/Bmi1−/− retina, although cGMP accumulation and TUNEL staining are detected at the onset of retinal degeneration (P12). This suggests that another process acts in parallel to overcome the initial loss of Rd1/Bmi1−/− photoreceptors. We demonstrate here that Bmi1 loss in the Rd1 retina enhances the activation of Müller glia by downregulation of p27kip1, that these cells migrate toward the ONL, and that some cells express the retinal progenitor marker Pax6 at the inner part of the ONL. These events are also observed, but to a lesser extent, in Rd1 and Rd10 retinas. At P12, EdU incorporation shows proliferating cells with atypical elongated nuclei at the inner border of the Rd1/Bmi1−/− ONL. Lineage tracing targeting Müller cells is in process and will determine the implication of this cell population in the maintenance of the Rd1/Bmi1−/− ONL thickness and whether downregulation of Bmi1 in Rd10 Müller cells equally stimulates their activation.

Conclusions: Our results show a dual role of Bmi1 in the rescue of photoreceptors in the Rd1/Bmi1−/− retina. Indeed, the loss of Bmi1 reduces Rd1 retinal degeneration, and as well, enhances the Müller glia activation. In addition, the emergence of cells expressing a retinal progenitor marker in the ONL suggests Bmi1 as a blockade to the regeneration of retinal cells in mammals.

Commercial Relationships: Karine Schouwney, None; Dusan Zencak, None; Maarten Van Lohuizen, None; Yvan Arsenijevic, None; Support: None

Program Number: 331 Poster Board Number: A572 Presentation Time: 8:30 AM - 10:15 AM Nano-size Particles Of Probiotics For Preventing And Treating Neuroinflammation

Janos Feher1, Erika Pinter2, Zsuzsanna Helyes2, Janos Szolcsanyi2, 1Dept of Visual Science, Sapientia University of Rome, Rome, Italy; 2Department of Pharmacology and Pharmacotherapy, University of Pecs, Pecs, Hungary.

Purpose: Rapidly growing evidence suggests that neuroinflammation may contribute to degenerative diseases of the nervous system, including the eye. Furthermore, chronic low-grade inflammation of the mucosal membranes may generate or aggravate neuroinflammation. Probiotics are widely recommended to restore microbiota-host symbiosis on mucous membranes and for treating inflammatory diseases of the gut, airways and vagina. Recently, several studies support that killed or non-motile (lysozyme killed) lactobacilli may also have anti-inflammatory and neuroprotective effects, but their effects on neuroinflammation have not yet been studied. Here we present our first results on the effects of probiotics lysate on reducing release of pro-inflammatory cytokines from LPS stimulated macrophages in vitro, and on attenuating LPS-induced neuroinflammation in vivo. Our theory is that fragmented or oligodispersed (lysozyme killed) probiotics may enhance bioavailability and efficacy of probiotic lysate.

Methods: Tyndallized probiotics were prepared from industrially grown Lactobacillus acidophilus and Bifidobacterium longum by heat treatment for 1 h at 70°C on three consecutive days. Using ultrasonication we prepared nano-size particles from probiotic lysate up to size less than 5.000nm. The influence of nano-size probiotics on TNFalpha and IL-1 beta release were studied in vitro and in vivo.

Results: Anti-inflammatory effects of nano-size probiotics were tested in three different models: (i) In vitro: Lysate of Lactobacillus acidophilus and Bifidobacterium longum dose and size-dependently reduced pro-inflammatory cytokine release of LPS stimulated myeloid macrophages of mouse, being the most effective the 1000 nanometer compared to the higher size. (ii) In vivo: Activation of microglia in brain and the retina was attenuated by nano-size probiotics animal model of sublethal sepsis and of inflammatory bowel disease. (iii) Case reports: Brain damage improved up to 2 years after ischemic stroke and traumatic brain injury treated with a composition of nano-size probiotics, cod liver oil and vitamin B complex.

Conclusions: Taking together these findings from our and other laboratories, we may conclude that nano-size particles of probiotic (i) may have anti-inflammatory effect in vitro and in vivo, (ii) may be formulated with other active and synergistic compounds, and finally (iii) administration of nano-size probiotics may open a new approach for preventing and treating neuroinflammation and related diseases of the nervous system and the eye. Ongoing clinical trials are destined to confirm this hypothesis.

Commercial Relationships: Janos Feher, Nutripharma Hungary Ltd, Budapest, Hungary (P); Erika Pinter, None; Zsuzsanna Helyes, None; Janos Szolcsanyi, None; Support: KOMP-1.1.4-11-B/2011-0024, Government of Hungary

Program Number: 332 Poster Board Number: A573 Presentation Time: 8:30 AM - 10:15 AM Development Of Single Step Nano-Optical Immunoassay As A Replacement For ELISA To Measure The Protein Biomarkers Upregulated In Dermal Stasis

Shraddha V. PrabhuKas1, Adam de la Zerda1, Sanjiv Gambhir2*, Richard Awdel1, 1Bascom Palmer Eye Institute, University of Miami, Miami, FL; 2Electrical Engineering and Radiology, 3Bioengineering & Materials Science and Engineering, 4Stanford University, Palo Alto, CA.

Purpose: This study focuses on the development of a rapid single-step, highly sensitive immunoassay for the measurement of protein biomarkers with a wide detection range. Gold nanorods (GNRs) were used as the signaling labels, as they are not affected by reagent chemistry or photobleaching, and exhibit unique optical properties such as tunable enhanced scattering/absorption of light and biocompatibility. Optical coherence tomography (OCT) was chosen as the tool to detect the GNR labels, utilizing their high scattering properties.

Methods: GNRs [size:15X60nm, SPR:840nm] were covalently attached to Glut-1 specific monoclonal antibodies via carbodiimide chemistry. Glut-1 protein (model
analyze) was directly attached to the surface of micro-titter plates by overnight incubation at 4°C. The Glut-1 monoclonal antibody functionalized GNRs were then allowed to incubate within the wells for 60 minutes at room temperature. The wells were then twice-rinsed with 1X PBS buffer to remove any unbound GNRs. 100µl of pH = 14, 1X PBS buffer was then infused into each well and the plates were sonicated for 45 minutes. The sonication and alkaline buffer causes the protein and gold nanorod conjugate to break up and float freely in the buffer solution. OCT imaging of the sonicated immunoassay plates containing functionalized GNRs + bound analyte dispersed in PBS solution was conducted using the Biophotonic Spectral Domain Ophthalmic Imaging System (SDOIS). A calibration curve was obtained by plotting the concentration of the Glut-1 protein initially incubated in each well versus the average signal intensity obtained from normalized OCT images.

**Results:** Our detection range using this nano-optical immunoassay was wide, ranging from 10ng/ml to 1ug/ml. The minimal detectable concentration based on the intercept of our calibration curve of Glut-1 was 2.8ng/ml.

**Conclusions:** This novel nano-optical immunoassay holds promise to act as a simple, selective, sensitive strategy for effective disease diagnosis, and offers advantages over traditional ELISA techniques, such as wide detection range, increased speed of analysis (due to fewer incubation/washing steps), and no label development required. Our future goal is to incorporate this strategy onto a microfluidic platform to be used as a point-of-care diagnostic tool.

**Commercial Relationships:** Shradha V. Prabhulkar. None; Adam de la Zerda. None; Sanjiv Gambhir. None; Richard Awdeh. None

**Support:** NIH/ NEI R21 – EY029 40

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**Program Number:** 333 Poster Board Number: A574

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

**TeleReA Tele-Rehabilitation Pilot Study for Artificial Vision Devices**


**Purpose:** The BrainPort™ (Wicab, Madison WI) pairs a camera to an electrode display which rests on the tongue. The device improves orientation and mobility for the blind by providing information about the proximal environment. Like all blind skills, practice is required to attain proficiency, but a lack of trained therapists, geographic and financial barriers are issues that must be overcome. Without a rehabilitation infrastructure to support long term training, rates of abandonment are likely to be high for artificial vision devices. Creative methods to overcome this deficiency must be urgently explored. The Rehabilitation Engineering Research Center at the University of Pittsburgh has developed a secure telehabilitation portal termed Visyter. The purpose of this pilot study was to: 1) develop a year-long rehabilitation protocol for advanced BrainPort training using existing Visyter infrastructure 2) determine the barriers to tele-rehabilitation in a blind cohort and 3) deploy a smartphone application allowing a sighted person to assist with training at home.

**Methods:** 3 adult, male blind subjects were enrolled in this pilot study. Baseline psychophysical and mobility tests used in previous BrainPort studies were conducted on day 1 and again after approximately 15 hours of training at the UPMC Eye Center. Upon completion, subjects were sent home with a BrainPort, a Droid smartphone (Verizon) allowing a sighted person to directly view images displayed on the tongue, a camera and a speaker. The clinical interactions between the subject and therapist consisted of increasingly advanced skills and homework tasks covered during 30 minute sessions for 6 months. Subjects used their own computers with a high speed internet connection. Our primary outcome measure included development of a prototype tele-rehabilitation capability.

**Results:** We successfully deployed the Visyter software program to all subjects. Barriers included the need for a sighted person to assist with hardware and software set up, and ideally be present for training sessions. Computers with high speed internet access are needed. All subjects and therapists felt the portal was useful and the smartphone application to be very helpful. All subjects and therapists felt the interaction was as productive as face to face interaction.

**Conclusions:** This study showed that telehabilitation represents a feasible strategy to overcome the barriers to successful long term rehabilitation with artificial vision devices. Future studies will explore use of smartphone cameras to allow for remote mobility training in outdoor settings as well as to investigate use of tele-rehabilitation portals for low vision populations.

**Commercial Relationships:** Amy C. Nau. None; Jacqueline Fisher. None

**Support:** Fine Foundation

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**Program Number:** 334 Poster Board Number: A575

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

**Suramin Is A Potent Stimulator Of Retinal Ganglion Cell Regeneration After Optic Nerve Injury**

Sue-Wai Yu, W.K. Wong, A.W.S. Cheung. E.Y.P. Cho. School of Biomedical Sciences, The Chinese University of Hong Kong, Shatin, Hong Kong.

**Purpose:** Suramin, an anti-parasitic drug and a non-specific P2 purinergic receptor antagonist, has been reported to have neuroprotective properties. However, whether suramin promotes regeneration of the central nervous system is not known. To address this, the influence of suramin on the survival and regeneration of retinal ganglion cells (RGCs) after optic nerve (ON) injury was studied.

**Methods:** The ON of adult hamster was transected and suramin or its vehicle (saline) was injected intravitreally. At 1 week post-ON cut, RGC survival was quantified by biHII-tubulin immunostaining, and surviving RGCs that expressed the growth-associated protein GAP-43, a marker that correlates with regenerative propensity, were counted. Peripheral nerve (PN) grafting to the cut ON was used to assess whether suramin could potentiate the regeneration of RGC axons into the graft. Intravitreal injection of ciliary neurotrophic factor (CNTF), a potent growth factor for RGC regeneration, PPADS (non-specific P2 antagonist), or suramin analogues (with greater specificity towards selective P2 subtypes), were performed to compare with suramin.

**Results:** Both Suramin and CNTF promoted moderate RGC survival (Fig.1A). Suramin, however, has a dramatic effect on RGC regeneration. The number of GAP-43 and regenerating RGCs were 400% and 800%, respectively, that of the control after suramin treatment (Figs.1B-C). Suramin also stimulated more GAP-43 expression (150%) and RGC axonal regeneration into the PN (155%) compared to CNTF (Figs.1B-C). In contrast, PPADS did not enhance GAP-43 expression and axonal regeneration of RGCs above that of control (Fig.3B-C), suggesting that suramin promoted regeneration independent of P2 antagonism. This was also reflected in studies with suramin analogues in which no clear-cut pattern was seen (Fig.3: NF157 and NF279 promoted GAP-43 expression and regeneration similar to suramin, while NF65 and NF110 resembled the control.**

**Conclusions:** Suramin promotes RGC axonal regeneration better than CNTF. The mechanism involved may be distinct from P2 blockade.

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Commercial Relationships: Sau-Wai Yu, None; W.K. Wong, None; A.W.S. Cheung, None; E.Y.P. Cho, None

Support: General Research Fund CUHK463309

Program Number: 335 Poster Board Number: A576

Presentation Time: 8:30 AM - 10:15 AM

Functional And Anatomic Evaluation Of Human Retinal Progenitor Cells Transplanted Into The Subretinal Space Of Rhodopsin Knockout Mice: Caihui Jiang, Petr Baranov, Ruilin Wang, Ximei Zhang, Michael Young. Ophthalmal-Scheiners Eye Rearch Inst, Harvard Medical School, Boston, MA.

Purpose: A leading cause of untreatable blindness is photoreceptor loss due to retinal degenerative diseases or injury. Replacement of photoreceptors has been suggested as a potential therapeutic approach for treatment of retinal degenerative diseases, such as retinitis pigmentosa and age-related macular degeneration. Here we investigate the functional and anatomic results of transplantation of human retinal progenitor cells (hRPCs) into the subretinal space of rhodopsin knockout mice.

Methods: hRPCs were isolated from 18 wk gestational age retina and expanded in vitro up to passage 9. Cells were characterized by flow cytometry for expression of progenitor markers. 100,000 live cells were transplanted into the subretinal space of rhodopsin -/- mice in HBSS + N-acetyl-cysteine (NAC). Mice in control group received subretinal injection of HBSS + NAC. Electroretinogram (ERG) and optical coherence tomography (OCT) were performed at various time after transplantation. Mice were sacrificed by CO2 inhalation 4 to 8 weeks after transplantation. Immunohistochemistry study was performed with the following primary antibodies against human mitochondria, nestin, Ki67, rhodopsin, recoverin. Retinal sections were viewed on a confocal microscope.

Results: The b-wave amplitudes of ERG in hRPC treated group were significantly higher than that in the HBSS + NAC control group. The outer nuclear layers (ONL) of retina in hRPC treatment group were significantly thicker than that in the HBSS + NAC control groups. 4 to 8 weeks after transplantation, a subset of hRPCs survived as xenografts in the mouse subretinal space without exogenous immune suppression. Grafted hRPCs migrated and incorporated into the recipient neural retina as was confirmed by human mitochondria staining. Integrated cells also express photoreceptor markers rhodopsin and recoverin.

Conclusions: Transplantation of hRPCs restores retinal structure and function in retinal degenerative mice. Transplanted hRPCs migrate and integrate into the mouse retina and differentiate into photoreceptors. These results suggest the potential of hRPC transplantation for treating retinal degenerative diseases.

Commercial Relationships: Caihui Jiang, None; Petr Baranov, None; Ruilin Wang, None; Ximei Zhang, None; Michael Young, None


Program Number: 336 Poster Board Number: A577

Presentation Time: 8:30 AM - 10:15 AM

Effect of Multi-walled Carbon Nanotubes on R28 retinal precursor Cell Survival: Thomas Baltz1, Christine Haselier1, Stephan Hess2, Frank Meinel2, Ingolf Endler2, Peter Walter2, Gabriele Thumann1. 1. Department of Ophthalmology, RWTH Aachen University, Aachen, Germany; 2. Fraunhofer Institute for Ceramic Technologies and Systems, Dresden, Germany.

Purpose: Interfacing neuronal tissue with extracellular electrodes is a promising approach to regain functionality in patients suffering from neuronal degeneration. For retinal degenerative diseases in which photoreceptors are degenerated, e.g. retinitis pigmentosa, prosthesis are being developed to restore vision by electrically stimulating surviving retinal cells. Nano modification of microelectrodes represents a valuable approach to optimize electrode properties, such as charge transfer capacity and signal to noise ratio. Aligned multiwalled carbon nanotubes (MWCNT) are an excellent candidate for interfacing them with neural systems. To assess whether MWCNT are applicable for retinal implants, in a first step MWCNT were synthesized on silicon wafers and the survival of R28 retinal precursor cells grown on these wafers was investigated.

Methods: Aligned MWCNT were synthesized on 4-inch silicon wafers by chemical vapour deposition. Substrates were deposited on a SiO2 buffer layer followed by an Al2O3 layer to improve CNT length and homogeneity. Iron particles, 3 mm in diameter, were deposited to act as catalyst for aligned CNT growth. The CNT synthesis was carried out at atmospheric pressure using Ar as carrier gas. R28 cells were seeded on silicon wafer pieces with and without MWCNT, as well as on plastic culture dishes. Cell survival was determined using the Fluorescein-Diacetat / Propidiumidol life-dead-assay.

Results: MWCNT exhibited a length of 14.8 µm and average inner and outer diameters of 5 and 10 nm, respectively. R28 cells proliferate and form confluent monolayers on wafers with and without MWCNT. Compared with uncoated wafers, however, cell death of R28 cells grown on MWCNT coated wafers was slightly increased, by average by an amount of 5.69% (p<0.05). The surviving rates were on average 98.50±6.84%, 97.67±4.231 and 91.99±1.61% (means±em.s.) for R28 cells grown on plastic dishes, uncoated and MWCNT coated wafers, respectively.

Conclusions: Our current data provide evidence that R28 retinal precursor cells are a sensitive indicator to examine putative toxic effects of biomaterials aimed to contact retinal tissue. MWCNT show a small but significant toxic effect on the survival of R28 cells. Optimization of synthesis parameters of MWCNT should further enhance biocompatibility.

Commercial Relationships: Thomas Baltz, None; Christine Haselier, None; Stephan Hess, None; Frank Meinel, None; Ingolf Endler, None; Peter Walter, None; Gabriele Thumann, None

Support: BMBF „Mikro-Nano-Integration“

Program Number: 337 Poster Board Number: A578

Presentation Time: 8:30 AM - 10:15 AM


Purpose: As a first step towards engineering an outer retina suitable for transplantation, we designed a biocompatible, biodegradable scaffold that will allow retinal progenitor cells (RPCs) to form flat, laminar structures. The scaffolds minimize the exposure of retinal progenitors to extracellular matrix components that are not found in retina, and will allow for coupling with the RPCs.

Methods: Scaffold sheets were formed from electroporuse fibers of polycaprolactone (PCL). Duration of collection determined polymer thickness and solvent concentration dictated porosity and pore distribution of the scaffold. The thickness and porosity of the sheets were varied to optimize cell culture and rate of degradation of the PCL. Finally, minimal defects were cut with a biopsy punch and coated with laminin. Retinal progenitors, derived from human embryonic stem cells (hESC-derived RPCs), were cultured to form neurospheres. The neurospheres were dissociated into single cell suspensions, seeded onto PCL sheets, and maintained in serum free medium. Fluorescence labeling and confocal microscopy were used to assess the morphology of the cultures.

Results: Individual PCL fibers measured ~3 µm in thickness. The thickness of the sheet could be varied from 20-200 µm. The porosity could be adjusted to form 5 to 100 µm diameter pores. The scaffolds degraded via surface erosion, within a slightly basic pH range at 7.4 to 7.9, over four to seven weeks, depending upon porosity. Scaffolds with pores 25 to 50 µm in diameter were subsequently chosen for culture experiments. Confocal imaging confirmed that the RPCs penetrated the thickness of the scaffold and continued to express retinal markers such as Pax6, recoverin, and N-cadherin. There was evidence of polarity, as the N-cadherin was expressed only along one surface of the polymer.

Conclusions: Electroporuse PCL polymers are biodegradable, biocompatible, and sustain the differentiated properties acquired by the neurospheres. It appears to be a suitable scaffold for reconstituting an outer retinal layer from hESC-derived RPCs that can enhance cell survival and delivery to a diseased sub-retinal space via transplantation.

Commercial Relationships: Lilanggi S. Eidrichkremru, None; Shaomin Peng, None; Nina Kristoff, None; Tawv A. van Zyl, None; Geleang Gan, None; Lin Li, None; Caihong Qiu, None; Mark Saltzman, None; Ron A. Adelman, None; Lawrence J. Rizzo, None

Support: HHMI-FRB Medical Research Fellows Program, Connecticut Innovations, 10SBC02, Leir Foundation, Newman’s Own Foundation, National Natural Science Foundation of China, 30772381

Program Number: 338 Poster Board Number: A579

Presentation Time: 8:30 AM - 10:15 AM

Characterizing the Tissue Engineered Cornea Stroma by CUDA GPU Accelerated Computing: Yougang Pang1, Xiaolu Wang2, Mingqiao Peng3, Bing Bo4, Charles S. Bouchard4, 1. Biomedical Engineering, 2. Bovine type I collagen hydrogel

Purpose: The unique aligned structure of collagen in corneal stroma plays a fundamental role in the optical properties of the cornea. A tissue engineered cornea using collagen hydrogel would also need an aligned architecture in order to generate a promising alternative. Effective characterization techniques are required in order to control the structures of an engineered cornea to resemble that of the normal human cornea. In the current project, we used a precisely controlled stretching technique to generate collagen hydrogel with an aligned structure and used our in-house developed CUDA GPU (graphic processing unit) accelerated image processing technique to characterize the dynamics of the collagen fiber alignment.

Methods: Bovine type I collagen hydrogel was polymerized and placed in a
A Prototype Instrument for Subretinal Transplantation of induced Pluripotent Stem (iPS) Cell-derived Retinal Pigment Epithelial Cell Sheets

Purpose: To develop surgical techniques and instruments for cell sheet transplantation into subretinal space.

Methods: Retinal pigment epithelial (RPE) cells were derived from human induced pluripotent stem cells and RPE cell sheets were prepared by the expansion of picked-up induced pluripotent stem (iPS) cell-derived RPE cell colonies. The pneumatic balloon actuator (PBA) was made of polydimethylsiloxane (PDMS) membrane and driven by pneumatic pressure. Employing the principle of PBA, a 3 x 3mm square shaped actuator was retracted into the cylindrical shaped cannula of 2mm diameter. We fabricated a prototype instrument for subretinal transplantation with a cannula which RPE cell sheets could be rounded in a cylindrical shape and inserted into the eye. By using the instrument, we tried to perform sequential motions so as to insert and transplant RPE cell sheets into the subretinal space of monkey eyes.

Results: The micro architecture of the collagen hydrogel was successfully imaged by reflection confocal microscopy and it changed from a random to an aligned pattern after stretching. The degree of alignment increased as stretching reached higher degrees. Recording the collagen hydrogel dynamics generated large amount of images, which were up to tens of Gigabytes in size. Conventional CPU (central processing unit) based image processing techniques were not capable of finishing the image processing in required time period. CUDA GPU accelerated image processing increased the alignment characterization speed by 50 times compared with CPU based image processing, which brought the alignment analysis to real-time level.

Conclusions: Controlled mechanical stretching showed promising results for engineering corneal stroma. Reflection confocal microscopy and CUDA GPU image processing are powerful tools to characterize the dynamics of collagen alignment.

Commercial Relationships: Yonggang Pang, None; Xiaoli Wang, None; Mingqiao Peng, None; Ping Bu, None; Charles S. Bouchard, None


Program Number: 339 Poster Board Number: A580
Presentation Time: 8:30 AM - 10:15 AM

A Preliminary Study Of An Active Micro Valve For Glaucoma

Methods: The valve is actuated by conducting polymers and fabricated by MEMS technology. The characterisation process is performed by numerical simulation using the finite element method and a circuit model, and by in vitro hydrodynamic testing.

Results: The hydraulic resistance of the designed GDD varies in the range of 13.08-0.36 mmHg min/ml with 3.38-0.43 mmHg min/ml for the Ahmed valve. The maximum displacement of the diaphragm in the vertical direction is 18.9 μm, and the strain in the plane is 2%. The proposed preliminary design allows to control the IOP by varying the hydraulic resistance in a greater range than the existing passive valves, and the numerical simulation facilitates the characterisation and the improvement of the design before its construction, reducing time and costs.

Conclusions: The microarchitecture of the valve actuator can be controlled to be used as a treatment for glaucoma.

Commercial Relationships: Fabio A. Guarnieri, Fernando Sasseti, Luciano Garelli, Bioengineering, CIMEC (INTEC-CONICET), Santa Fe, Argentina.

Support: Fundacion Argentina de Nanotecnologia

Program Number: 340 Poster Board Number: A581
Presentation Time: 8:30 AM - 10:15 AM

ARVO 2012 Annual Meeting Abstracts by Scientific Section/Group – Nanotechnology and Regenerative Medicine Group (NT)

Pluripotent Stem (iPS) Cell-derived Retinal Pigment Epithelial Cell Sheets

Methods: Embryoid bodies were formed by the hanging drop method, plated for differentiation, and characterized by RT-PCR to assess for differentiation into three germ layers. Embryoid bodies were formed by the hanging drop method, plated for differentiation, and characterized by RT-PCR to assess for differentiation into three germ layers. In vivo differentiation was assessed by teratoma formation after subcutaneous injection of iPS cells into immune deficient mice. iPS cells were injected into blastocysts to assess contribution in chimera mice.

Results: Both three and four factor (3F and 4F) lenti-viral induction methods to form iPS cells demonstrated endogenous expression of Oct4, Sox2, Klf4, and c-Myc similar to mouse ES cells. 3F and 4F iPS cells were more efficient than ES cells. In vitro and in vivo differentiation of iPS cells generated cells in all three germ layers.

Conclusions: We are the first group to demonstrate that CB cells can be reprogrammed into iPS, and that three factors are sufficient to generate iPS cells from the CB. Dispersing c-Myc from a known oncogene for safer cell transplant.

Support: V.N.R. NIH T35-DK007386; A.N. None; S.H.C. Hope for Vision, Research to Prevent Blindness, K-08 Career Development Award

Program Number: 342 Poster Board Number: A583
Presentation Time: 8:30 AM - 10:15 AM

Adult Dental Pulp Stem Cells Present Similarities Between Dental Pulp From Deciduous Teeth and Human Limbal

Purpose: Expression profile of ADPSC was studied using markers for limbal stem cells (LSC) and corneal epithelia such as ABCG2, connexin 43, vimentin, p63, keratin-3/12. The expression was analyzed by immunochemistry and RT-PCR.

Results: Expression profile of ADPSC was studied using markers for limbal stem cells (LSC) and corneal epithelia such as ABCG2, connexin 43, vimentin, p63, keratin-3/12. The expression was analyzed by immunochemistry and RT-PCR.

Support: None; Aiguo Ni, None; Sai H. Chavala, None

Program Number: 341 Poster Board Number: A582
Presentation Time: 8:30 AM - 10:15 AM

Oc4t, Sox2, Klf4 are Sufficient to Reprogram Ciliary Body Cells into Induced Pluripotent Stem Cells

Purpose: Atrophic age-related macular degeneration (AMD) is caused by retinal pigment epithelial (RPE) degeneration. Induced pluripotent stem (iPS) cells, essentially undistinguishable from embryonic stem (ES) cells, have been derived from skin fibroblasts using four reprogramming factors, Oct4, Sox2, Klf4, and c-Myc. Fibroblast derived iPS cells have been differentiated into RPE cells and present a viable option to replace damaged RPE cells. Ciliary body (CB) cells may be a favorable cell source for iPS because they possess the same embryonic lineage as RPE cells. Since c-Myc is a known oncogene, reprogramming using only three factors is favorable for cell replacement therapy. The study focused on whether CB cells can be reprogrammed into iPS cells, and if three factors are sufficient for pluripotency.

Methods: The CB region of Sox2-GFP reporter mice eyes were dissected and cultured as a monolayer after enzymatic and mechanical trituration. Passage 2 CB cells were seeded onto mouse embryonic fibroblasts after lenti-viral transfection of Oct4, Klf4, Sox2, and without c-myc to generate iPS. Resulting iPS cells were characterized by immunostaining and RT-PCR for embryonic stem cell markers. Embryoid bodies were formed by the hanging drop method, plated for differentiation, and characterized by RT-PCR to assess for differentiation into three germ layers. In vivo differentiation was assessed by teratoma formation after subcutaneous injection of iPS cells into immune deficient mice. iPS cells were injected into blastocysts to assess contribution in chimera mice.

Results: Both three and four factor (3F and 4F) lenti-viral induction methods to form iPS cells demonstrated endogenous expression of Oct4, Sox2, Klf4, and c-Myc similar to mouse ES cells. 3F and 4F iPS cells were more efficient than ES cells. In vitro and in vivo differentiation of iPS cells generated cells in all three germ layers.

Conclusions: We are the first group to demonstrate that CB cells can be reprogrammed into iPS, and that three factors are sufficient to generate iPS cells from the CB. Dispersing c-Myc from a known oncogene for safer cell transplant.

Support: None; Aiguo Ni, None; Sai H. Chavala, None
immunocitochemistry analysis, with the same aforementioned markers.

**Results:** RT-PCR results showed that ADPSC present similarities between LSC markers and dental pulp from deciduous teeth, which was demonstrated by immunocitochemistry. The differentiation of ADPSC into corneal epithelium was confirmed by immunocitochemistry analysis which reacted positively with K3/12. In control group, we could not observe positive reaction.

**Conclusions:** We concluded that ADPSC present gene expression profile similar to those observed in LSC and dental pulp from deciduous teeth. Moreover, ADPSC showed able to differentiate into corneal epithelium cells. These similarities suggest these cells are a strong candidate to be used in corneal epithelium reconstruction in cases of limbal stem cells deficiency.

**Commercial Relationships:** Priscila C. Cristovam, None; Babylia G. Monteiro, None; Nelson F. Lizer, None; Renata R. Loureiro, None; Joyce L. Covre, None; Juliana A. Sobrinho, None; Irina Kerkis, None; José Álvaro P. Gomes, None

Support: FAPESP

Program Number: 343 Poster Board Number: AS84
Presentation Time: 8:30 AM - 10:15 AM

**Sub-Retinal Injection of Human Adult Stem Cells Preserves ERG Response in RCS Rats**

**Adi Tzameret**, Michael Belkin, Avraham J. Treves, Ziva Rosenthal-Galili, Arnon Nagler, Ygal Rotenstreich, Goldschleger Eye Research Institute, Tel-Hashomer, Israel; Cancer Research Center, Tel-Hashomer, Israel; Hematology Division, Tel-Hashomer, Israel.

**Purpose:** To investigate the effect of subretinal injection of allogeneic human-derived bone marrow mesenchymal stem cell population (hBM-MSCs) on retinal functions. measured electroretinographically, and retinal structure of Royal College of Surgeons (RCS) rats.

**Methods:** hBM-MSCs (CD73+; CD90+, CD105+, CD45-) from healthy human donors were expanded ex-vivo up to for four passages. 0.25 Million cells in 5µl were transplanted into the sub-retinal space of one eye each of 54 4 weeks old RCS rats. Ten RCS rats were injected subretinally with saline as control. The ERG responses of both eyes of all the animals was tested before the injections and afterwards for ten weeks. Animals were dark-adapted for minimum of six hours prior to the ERG measurements. Scotopic and photopic ERGs were recorded from both eyes simultaneously using corneal golden wire loops. The eyes were then enucleated and processed for histology.

**Results:** Four weeks after injection (figure 1), the b-wave amplitude responses of the scotopic and photopic ERG showed 74% deterioration from baseline compared to 94% deterioration (p<0.05) in the control groups (not injected eyes and saline injected eyes). These significant differences (p<0.05) were found up to the tenth week.

**Conclusions:** In this study we have shown for the first time that transplanting hBM-MSCs as a thin homogenous sub-retinal layer slows significantly the retinal deterioration rate as measured by ERG in RCS rats up to ten weeks. Subretinal injection of autologous hBM-MSCs may possibly be a therapeutic modality in patients with retinal degeneration.

Commercial Relationships: Adi Tzameret, None; Michael Belkin, None; Avraham J. Treves, None; Ziva Rosenthal-Galili, None; Arnon Nagler, None; Ygal Rotenstreich, None

Support: None

358 Nanotechnology and Regenerative Medicine Cross-Section Papers
Tuesday, May 8, 2012, 3:45 PM - 5:30 PM
Florida A Paper Session
Program #/Board #: 3635-3641

**Organizing Section:** Nanotechnology and Regenerative Medicine Group

**Program Number:** 3635 Presentation Time: 3:45 PM - 4:00 PM
**Cellular Therapy For Open Angle Glaucoma: Mechanism Of Tissue Regeneration By Mesenchymal Stem Cells**

**Renaud Manuguerra**, Denis-Claude Roy, Mark Lesk, University of Montreal, Montreal, QC, Canada; HMR research center, Montreal, QC, Canada.

**Purpose:** Elevated intraocular pressure (IOP) in open angle glaucoma is often related to a dysfunction of the trabecular meshwork (TM) of the anterior chamber angle. A large number of studies involving multipotent mesenchymal stem cells (MSC) have demonstrated their ability to induce regeneration of damaged tissues and organs. The purpose of this project is to evaluate the ability of MSCs to induce regeneration of the trabecular meshwork and to assess their ability to lower IOP in open angle glaucoma.

**Methods:** MSC obtained from B6 mouse bone marrow were co-cultured with rat TM cells to study their differentiation potential. MSC (1X10^5 cells) were also injected into the anterior chamber of rat eyes harbouring experimental glaucoma caused by laser photocoagulation to the TM. Control rats received a similar injection of mouse T lymphocytes (1X10^5 cells) or no injection at all. The homing and repair potential of these cells and their ability to restore baseline IOP was assessed using immunohistochemistry and tonometry over 6 weeks.

**Results:** After 7 days in vitro, MSC were not found to upregulate TM cell markers, such as Aquaporin-1, Pax-6, Laminin and Fibronectin. In vivo, MSC were found scattered throughout the anterior chamber angle, but migrated in significantly higher numbers towards the area of laser damage (N=15). The MSC remained in the eye for only 4 days as detected by immunofluorescence. Interestingly, the IOP of rats who received an MSC graft (1X10^5 cells) in the anterior chamber began to decrease significantly on day 4 when compared to the control rats (p<0.01) in a blinded study. This drop in IOP continued until ocular pressure was restored to baseline at the end of the first week in the MSC treated group (p<0.001). Baseline IOP was attained only at day 30 in the control groups. Additionally, the anterior chamber histology was restored to normal after 1 month in the MSC treated group, while demonstrating clear signs of scarring in the control groups at the same time period.

**Conclusions:** The impressive regenerative effects of MSC, along with their apparent lack of differentiation and their rapid clearance out of the anterior chamber implies a mechanism of action linked to secreted factors. This could represent a novel and promising approach in the management of open angle glaucoma.

**Commercial Relationships:** Renaud Manuguerra, None; Denis-Claude Roy, None; Mark Lesk, None

Support: None

Program Number: 3636 Presentation Time: 4:00 PM - 4:15 PM
**Bioengineered Corneal Endothelium for Transplantation**

Matthew Giegengack, Faruka Khan, Keith Walter, Jin San Choi, Min Jeong Kim, Gauggung Niu, Shuy Soker, Ophthalmology, Wake Forest Institute of Regenerative Medicine, Wake Forest University, Winston-Salem, NC.

**Purpose:** The objectives of this study are to fabricate gelatin-based scaffold as a carrier of human corneal endothelial cells (HCEC) and to characterize gelatin-based bioengineered cornea, containing HCECs, for endothelium transplantation. We hypothesize that we may overcome the supply issue in Descemet’s stripping endothelial keratoplasty (DSEK) by creating a process whereby a single high quality donor can be used to create many high quality corneal donor grafts.

**Methods:** Human CEC were obtained from discarded corneas of eye donors by digestion in collagenase II (2 mg/mL D-PBS) for 40 min at 37°C. The cells were cultured in EGM-2 with 10% FBS and thoroughly characterized. Corneal scaffolds were made using a 10% gelatin solution that was subsequently stabilized through N-ethyl-N’-[3-dimethylaminopropyl]carbodiimide/N-hydroxy succinimide (EDC/NHS) cross-linking. The EDC/NHS was then washed out for 2 days using de-ionized water. Approximately 130, 600, and 3000 cells/mm2 were seeded on the gelatin-based scaffolds. The constructs were placed in EGM-2 + 10% FBS for 7 days, then evaluated cell-coverage of surface on the film by alizarin red S.

**Results:** The modulus of the gelatin gel is around 3.0 Mpa and the tensile strain is about 70%. The gelatin gel has good transparency with visible light scope (>90%); this is higher than natural corneal stroma, transparency (80-90%). There was no significant difference in gelatin gel permeability with or without cells. A 1.5 molar ratio between gelatin’s amine and EDC/NHS, rather than 1:10, provided higher rates of diffusion and permeability. Initially cells grew better in TCP; however, after 7 days cell growth was similar between TCP and the gelatin-based scaffold. Alizarin red staining shows good cell integrity with confluent HCECs growth on gelatin-based scaffold.

**Conclusions:** We obtained a highly transparent gelatin gel with proper mechanical properties. The gelatin gel is easy to handle and can withstand manipulation.
porous structure of the gelatin gel enables efficient diffusion of nutrients through the gelatin-based scaffold. HCECs can be seeded successfully on the gelatin-based scaffolds and show high levels of cell proliferation. Currently, a rabbit model is being used as an in vivo test for the gelatin-based scaffold seeded with HCECs. The gelatin-based scaffold is a potential scaffolding candidate for endothelial cell transplantation.

**Commercial Relationships:** Matthew Giegengack, None; Faraaz Khan, None; Keith Walter, Ocular Systems Incorporated (C); Jin San Choi, Ocular Systems Incorporated (E); Min Jeong Kim, None; Guoguang Niu, None; Shay Soker, None

**Support:** None

**Program Number:** 3639

**Presentation Time:** 4:15 PM - 4:30 PM

**Sustained Therapeutic Effects of Nanoceria In a Mouse Model For AMD**

E17 and p0 retina tended to form rosettes strongly expressing rhodopsin whereas OLM-like structure labeled with ZO-1 and GS.

**Purpose:** To characterize the 3-dimensional differentiation of the mES and mPS derived retinal cells sheets derived from Rx-GFP mice and Nrl-GFP mice, and to evaluate the viability and maturation of retinal sheets transplanted into the wild type and degenerative mouse (rd1) retina.

**Methods:** Rx-GFP mES and Nrl-GFP mPS were differentiated in three-dimensional culture into retina tissue by a modified method by Eiraku et al (Nature, April 2011). The retinal progenitors were evaluated immunohistochemically along differentiation. The embryonic retina (E17) and postnatal retina (p0, p4) and mES or mPS derived retina were also transplanted into the subretinal space of wild type (B6) and rd1 retina. Their survival and maturation was immunohistochemically evaluated.

**Results:** The three-dimensional culture of mES or mPS cells produced optic vesicle-like structures strongly expressing Rx-GFP (ES line) co-labeled with Pax6 on d.d.9. These vesicles formed well organized retinal neuroepithelial like layers positive for Cx4 and Recoverin on d.d.16, followed by rhodopsin expression around d.d.25. These retinal-like structure partially presented outer limiting membrane (OLM) like structure labeled with ZO-1 and GS. E17 and p0 retina tended to form rosettes strongly expressing rhodopsin whereas p4 retina often lost structure but ingressed into the OLN of wild type host retina. Retina-like sheets of d.d.20 derived from mES and mPS also tended to form rosette, and seemed to mature in subretinal space.

**Conclusions:** Retina-like sheets derived from mES and Nrl-GFP mPS could serve as graft in retina sheets transplantation.

**Commercial Relationships:** Juthaporn Assawachanondam1, None; Michiko Mandai1, None; Satoshi Okamoto, None; Jun Kaneko1, None; Mototsugu Eirak1, None; Yoshiaki Sasa1, None; Masayo Takahashi, None

**Support:** None

**Program Number:** 3638

**Presentation Time:** 4:30 PM - 4:45 PM

**Sustained Therapeutic Effects of Nanoceria In a Mouse Model For AMD**

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**Conclusions:** Retina-like sheets derived from mES and Nrl-GFP mPS could serve as graft in retina sheets transplantation.

**Commercial Relationships:** Juthaporn Assawachanondam1, None; Michiko Mandai1, None; Satoshi Okamoto, None; Jun Kaneko1, None; Mototsugu Eirak1, None; Yoshiaki Sasa1, None; Masayo Takahashi, None

**Support:** None

**Program Number:** 3640

**Presentation Time:** 5:00 PM - 5:15 PM

**An In Vivo Model for Fuchs Endothelial Corneal Dystrophy**

M. Nour Haydari1,2, Marie-Claude Perron, André Deveault, Myriam Barbellion, Benjamin Goyer, Olivier Roy, Simon Laprise, J. Douglas Cameron, Stéphanie Proulx, Isabelle Brunette1,2. Ophthalmology, University of Montreal, Montreal, QC, Canada; 1Masai Medical Center, Kanazawa, Japan; 2Centre LOEX de l'Université Laval, Génie tissulaire et régénérat, Centre de recherche FRSQ du Centre hospitalier affilié universitaire de Québec and Département d’ophthalmologie et d’oto-rhino-laryngologie, Faculté de médecine, Université Laval, Québec, QC, Canada; 3University of Minnesota, Minneapolis, MN, USA.

**Purpose:** To evaluate the in vivo functionality of a tissue-engineered corneal endothelium reconstructed using corneal endothelial cells from human patients with Fuchs endothelial corneal dystrophy (FECD).

**Methods:** Sixteen healthy cats underwent full-thickness cornea transplantation. Eight animals were grafted with a tissue-engineered (TE) corneal endothelium reconstructed using cultured endothelial cells from patients with FECD (TE-FECD). Two control animals were grafted with a TE corneal endothelium reconstructed using cultured endothelial cells from normal eye bank corneas (TE-normal). Two control animals received a native human cornea. Four other controls were grafted with the stromal carrier only (without endothelial cells). Outcome parameters included graft survival (0 (opaque) to 4 (clear)), central pachymetry, optical coherence tomography, endothelial cell morphometry, transmission electron microscopy (TEM) and immunostainings of function-related proteins.

**Results:** Seven days after transplantation, 6 of 8 TE-FECD grafts, all TE-normal grafts and all native normal control grafts were clear (transparency score greater than 3), while all carrier only control grafts were opaque (transparency score of less than 1). The mean central pachymetry was 755±141 μm for TE-FECD, 524±7 μm for TE-normal.
for TE-normal, 555±32 µm for native normal and 1188 ±245 µm for carrier only. TEM showed subendothelial loose fibrillar material deposition in all TE grafts. Other typical but nonspecific findings included: intracellular filaments, cytoplasmic processes, enlarged rough endoplasmic reticulum and lysosomes. No corneal guttae were observed in this early postoperative period. The TE endothelium expressed Na⁺-K⁺/ATPase and Na⁺/HCO₃⁻. Clusterin immunostaining was faint and similarly expressed in TE-FECD, TE-normal and native grafts.

Conclusions: Restoration of corneal thickness and transparency demonstrated that the TE-FECD grafts were functional in vivo. This novel TE approach opens the door to future studies on FECD cell rehabilitation.

Commercial Relationships: M. Nour Haydari, None; Marie-Claude Perron, None; André Deveault, None; Myriam Bareille, None; Benjamin Goyer, None; Olivier Roy, None; Simon Laprise, None; J. Douglas Cameron, None; Stéphanie Proulx, None; Isabelle Brunette, None

Support: CIHR, FRQS Research in Vision Network and Fondation du CHA.

Program Number: 3641 Presentation Time: 5:15 PM - 5:30 PM

A porous poly(ε-caprolactone) tissue engineering scaffold for RPE Transplantation

Kevin J. McHugh¹,², Carrie Spencer¹, Patricia A. D’Amore³,⁴, Sarah L. Tao⁵, Magali Saint-Geniez³,⁵, ¹Schepens Eye Research Institute, Boston, MA; ²Biomedical Engineering, Boston University, Boston, MA; ³Ophthalmology, Harvard Medical School, Boston, MA; ⁴The Charles Stark Draper Laboratory, Inc., Cambridge, MA; ⁵Advanced Development Center, Current Affiliation: CooperVision, Inc., Pleasanton, CA.

Purpose: Transplantation of retinal pigment epithelium (RPE) is a promising approach for the treatment of retinal diseases including dry age-related macular degeneration (AMD). Past replacement strategies using RPE cell suspensions or sheets have failed largely due to poor adhesion or lack of organization on a Bruch’s Membrane (BrM) that is aged or itself diseased. In this study, we developed and tested a thin film, porous poly(ε-caprolactone) (PCL) scaffold to act as a biomimetic BrM substitute for RPE culture and potential implantation into the sub-retinal space.

Methods: A nanopatterned mold was created using a combined process of photolithography and reactive ion etching. Porous PCL scaffolds were then produced from the mold by spin-assisted templating and mounted on transwell inserts. Fetal human RPE cells (fhRPE) were maintained at confluence on the scaffolds for up to 8 weeks and compared to non-porous PCL and porous polyester (PET) transwells (Costar) using transepithelial resistance, gene expression, phagocytic capacity, and immunohistochemistry.

Results: fhRPE transepithelial resistance significantly increased on porous PCL compared to PET transwells beginning at 5 days and persisting through the end of the 8 week experiment. Cells on porous PCL also displayed significant upregulation of key RPE proteins including a 6.45-fold increase in RPE65 and 2.19-fold increase in CRALBP compared to the PET control at 4 weeks. Alternately, cells on non-porous PCL fail to show a significant change in major RPE markers compared to the control. fhRPE cultured on porous PCL and PET displayed similar levels of binding and phagocytosis of fluorescently-labeled photoreceptor outer segments after four weeks of culture. In addition, the greatest degree of pigmentation was observed in cells on the porous PCL followed by non-porous PCL, and finally PET.

Conclusions: Both material and porosity are important factors in RPE maturation and function in vitro. Our porous PCL thin film scaffold exploits both characteristics to promote the development of a mature RPE monolayer as evaluated by gene expression profile, organization, and polarization while allowing transport across the membrane.

Commercial Relationships: Kevin J. McHugh, None; Carrie Spencer, None; Patricia A. D’Amore, 61/499,909 (P); Sarah L. Tao, 61/499,909 (P); Magali Saint-Geniez, 61/499,909 (P)

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