**448 Stem cells and tissue engineering for retinal repair**
Wednesday, May 10, 2017 11:00 AM–12:45 PM
Exhibit/Poster Hall Poster Session
**Program #/Board # Range:** 4556–4586/B0211–B0241
**Organizing Section:** Retinal Cell Biology

**Program Number:** 4556 **Poster Board Number:** B0211
**Presentation Time:** 11:00 AM–12:45 PM
**Development of an automated platform for large-scale fluorescence screening of 3-D retinal organoids**
*M Natalia Vergara[1], MIGUEL FLORES-BELLVER[1], Silvia Aparicio Domingo[1], Minda M. McNally[1], Karl J. Wahlin[2], Meera Saxena[1], Jeff S. Mumm[1], Valeria Canto Soler[1]*. [1]The Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, MD; [2]Shiley Eye Institute, University of California, San Diego, San Diego, CA.
**Purpose:** The development of stem cell-derived retinal organoid systems has opened previously unimaginable possibilities for the study and potential treatment of retinal degenerative diseases. However their variability and the lack of high throughput platforms for their analysis pose severe limitations for their use in drug development applications. Hence, the purpose of this work was to develop a platform (3D- ARQ) that enables fluorescence quantification-based large-scale drug screening in complex human iPSC-derived retinal organoids, while providing the speed, sensitivity and reproducibility necessary for this kind of assays.
**Methods:** Transgenic hiPSC lines harboring GFP or YFP fluorescent reporters were generated and induced to differentiate into retinal organoids. Wild type retinal organoids were stained with various fluorescent dyes and used for parameter optimization purposes. Non-stained organoids were used as controls in all experiments. Organoids were seeded into v-bottom 96 well plates and scanned using a fluorescence microplate reader that allows XYZ-dimensional detection and fine-tuned wavelength selection. Results were confirmed byconfocal microscopy.
**Results:** Using this approach we: i) optimized the parameters for detecting fluorescent reporter signal in retinal organoids, ii) characterized autofluorescent background profiles, iii) determined the most favorable reporter emission ranges for 3D-ARQ-based quantification of retinal organoids, iv) established co-labeling methods to compensate for size variability between individual samples, and v) determined the quality of the assay for HTS applications by evaluating performance and sensitivity parameters. We validated the quantitative power of 3D-ARQ by measuring fluorescence intensity in global transgenic vs. chimeric YFP-expressing retinal organoids; demonstrated the applicability of this technology to track developmental progression by quantifying immunofluorescence staining for Otx2 at different weeks of differentiation; and exemplified the capacity of 3D-ARQ to track changes in physiological state by using the system to measure ROS accumulation following an oxidative insult.
**Conclusions:** We have developed and validated a technology that enables the rapid and robust quantification of fluorescence reporters in hiPSC-derived retinal organoids to a level that meets HTS requirements for drug discovery applications.
**Commercial Relationships: M Natalia Vergara**, None; **MIGUEL FLORES-BELLVER**, None; **Silvia Aparicio Domingo**, None; **Minda M. McNally**, None; **Karl J. Wahlin**, None; **Meera Saxena**, None; **Jeff S. Mumm**, None; **Valeria Canto Soler**, None
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**Program Number:** 4557 **Poster Board Number:** B0212
**Presentation Time:** 11:00 AM–12:45 PM
**Towards culturing retinal progenitor cells as a planar sheet**
*Tina Xia[1], Deepti Singh[2, 3], Laurel Tainsh[1], Ron A. Adelman[3], Lawrence J. Rizzolo[2, 3]*. [1]Yale School of Medicine, New Haven, CT; [2]Department of Surgery, Yale School of Medicine, New Haven, CT; [3]Department of Ophthalmology and Visual Sciences, Yale School of Medicine, New Haven, CT.
**Purpose:** Retinal progenitor cells (RPC) can be successfully differentiated into spherical organoids with retina-like lamination, but planar sheets of retinal tissue would have advantages for experimentation and transplantation. This study explores methods to culture RPC on planar scaffolds with and without laminin-521, a component of the inner limiting membrane.
**Methods:** Retinal cups (RC) were derived from human iPSC and ESC cells using published protocols. After 21 days of differentiation (D21) RC formed containing retinal progenitor cells (RPC). Whole retinal cups, or RC dissociated with trypsin, were seeded on 60μm thick scaffold of gelatin-chondroitin sulfate-hyaluronic acid. For laminin-coating experiments, early stage embryoid bodies were seeded on scaffold coated with 2μg/cm$^2$ of laminin-521. Differentiation was monitored by quantitative RT-PCR and immunofluorescence for expression of early eye field genes and subsequent markers of differentiation.
**Results:** Whole retinal cups adhered to the scaffold. Some cells invaded the scaffold, but the majority spread as thick clusters along the surface. In comparison, dissociated RC cells also invaded the scaffold, but attachment efficiency was low. On D29 of differentiation, dissociated RC expressed lower levels of early eye field genes PAX6, LHX2, and SIX3. But by D51, gene expression in dissociated and undissociated RC were equivalent. In neither culture did the cells become confluent on the scaffold. For embryoid bodies, there was 57% ±10% ($p < 0.05$) higher attachment on laminin-coated scaffolds compared to non-coated ones. On imaging, cells from embryoid bodies invaded and were uniformly distributed across and within the scaffolds. However, embryoid bodies did not readily differentiate into retinal lineage under these conditions. By D9, stem cells marker, NANOG, was downregulated but OCT4 expression persisted. Expression of early genes RAX, PAX6, and LHX2 were evident at low levels.
**Conclusions:** Early RC can be dissociated and seeded on scaffolds with low efficiency, and after a delay continued to differentiate. Laminin scaffold coating promotes higher and uniform cell adhesion of embryoid bodies, but retinal differentiation is slower in this condition. Besides being a part of the inner limiting membrane, laminin-521 provides a niche for pluripotent stem cells. In view of these results, current experiments focus on seeding dissociated RC on laminin-coated scaffolds.
**Commercial Relationships: Tina Xia**, None; **Deepti Singh**, None; **Laurel Tainsh**, None; **Ron A. Adelman**, None; **Lawrence J. Rizzolo**, None
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**Program Number:** 4558 **Poster Board Number:** B0213
**Presentation Time:** 11:00 AM–12:45 PM
**Characterization of hiPSC-derived 3D mini retinas in long term culture**
*Silvia Aparicio Domingo[1], MIGUEL FLORES-BELLVER[1], M Natalia Vergara[1], Minda M. McNally[1], Joseph A. Brzezinski[2], Valeria Canto Soler[1]*. [1]Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD; [2]Ophthalmology, University of Colorado, Aurora, CO.
**Purpose:** Retinal degenerative diseases cause the dysfunction and death of retinal photoreceptor cells leading to vision loss, and eventually, blindness. We recently established an efficient strategy for inducing human iPS cells to differentiate into fully laminated 3D mini retinas containing light-sensitive photoreceptors. The purpose of this study is to characterize the viability and differentiation capacity of our 3D mini retinas upon long-term culture in vitro, as a further step towards evaluating their potential for modeling retinal degenerative diseases as well as for their use as cellular products for retinal transplantation.
**Methods:** Differentiation of hiPSC into 3D mini retinas was conducted as previously described (Zhong et al., 2014). hiPSC-derived mini retinas were collected from week 15 to 70 (~4 to 17 months) of differentiation. Cellular composition and three-dimensional organization were evaluated by immunofluorescence detection of molecules associated with differentiation and function of various retinal cell types and three-dimensional confocal imaging. Functional differentiation and maturation was further evaluated at the ultrastructural level by transmission electron microscopy.
**Results:** hiPSC-derived mini retinas showed highly organized laminae throughout the culture period. Over time however, cells in the inner nuclear layers (i.e. ganglion and amacrine cells) were gradually lost due to apoptotic cell death. The remaining bipolar cell layer and ONL maintained their cellular organization and showed evidence of further maturation, featuring highly differentiated rods and cones of all subtypes, as well as the presence of all major bipolar cell subtypes, including rod, cone ON and cone OFF bipolar cells. Furthermore, we observed evidence of synaptic formation between photoreceptors and cells located in the bipolar cell layer.
**Conclusions:** Our results demonstrate the feasibility of culturing hiPSC-derived 3D mini retinas for significantly long periods of time. These long-term mini retinas consist of a well-organized ONL and a bipolar cell layer, including their corresponding major cellular subtypes. Our system provides a model to study the mechanisms of human photoreceptor differentiation over a period equivalent to at least 6-8 months after birth. Furthermore, it facilitates evaluating strategies for cell-based therapies involving transplantation of hiPSC-derived photoreceptor sheets.
**Commercial Relationships: Silvia Aparicio Domingo**, None; **MIGUEL FLORES-BELLVER**, None; **M Natalia Vergara**, None; **Minda M. McNally**, None; **Joseph A. Brzezinski**, None; **Valeria Canto Soler**, None
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**Program Number:** 4559 **Poster Board Number:** B0214
**Presentation Time:** 11:00 AM–12:45 PM
**Influence of basement membrane components on the *in vitro* maturation of hESC-RPE cells**
*Tanja Ilmarinen[1], Pyry Grönroos[1], Heidi Hongisto[1], Anni Sorkio[1], Soile Nymark[2], Heli Skottman[1]*. [1]BioMediTech, University of Tampere, Tampere, Finland; [2]BioMediTech, Tampere University of Technology, Tampere, Finland.
**Purpose:** Human pluripotent stem cell derived retinal pigment epithelial cell (hPSC-RPE) transplantation is currently under clinical evaluation as treatment for macular degeneration. For cell therapy, RPE of uniform quality and consistent level of maturation is required with potential implications to functional cell integration and survival after transplantation. We have previously shown, that the culture substrate dramatically affects human embryonic stem cell derived RPE (hESC-RPE) basal lamina production and maturation, including barrier properties. In the present study, we aimed to further identify the role of abundant RPE basement membrane (BM) proteins, type IV collagen, laminin, and nidogen-1, to the maturation and quality of hPSC-RPE.
**Methods:** Human ESC-RPE were seeded (2 x $10^5$ cells/$cm_2$) on 1 μm PET culture inserts coated with collagen IV or laminin alone, or in combination with and without nidogen-1. Both freshly differentiated and cryopreserved cells were used. The cells were matured for eight to ten weeks in serum-free culture conditions and analyzed for transepithelial electrical resistance (TER), phagocytosis activity, ion transport and markers for tight junctions and visual cycle.
**Results:** The presence of laminin improved the overall consistency of the epithelium producing higher TER. Especially after cryopreservation, collagen IV coating alone resulted in a poor quality epithelium on PET inserts compared to the combination of collagen IV and laminin. Addition of nidogen did not influence the expression or polarization of Na/K-ATPase and ZO-1, but increased the expression of visual cycle proteins RPE65 and CRALBP, as well as tight junction protein claudin-19.
**Conclusions:** Collagen IV and laminin are typically used individually for coating cell culture surfaces for hPSC-RPE culture. Our results indicate, that it would be advantageous to combine these BM proteins and use extracellular matrix linker molecules such as nidogen to obtain high quality hPSC-RPE. After cryopreservation of hPSC-RPE, the culture substrate is even more relevant, with possible implications to clinical use of these cells after cell banking.
**Commercial Relationships: Tanja Ilmarinen**, None; **Pyry Grönroos**, None; **Heidi Hongisto**, None; **Anni Sorkio**, None; **Soile Nymark**, None; **Heli Skottman**, None

**Program Number:** 4560 **Poster Board Number:** B0215
**Presentation Time:** 11:00 AM–12:45 PM
**Xenotransplantation of human embryonic stem cell-derived RPE cells, MA09-hPRE, to the subretinal space of minipigs and various methods available for detection of adverse changes in eye**
*Joo Yong Lee[1], You Na Kim[1], Sung-Min Cho[2], Junyeop LEE[3], Woo-Chan Son[2]*. [1]Ophthalmology, Asan Medical Center, Seoul, Korea (the Republic of); [2]Asan Institute for Life Sciences and Department of Pathology, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea (the Republic of); [3]Department of Ophthalmology, Yeungnam University, College of Medicine, Daegu, Korea (the Republic of).
**Purpose:** The purpose of this study was to determine the safety and tolerability of the subretinal injection of hESC-derived RPE cells in the laboratory minipig.
**Methods:** A volume of 150 μl of RPE reconstituted in BSS Plus was injected into the subretinal space of 16-month old laboratory minipigs (n=10) via a 23-G subretinal cannula, delivering the dose of 60 x $10^4$ RPE cells and 120 x $10^4$ RPE cells. Postoperative examinations were performed on postoperative day 1, week 1 and then weekly until the last follow-up using fundus photography and spectral-domain optical coherence tomography. Histologic and immunohistochemistry examination using CD3, CD 45 antibody was performed after enucleation.

**Results:** There was no serious systemic and ocular adverse event during the follow up (9 days – 12 weeks) Fundus photography revealed increased subretinal pigmentation after 4-6 weeks after subretinal injection. Spectral-domain OCT showed no significant morphologic changes during the follow-up. On immunohistochemistry examination, CD3 (+) and CD 45 (+) inflammatory cells were identified in the subretinal space.
**Conclusions:** Subretinal injection of high dose hESC-derived RPE cells was well tolerated in the preclinical study using minipig. These data suggest that high-dose hESC-derived RPE cells could be used for the treatment of degenerative macular diseases in the human trials.
**Commercial Relationships: Joo Yong Lee**, None; **You Na Kim**, None; **Sung-Min Cho**, None; **Junyeop LEE**, None; **Woo-Chan Son**, None

**Program Number:** 4561 **Poster Board Number:** B0216
**Presentation Time:** 11:00 AM–12:45 PM
**Exploring Cytoprotective Molecules for Polarized HESC-RPE Cells**
*Danhong Zhu[1], Jon takemoto[2], Sreekumar Parameswaran[3], David R. Hinton[1]*. [1]The Department of pathology, The Department of Ophthomology, Univ of Southern California, Los Angeles, CA; [2]Biology, Utah state university, Logan, UT; [3]Arnold & Mabel Beckman Center, Doheny Eye Institute, Los Angeles, CA.
**Purpose:** The implantation of a polarized retinal pigment epithelial (RPE) cell sheet derived from human embryonic stem cells is a promising treatment under investigation for RPE dysfunction related diseases, including age related macular degeneration (AMD). However, long-term culture of RPE cells under high oxygen exposure may cause RPE cell stress and senescence. Mesobiliverdin, an analog of biliverdin, and the peptide Humanin were reported to exhibit cytoprotective effects on pancreatic cells and neurons. This study aimed at investigating whether these molecules have cytoprotective effects on polarized HESC-RPE cells as well.
**Methods:** 1). RPE cells derived from H9 human embryonic stem cells were cultured on vitronectin coated transwell or 24-well culture plates for 4 weeks to form polarized monolayers. 2). The polarized RPE monolayers were treated with different concentrations of Mesobiliverdin or Humanin. 3). The treated RPE cells were measured for cell viability. 4). Western blot, immunostaining, qPCR, and senescence associated beta-gal (SA beta-gal) staining were used to detect the oxidative stress- and senescence-related gene and protein expression.
**Results:** 1. All control and mesobiliverdin as well as humanin treated HESC-RPE cells exhibited excellent cell viability but did not showed any further increase in viability after treatment with mesobiliverdin and humanin, as compared to the non-treated control cells.
2. Both mesobiliverdin and humanin treatments have modestly increase HESC-RPE cell viability under oxidant stress.
3. Moreover, mesobiliverdin and humanin decreased the senescence of HESC-RPE cells after long-term culture under normal cell culture conditions: Humanin treated HESC-RPE cells displayed only about 30% SA-beta-gal stained positive cells, mesobiliverdin treated cells showed about 60% SA positive cells, and as comparison, the non-treated control cells had about 90% positive cells.
**Conclusions:** Neither mesobiliverdin nor humanin increased HESC-RPE cell viability when grown as a monolayer; however, untreated cells already demonstrated excellent viability. Both mesobiliverdin and humanin exhibited protective effects on HESC-RPE cells against oxidative stress, and humanin had an even stronger ability in preventing the polarized HESC-RPE cells from undergoing senescence in vitro. Future studies will focus on the mechanism of Humanin in the inhibition of HESC-RPE cell senescence.
**Commercial Relationships: Danhong Zhu**, None; **Jon takemoto**; **Sreekumar Parameswaran**, None; **David R. Hinton**, None
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**Presentation Time:** 11:00 AM–12:45 PM
**RPE Differentiation from hESC and hiPSC on Electrospun Scaffolding**
*Dawn Landis*. NEI, NIH, Bethesda, MD.
**Purpose:** A major objective of current research on stem cell-derived photoreceptors is to produce cells that are well differentiated and form outer segments(OS) for disease studies, drug discoveries, and donor tissues for transplantation. Absent in current 3D retinal differentiation systems is an RPE layer, which is critical for OS development and maintenance through nourishment of photoreceptors, buffering of ions, and production of visual compounds. We hypothesize an electrospun polymer that sharing physical characteristics with Bruch's membrane may be a better substrate for RPE differentiation.
**Methods:** In order to support photoreceptors we have developed techniques using various factors and culture conditions to differentiate RPE from stem cells (H9) and patient iPSCs on matrigel (a previously established protocol) and electrospun polymer scaffolding made of slowly biodegradable material (PCL). RPE cultures were assessed by pigment production, IHC for tight junction markers, apical basal polarization, and mature RPE cellular markers, and morphological measurements.
**Results:** We developed a PCL based electrospun scaffold mimicing the Bruch's membrane to differentiate stem cells into RPE on. H9 ESCs differentiated into RPE on scaffolding mimic adult RPE (similarly to previously protocols with matrigel) evidenced through expression of typical proteins of mature RPE cells (MITF, CRALBP, EZRIN, & ZO-1), and distinctive RPE morphology such as: tight junctions between cells required for epithelial monolayer, hexagonal shape, and microvilli projections. iPSCs were also differentiated into RPE and developed similar characteristics as ESC. We tested patient iPSCs with a CRX mutation CRX for defects in differentiation and function. Control and CRX mutant iPSCs differentiated into RPE. Further experiments need to be done to determine if differences in RPE function exist.
**Conclusions:** We have differentiated RPE from ESC and iPSC cells from control and CRX mutant patients. Our culture system on the PCL based electrospun scaffold is comparable to current 2D matrigel protocols and may be more suitable for use in co-culture systems with 3D retinal differentiation. Using this system to test CRX mutant cells, it appears differentiation occurs similarly in control and mutant iPSCs although further tests are necessary. These culture systems could be beneficial in photoreceptor and RPE development assays, small molecule screening, and donor tissue.
**Commercial Relationships: Dawn Landis**, None

**Program Number:** 4563 **Poster Board Number:** B0218
**Presentation Time:** 11:00 AM–12:45 PM
**low Integrin Expression Levels of ips-derived human retinal pigment epithelial Cells Correlates with Their Lower Reattachment Rates Onto Human Bruch's Membrane**
*Qun Zeng, Ahmet Hondur, yao li, Stephen Tsang, Tongalp H. Tezel*. Ophthalmology, Columbia University, New York, NY.
**Purpose:** To compare the integrin a3, a5, and b1 expression of iPS-derived human RPE (iPS-RPE) and ARPE-19 cells and test the

efficacy of several known strategies to boost up iPS-derived human RPE integrin expression.
**Methods:** Three iPS-derived human RPE cell lines were generated according to Yamanaka protocol. Expression of integrin a3, a5, and b1 on passage #2-4 iPS-RPE cells were determined using flow cytometry and compared with the expression of integrins on ARPE19 cells. Several strategies were also applied to preserve and/or boost up the integrin expression of the iPS-RPE, including: (1) Incubation with glucocorticoids (cortisol: 10 nM -10 mM; dexamethasone: 100 nM-500 nM) for 12 days; (2) Incubation with extracellular matrix molecules (laminin, 1 ug/ml and fibronectin, 20 ug/ml); (3) Different harvesting techniques (trypsinization vs. scraping), and (4) Plating cells on different matrices (Matrigel®, non-treated tissue culture plastic and human Bruch's membrane). ARPE-19 cells treated similarly was taken as control.
**Results:** Integrin a3, a5, and b1 expression of all three iPS-RPE cell lines were significantly lower than ARPE-19 cells. Glucocorticoids did not increase the a3, a5, and b1 integrin expression of iPS-RPE cells; however, cortisol and dexamethasone at concentrations higher than 100 nM induced mild increase in a-5 expression of ARPE-19 cells. Overnight incubation of iPS-RPE and ARPE-19 cells in the presence of laminin and fibronectin or adding soluble laminin and fibronectin to their culture media did not alter the integrin expression. Harvesting both iPS-RPE and ARPE-19 cells with trypsinization resulted in better preservation of a3, a5, and b1 integrins. iPS-RPE cells plated on Matrigel® gradually expressed more a-3 integrin; however, plating them on human Bruch's membrane significantly downregulated the a-3 integrin expression.
**Conclusions:** Integrin a3, a5, and b1expression of iPS-RPE cells is significantly lower than ARPE-19 cells. This correlates with their lower reattachment and survival rates on human Bruch's membrane. Strategies should be developed to increase integrin expression of iPS-RPE prior considering to transplant these cells for repairing the damaged human RPE matrix.
**Commercial Relationships: Qun Zeng**, None; **Ahmet Hondur**, None; **yao li**, None; **Stephen Tsang**, None; **Tongalp H. Tezel**, None
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**Program Number:** 4564 **Poster Board Number:** B0219
**Presentation Time:** 11:00 AM–12:45 PM
**Decellularized Retina A Natural Scaffold for Culturing Retinal Progenitor Cells Derived from Normal Human Corneal iPSC**
*Roy Joseph[1], Om P. Srivastava[1], Roswell R. Pfister[2]*. [1]Department of Vision Sciences, Univ of Alabama at Birmingham, Birmingham, AL; [2]Eye Research Foundation, Birmingham, AL.
**Purpose:** To study the effect of decellularized retina on the attachment, proliferation and differentiation of retinal progenitor cells derived from normal human corneal induced pluripotent stem cells.
**Methods:** Normal human corneal fibroblasts from three donors were reprogramed directly to pluripotent stem cells (PSC) by delivering reprogramming factors in a single virus using 2A "self-cleaving" peptides, using a single polycistronic lentiviral vector co-expressing four transcription factors (Oct 4, Sox2, Klf4 and Myc) to yield induced pluripotent stem cells (iPSC). Phase-contrast images were obtained using our Olympus 1X71 inverted microscope. These iPS cells were immunofluorescentally characterized for stem cell markers (SSEA4, Oct4 and Sox2). The iPS cells were differentiated in retinal differentiation media [(DMEM F-12 medium containing 10% knockout serum replacement 2% B27 supplement 1% N2 supplement, 1% L-glutamine, 1% 100X NEAA, 1% penicillin/streptomycin, 1 ng/ml noggin, 1 ng/ml DKK-1, 1 ng/ml IGF-1 and 0.5 ng/ml bFGF]. Immunohistochemical analyses of the differentiated cells were done using immunofluorescence microscope. Human retina obtained from Eye bank was decellularized using 1% SDS solution. After several washes the decellularized retina was used as a natural scaffold to culture retinal progenitor cells.
**Results:** Immunofluorescence analysis of the colonies showed that they were positive for all the three stem cell markers (SSEA4, Oct4 and Sox2). The iPS cells were grown to differentiate for 33 days, and then immunocytochemical analysis of the cells showed expression of retinal photoreceptor markers, recoverin and rhodopsin. The retinal progenitor cells attached to the decellularized retina as seen by immunohistochemical methods.
**Conclusions:** We successfully reprogramed normal human corneal fibroblasts using single polycistronic virus co-expressing four transcription factors. We were also able to differentiate the iPSC to retinal precursor cells which expressed photoreceptor markers, rhodopsin and recoverin. These retinal precursor cells are a valuable source which could be used for transplantation in retinal degenerative diseases.
**Commercial Relationships: Roy Joseph**, None; **Om P. Srivastava**, None; **Roswell R. Pfister**, None

**Program Number:** 4565 **Poster Board Number:** B0220
**Presentation Time:** 11:00 AM–12:45 PM
**Evaluation of neurotrophic factors secreted by mesenchymal stem cells in co-culture with porcine neuroretinas**
*Sonia Labrador[1], Girish K. Srivastava[1, 3], Maria Teresa Garcia-Gutierrez[1], Soraya Tabera-Bartolome[2], Jose-Carlos Pastor[1, 4], Ivan Fernandez-Bueno[1, 4]*. [1]Instituto Universitario de Oftalmobiología Aplicada (IOBA), University of Valladolid, Valladolid, Spain; [2]Instituto de Biología y Genética Molecular (IBGM), University of Valladolid, Valladolid, Spain; [3]Centro en Red de Medicina Regenerativa y Terapia Celular, Junta de castilla y León, Valladolid, Spain; [4]Red Temática de Investigación Cooperativa Sanitaria (RETICS), Instituto de Salud Carlos III (OFTARED), Valladolid, Spain.
**Purpose:** Retinal ganglion cells (RGCs) could be neuroprotected by a variety of neurotrophic factors potentially secreted by Mesenchymal Stem Cells (MSCs). Our proposal is to evaluate the effect of Brain-Derived Neurotrophic Factor (BDNF), Ciliary Neurotrophic Factor (CNTF) and Glial Derived Neurotrophic Factor (GDNF), secreted by MSCs in a porcine neuroretina (NR) culture.
**Methods:** Six NR explants were obtained from the area centralis of three adult porcine eyes and cultured alone (controls) on culture membranes or supplemented with MSC in the same wells but physically separated by the culture membrane. Human Bone Marrow Mesenchymal Stem Cells (hBMMSC) were commercially provided by Citospin S.L. (Valladolid, Spain). Co-cultures were maintained during 72 hours. Culture supernatants were collected at culture media exchange at 24, 48 and 72 hours. The presence and concentration of BDNF, CNTF and GDNF was determined in the culture supernatants. Neurotrophic factor levels were measured with quantitative enzyme-linked immunosorbent assays (ELISA) specific for Human BDNF (RayBiotech, Norcross, GA), Human CNTF (Cloud-Clone Corp., Houston, TX) and Human GDNF (RayBiotech), respectively. HBMMSC immunophenotyping after co-culture was performed with MSCs characterization kit (Millipore, Billerica, MA). Data were analyzed using SPSS (version 24.0, SPSS, Inc., Chicago) Analysis of means were performed by repeated measures analysis of variance (ANOVA). The statistical significance level was set at 5%.
**Results:** NR co-cultured with MSCs preserved their structure better than NR cultured alone. hBMMSC maintained their immunophenotype (immunopositive for CD44, CD90, STRO-1 and

CD146, and negative for CD14 and CD19) 72 hours after co-culture with porcine NR. BDNF levels in co-culture supernatants were significantly higher than controls at all time points studied ($p<0.05$). CNTF levels in the co-cultures were significantly higher than controls at 72 hours ($p<0.05$). GDNF was not detected in any culture supernatants.
**Conclusions:** MSCS preserved porcine neuroretina from spontaneous degenerative changes. MSCs secreted neurotrophic factors (BDNF and CNTF) relevant to RGC survival in co-culture with porcine NR. The next step of this study will compare the survival of the RGC on the porcine NR to determine the potential neuroprotective effects of these hBMMSC.
**Commercial Relationships: Sonia Labrador**, None; **Girish K. Srivastava**, None; **Maria Teresa Garcia-Gutierrez**, None; **Soraya Tabera-Bartolome**, None; **Jose-Carlos Pastor**, None; **Ivan Fernandez-Bueno**, None
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**Program Number:** 4566 **Poster Board Number:** B0221
**Presentation Time:** 11:00 AM–12:45 PM
**Retinal progenitor cells (RPC) cultured on planar scaffolds can integrate into the degenerated retina of rd10 mice and retain markers of differentiation**
*Deepti Singh[1], Shao-Bin Wang[2], Tina Xia[1], Laurel Tainsh[1], Maryam Ghiassi-Nejad[1], Ron A. Adelman[2], Lawrence J. Rizzolo[1].*
[1]Surgery/Ophthalmology, Yale University, NEW HAVEN, CT; [2]Department of Ophthalmology, Yale University, NEW HAVEN, CT.
**Purpose:** To restore vision that has already been lost, RPC need to integrate with the remaining host retina after implantation into subretinal space. We tested whether RPC grown as thick, planar sheets, alone or co-cultured with RPE in their native geometry, could re-populate the outer nuclear layer (ONL) with photoreceptor precursors.
**Methods:** H9 human embryonic stem cells were differentiated for 2 weeks to early RPC and seeded on a 60 µm thick, planar scaffold fabricated from gelatin, chondritin sulfate, and hyaluronic acid. Some scaffolds were layered on a monolayer of human fetal RPE (hfRPE) and culture continued for 4 weeks. Metabolic activity was monitored by alamar blue and, retinal markers by quantitative RT-PCR (qPCR) and immunocytochemistry. For transplantation, 30-day-old rd10 mice were used, when the photoreceptor layer would be 60-70 % degenerated. Co-cultured or mono-cultured RPC were engrafted into the subretinal space and analyzed 6 weeks later by mfERG and immunocytochemistry. Antibodies IBA-1 and IL-6 were used to assess evidence of an immune response.
**Results:** Co-culture showed higher metabolically active cells (83.3% ± 0.5%) in comparison with mono-culture system. qPCR demonstrated that in presence of hfRPE, stem cells differentiated more efficiently than without hfRPE *in vitro* with 5 to 15-fold increase in early eye field gene like RAX, SIX3, LHX2, CHX10, 7 to 10-fold increase in photoreceptor markers such as PROX1, Recoverin, CRX, PRPH2 and NR2E3. Six weeks post-implantation, the scaffold was degraded, and TRA-1-85 (human cell marker) positive cells lined the host ONL. There was no evidence of an immune response. TRA-1-85+ cells that lined the outer nuclear layer continued to express recoverin. Pigmented TRA-1-85+ cells were found in the RPE layer. TRA-1-85+ cells with long processes were found in the inner plexiform layer. The mfERG recordings from co-culture mice revealed a modest increase in the P1 wave from 4.86±0.07 nV/deg$^2$ (control) to 7.0±1.0 nV/deg$^2$ (co-culture).
**Conclusions:** Planar sheets of RPC can retain differentiated properties after transplantation into the subretinal space. The data provide preliminary evidence that function can be restored in mice with late-stage retinal degeneration.
**Commercial Relationships: Deepti Singh**, None; **Shao-Bin Wang**, None; **Tina Xia**, None; **Laurel Tainsh**, None; **Maryam Ghiassi-Nejad**, None; **Ron A. Adelman**, None; **Lawrence J. Rizzolo**, None
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**Program Number:** 4567 **Poster Board Number:** B0222
**Presentation Time:** 11:00 AM–12:45 PM
**Injectable Hydrogels for Stem Cell Based Regenerative Treatment of Diabetic Retinopathy**
*Tao Lowe, Sri Chandana Damera*. Pharmaceutical Sciences, University of Tennessee Health Science Center, Memphis, TN.
**Purpose:** Intravitreally injected adipose derived stem cells (ADSC) have shown potential to rescue the neural retina from hyperglycemia-induced degeneration, resulting in improved visual function. Hydrogel biomaterials have shown to improve the cell attachment, viability/proliferation, and differentiation of stem cells when used as scaffolds in various regenerative treatment studies. However, currently no injectable hydrogels scaffolds for ADSCs for the ocular application are available. The purpose of this study is to design injectable hydrogels as scaffolds for enhanced performance of ADSC based regenerative therapy for DR.
**Methods:** A series of thermoresponsive and biodegradable hydrogels were synthesized by photo-polymerization of N-isopropylacrylamide monomer and oligolactate 2-hydroxyethyl methacrylate macromer. Different weight ratios of the reactants (19:1~16:4, w:w), and amounts of Irgacure 2959 photo initiator (0.0125, 0.025, 0.05 wt%), UV intensity (100-750 mW/cm$^2$), and reaction time (3, 10 min) were used to synthesize the hydrogels. The chemical structure and thermoresponsive property of the hydrogels were characterized by FTIR and UV-Vis spectrometer, respectively. The time dependent hydrolytic degradation of the hydrogels was evaluated by FTIR and dynamic mechanical analyzer. The rheological property including storage and loss moduli of the hydrogels was measured by rheometer. The cytotoxicity of the hydrogels to ADSCs at 37 °C was assessed by MTT assay. *In vivo* retinal histopathological studies of the ADSCs-loaded injectable hydrogels for the decrease in vascular leakage and apoptotic cells in the diabetic rat eyes are under investigation.
**Results:** FTIR measurements confirmed the successful synthesis of the hydrogels. The thermo-responsive, biodegradable and rheological properties of the hydrogels strongly depended on the hydrogel composition and reaction conditions. The higher were the amount of the Irgacure 2959 initiator, UV intensity, reaction time, and the reactants, the higher were the storage and loss moduli of the hydrogels at 37 °C. The hydrogels were not cytotoxic to ADSCs at concentration up to at least 10 mg per mL.
**Conclusions:** The developed hydrogels can be tuned to have different degradation and mechanical properties to promote ADSC growth inside their matrices. Successful completion of this project will have significant impact on development of injectable biomaterials to treat DR.
**Commercial Relationships: Tao Lowe**, None; **Sri Chandana Damera**, None

**Program Number:** 4568 **Poster Board Number:** B0223
**Presentation Time:** 11:00 AM–12:45 PM
**Secretome of induced pluripotent stem cells-derived optic vesicles**
*Ramesh Kaini, Whitney Greene, Heuy-Ching H. Wang*. Ocular Trauma, United States Army Inst of Surgical Rsrch, Rowlett, TX.
**Purpose:** The paracrine factors secreted in the conditioned medium (CdM) of pluripotent stem cells have shown a promising prospect as a therapeutic agent in regenerative medicine. We hypothesize that pluripotent stem cells differentiated towards retinal lineage secrete neuroprotective and neuroregenerative factors. In this study, we sought to differentiate human induced-pluripotent stem (iPS) cells towards neural retinal lineage and study the factors secreted in the CdM.
**Methods:** 3D1 iPS cells were differentiated toward retinal lineage by a modified protocol. Embryoid bodies (EBs) of 9000 cells were generated using Aggrewells. EBs were transitioned to neural induction medium on day 3 of differentiation and maintained in a suspension culture system. On Day 17, neuro-rosettes were manually separated and transferred to suspension culture. Fifty rosettes were cultured in each well of 6 well ultralow attachment plate with 2 ml of DMEM/F12 (3:1) medium supplemented with 2% B27. Media was harvested every 72 hours and replaced with fresh 2 ml medium. Harvested CdM were centrifuged at 5k rpm for 5 minutes and stored at -80$^0$F.
Differentiation of iPS cells towards retinal lineage was confirmed by immunofluorescence analysis of neural (OTX 2, SOX1), eye field (LHX2, SIX6), and retinal precursor marker (CHX10, PAX6). Multiplex luminex Immunoassays were performed for expression of several neuroprotective factors.
**Results:** EBs derived from 3D1 cells expressed neuronal markers (OTX2, SOX1), eye field markers (SIX6, LHX2) and retinal precursor markers (co-expression of CHX10 and PAX6) at different time points which confirmed to previous studies on step-wise differentiation towards optic vesicle formation. We observed secretion of several neuroprotective factors including Osteopontin, Hepatocyte growth factor (HGF), Stromal cell-derived factor 1 (SDF-1), Insulin-like growth factor 1 (IGF-1), and Mesencephalic astrocyte-derived neurotrophic factor (MANF).
**Conclusions:** In this study, we observed that iPS-derived optic vesicles secrete several growth factors that are previously described to protect neurons.
**Commercial Relationships: Ramesh Kaini**, None; **Whitney Greene**, None; **Heuy-Ching H. Wang**, None
**Support:** ORISE Fellowship to RRK; NRC Research Associateship to WAG; MRMC internal funding

**Program Number:** 4569 **Poster Board Number:** B0224
**Presentation Time:** 11:00 AM–12:45 PM
**Derivation and Characterization of induced-primary RPE culture (ipRPE) from 3D mini retinas**
*MIGUEL FLORES-BELLVER, Silvia Aparicio Domingo, M Natalia Vergara, Minda M. McNally, Valeria Canto Soler*. WILMER EYE INSTITUTE, JOHNS HOPKINS UNIVERSITY, BALTIMORE, MD.
**Purpose:** Retinal pigmented epithelium (RPE) derived from human induced pluripotent stem (iPS) cells is a promising source of cells for transplantation, disease modeling and drug screening. Successful generation of RPE from heterogeneous cultures of differentiating human iPS cells occurs with varying efficiency. Recently, our team established a simple and efficient strategy for inducing human iPS cells to differentiate into 3D mini retinas in vitro, with spatial and temporal features that replicate the development of the human retina in vivo, including the formation of RPE tissue. The goal of the present study was to establish a novel methodology for deriving RPE cells from the RPE tissue present in 3D mini retinas.
**Methods:** Similarly to the approach generally followed for deriving cultures of primary RPE cells, RPE tissue is dissected from the 3D mini retinas, dissociated into single cells and seeded on matrigel-coated plates where they form characteristic RPE monolayers. Expression of specific molecules associated with differentiation and function of the RPE cultures was evaluated by RT-PCR, immunofluorescence, and western blot (MITF, ZO-1, EZRIN, RPE65, CRALBP, LRAT, TYR, among others).
**Results:** Expression of the key RPE genes evaluated in our cultures was in accordance with patterns previously reported for adult human RPE cells in vivo, as well as human primary RPE cultures, and RPE cells derived from pluripotent stem cells. Interestingly, RPE monolayers also showed formation of epithelial domes, suggesting that the cells are functional and engaged in active fluid transport, as is known to occur through tight junction in mature RPE cells.
**Conclusions:** Therefore, we propose this new pseudo human primary RPE culture, referred by us as induced-primary RPE (ipRPE), as an alternative and promising source of pure hiPSC-derived RPE cells.
**Commercial Relationships: MIGUEL FLORES-BELLVER**; **Silvia Aparicio Domingo**, None; **M Natalia Vergara**, None; **Minda M. McNally**, None; **Valeria Canto Soler**, None
**Support:** 90069812 - 2016-MSCRFF-2849

**Program Number:** 4570 **Poster Board Number:** B0225
**Presentation Time:** 11:00 AM–12:45 PM
**RPE-like cells emerging from chick glial cell culture**
*Run-Tao Yan, Li He, Shu-Zhen Wang*. Ophthalmology, Univ of Alabama at Birmingham, Birmingham, AL.
**Purpose:** The demonstrations of Müller-originated retinal regeneration in fish and of glia-originated neurogenesis in adult mammalian brain have heightened the expectation for Müller glia being endogenous stem/progenitor cells for retinal regeneration in high vertebrates. This study tests whether the presumptive stemness/progenitorness of Müller glia would include the potential to develop towards RPE cells.
**Methods:** Primary Müller glial cell cultures were established using E13 and E14 chick retinas. These specific ages were used for two reasons: (i) the neural retina can still be readily separated from the RPE during dissection so as to avoid RPE carryover in harvested tissues; and (ii) retinogenesis has ceased in the central and central-peripheral regions so as to minimize the population of uncommitted cells. The peripheral region of retina was excluded to minimize potential contamination by progenitor cells. Trypsin/EDTA was used to dissociate cells of the harvested tissue, and the disassociated cells were cultured in 75-mL flasks. Frequency of medium replacements was reduced to promote neuronal cell death from malnutrition. Microscopy and immunocytochemistry were used to assess Müller-to-RPE transition.
**Results:** After 1 week in culture, Müller glial cells started to constitute a significant portion of the cell population as they proliferated while neurons died off. This trend continued as culture time lengthened. At 2 weeks, the culture became confluent with glial cells, and the vast majority of the retinal neurons had died. At this point, sporadic, pigment granules-containing (RPE-like) cells were visible. As the culture aged, more cells exhibited pigment granules and the pigmentation enhanced. By 3 weeks, the culture contained many patches, large and small in size, of darkly-pigmented cells. By 4 weeks, some of the darkly-pigmented cells were cobblestone-like, similar to that of RPE cells. After 7 weeks in culture, pigmented cells appeared to cover the entire surface of the vessel, and some of the cells were immunopositive for RPE protein RPE65.

**Conclusions:** These observations indicated a Müller-to-RPE transition occurring in the culture and imply that those Müller glial cells retained certain stemness/progenitorness with default pathways that included RPE, similar to that of pluripotent stem cells, such as ESCs and iPSCs.
**Commercial Relationships: Run-Tao Yan**, None; **Li He**, None; **Shu-Zhen Wang**, None
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**Program Number:** 4571 **Poster Board Number:** B0226
**Presentation Time:** 11:00 AM–12:45 PM
**Adipose tissue derived mesenchymal stem cells differentiate towards RPE and rescue apoptotic RPE under oxidative stress, in vitro and in vivo**
*Aya Barzelay[1, 2], shira wheisthal[1, 2], sebastian katz[1, 2], moshe ben hemo[1, 2], anat nitzan[1, 2], Mark Krauthammer[1, 2], Anat Loewenstein[1, 2], Adiel Barak[1, 2]*. [1]Ophthalmology, Ophthalmology stem cells laboratory, Tel Aviv, Israel; [2]Medicine, Tel Aviv University, Tel Aviv, Israel.
**Purpose:** Oxidative stress leads to degeneration and apoptosis of Retinal pigment epithelial cells (RPE) in age related macular degeneration (AMD). Subcutaneous adipose derived mesenchymal stem cells (ASCs) may serve a therapeutic tool for regenerating RPE. Here we evaluated ASCs' protective effect on apoptosis of RPE, the differentiation potential of ASCs towards RPE, and the efficacy of sub-retinal transplantations of ASCs in a mouse model of AMD.
**Methods:** Human ASCs were harvested from subcutaneous fat of patients undergoing abdominoplasty. A co-culture of ASCs and human RPE, or human RPE alone, was subjected to oxidative stress by exposure to 1.5mM hydrogen peroxide (H2O2). H2O2 induced RPE apoptosis was measured by Annexin V/ propidium iodide staining and flow cytometry analysis. The differentiation potential of ASCs towards RPE was evaluated using td-tomato marked RPE and GFP marked ASCs seeded in a co-culture. GFP marked ASCs were then isolated by FACS sorter and analyzed for RPE and eyefield markers (BF1, Rx, MITF, PAX6, RPE65) by qRT-PCR and immunostaining. Finally, GFP marked ASCs were transplanted in the subretinal space of sodium iodate (NAIO3) treated mice compared to controls of subretinal saline injection. Evaluation of transplanted ASCs and endogenous RPE was studied by immunostaining for GFP and RPE65 of frozen sections at day 0, 7, 14, and 21 days post injection.
**Results:** Treatment of RPE with ASCs prevented H2O2 induced apoptosis (70% decrease, $p < 0.05$). After 7 days in co-culture, ASCs upregulated RPE and eyefeild markers (BF1 1.9+ 0.2 Rx 4.5+0.8 MITF 1.9+0.17 PAX6 5.5+0.2 RPE65 7.7+2.6, folds of control). In vivo, transplanted ASCs were located in the subretinal space of NAIO3 mice at days 0, 7, 14, and 21 post injections. Level of RPE65 was higher in ASCs treated mice at day 14 (4 folds increase in mean florescence of RPE65 compared to controls).
**Conclusions:** Treatment of apoptotic RPE with ASCs reduced RPE apoptosis in vitro. ASCs demonstrate a differentiation potential into RPE, evident by upregulation of RPE and eye field markers. Transplantation of ASCs in the subretinal space of NAIO3 mice resulted in an increase in RPE cell count compared to controls. ASCs may have therapeutic potential in regenerating RPE.
**Commercial Relationships: Aya Barzelay**, None; **shira wheisthal**, None; **sebastian katz**, None; **moshe ben hemo**, None; **anat nitzan**, None; **Mark Krauthammer**; **Anat Loewenstein**, None; **Adiel Barak**, None

**Program Number:** 4572 **Poster Board Number:** B0227
**Presentation Time:** 11:00 AM–12:45 PM
**Generation of a VSX2::EGFP reporter cell line for modeling human retinal differentiation**
*Wei Liu, Albert Lowe, Ales Cvekl*. Ophthal & Visual Sciences, Albert Einstein College of Med, New York, NY.
**Purpose:** In murine retinal development, homeodomain transcription factor Vsx2 (also known as Chx10) is a specific marker for newly specified retinal progenitor cells (RPCs) in the inner layer of optic cups. In our in vitro retinal differentiation system, newly specified $VSX2^+$ RPCs self-organize into apically convex epithelium, and substantially pure $VSX2^+$ epithelium has an intrinsic propensity to differentiate into stratified neuroretina in floating cultures (Lowe et al., 2016). Accordingly, the generation and isolation of $VSX2^+$ epithelium is the critical step in retinal differentiation in vitro. This study aims to generate VSX2::EGFP H1 hESCs to investigate human retinal differentiation.
**Methods:** Genetic engineering of undifferentiated H1 hESCs using CRISPR/Cas9 technology was performed. The engineered hESCs were used for retinal differentiation following a novel procedure that was recently established in our laboratory.
**Results:** VSX2::EGFP-Puro H1 hESCs were generated by nucleofection with a homology directed repair (HDR) vector containing a 2A-EGFP-Puro cassette targeting the VSX2 locus immediately before the stop codon and a PX458 vector containing sgRNA. Puromycin was used for selecting electroporated H1 hESCs. Targeted hESCs were identified using PCR with primer pairs detecting homologous recombination at the left and right homology arm, respectively. Homozygous VSX2::EGFP-Puro H1 hESCs were identified when the native allele was absent. Then, VSX2::EGFP hESCs were generated after CRE-mediated removal of Puro cassette. VSX2::EGFP H1 hESCs were used for retinal differentiation following our procedure. In the retinal cultures, VSX2::EGFP recapitulates VSX2 expression, which is revealed by dual immunostaining. Live imaging shows that VSX2::EGFP is specifically present in retinal organoids following Dispase-mediated cell detachment, confirming self-organization of $VSX2^+$ epithelium we described recently (Lowe et al., 2016).
**Conclusions:** We have generated a VSX2::EGFP reporter cell line that has multiple applications in modeling human retinal differentiation and disease.
**Commercial Relationships: Wei Liu**; **Albert Lowe**, None; **Ales Cvekl**, None
**Support:** NH Grant EY022645, EY012200, RPB unrestricted grant

**Program Number:** 4573 **Poster Board Number:** B0228
**Presentation Time:** 11:00 AM–12:45 PM
**Differentiation competence of human adipose tissue-derived stem cells toward retinal lineage**
*Yuqiang Huang, Chongbo Chen, Jiajian Liang, Mingzhi Zhang*. Joint Shantou International Eye Center (JSIEC) of Shantou University and the Chinese University of Hong Kong, Shantou, China.
**Purpose:** To investigate the differentiation competence of human adipose tissue-derived stem cells (hADSCs) toward retinal lineage by inhibiting BMP and Wnt signaling.
**Methods:** hADSCs were divided into induction group and negative control group (NC). hADSCs of induction group were induced toward retinal lineage with a 2-step induction procedure using media containing Noggin and Dkk1, antagonist of BMP signaling and Wnt/β-catenin signaling respectively. Step 1: hADSCs were induced into neurospheres on ultra-low attachment culture plates for 3 days. Step 2: Neurospheres were transferred onto matrigel-coated culture plates and induced to differentiate for up to 24 day. hADSCs of

negative control group were cultured using media only containing DF12 and 10%FBS. Cells at various time points of induction were collected and expression of markers labeling retinal progenitor cells (RPCs), photoreceptor cells (PRCs) and retinal ganglion cells (RGCs) were revealed by RT-PCR, real-time PCR and Western-blot.
**Results:** The hADSCs-derived differentiated cells in the induction groups showed bright spots around nucleus, while cells in the NC group showed fibroblast-like morphology. The markers for RPCs (PAX6), PRCs (CRX, NRL and RHO), RGCs (ATOH7 and THY1), and neural precursor (NES and TUBB3) were significantly up-regulated after induction compared to negative control group.
**Conclusions:** hADSCs possess the potential to differentiate toward retinal lineage by inhibiting BMP and Wnt signaling.
**Commercial Relationships: Yuqiang Huang**, None; **Chongbo Chen**, None; **Jiajian Liang**, None; **Mingzhi Zhang**, None
**Support:** Natural Science Foundation of Guangdong Province of China (2016A030313063)

**Program Number:** 4574 **Poster Board Number:** B0229
**Presentation Time:** 11:00 AM–12:45 PM
**A simple approach to single cell RNAseq analysis facilitates virtual cell sorting of retinal cell subtypes derived from human pluripotent stem cells**
*Joe Phillips[1, 2], Peng Jiang[1], Patrick Barney[1], Jee Min[1], Shivani Jain[1], Tasnia Tabassum[1], Katherine Barlow[1], James Thomson[1], David M. Gamm[1, 2].* [1]University of Wisconsin--Madison, Madison, WI; [2]McPherson Eye Research Institute, Madison, WI.
**Purpose:** To probe the authenticity of human pluripotent stem cell (hPSC)-derived photoreceptors (PRs) and other retinal subtypes generated within 3D hPSC-optic vesicle (OV) like structures using single cell RNA-seq (scRNAseq) analysis. While scRNAseq provides a powerful tool to profile individual cell gene expression, the datasets are challenging to interpret, particularly when profiling heterogenous cell populations. Thus, we sought to develop a straightforward strategy to mine scRNAseq data to extract reliable information on the multitude of cell populations in differentiating hPSC-OVs.
**Methods:** hPSC-OVs were generated from a custom *CRX*-tdTomato hPSC reporter line using our established protocols. RT-PCR and immunocytochemistry (ICC) were performed at multiple time points to assess the differentiation status of retinal cell types, focusing on PR production and maturation. Thereafter, we performed scRNAseq analysis on dissociated hPSC-OVs at 70 (n=157 cells) and 218 (n=195 cells) days of differentiation using the Fluidigm C1 system and the Illumina HiSeq 2500 sequencing platform.
**Results:** Molecular and ICC analyses showed robust tdTomato expression in PRs, as well as RPE cells, beginning at early stages of differentiation. Production of all other retinal cell types was also confirmed. Subsequent scRNAseq analyses using commonly employed, unbiased algorithms were unfruitful in clearly identifying cell types. Therefore, we developed a simple and versatile approach to generate transcriptome datasets akin to those produced following high throughput analysis of FAC-sorted gene reporter lines. This method takes into account the rank order of expression of a given target (or "bait") gene across the entire scRNAseq dataset and searches for genes with the highest correlations in an unbiased fashion. Moreover, using this method, we could input multiple "bait" genes simultaneously to refine datasets that more reliably reflect specific retinal cell subtypes, demonstrating the expression of known and novel retinal cell subtype specific genes. Novel gene expression was then validated by ICC on hPSC-OV and human retinal sections.
**Conclusions:** Using our novel scRNAseq analysis method, we provide further evidence of the authentic nature of hPSC-derived PR precursors and uncover previously unknown genes that are expressed in differentiating human retinal cell subtypes.
**Commercial Relationships: Joe Phillips**; **Peng Jiang**, None; **Patrick Barney**, None; **Jee Min**, None; **Shivani Jain**, None; **Tasnia Tabassum**, None; **Katherine Barlow**, None; **James Thomson**, None; **David M. Gamm**, Opsis Therapeutics (I)
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**Program Number:** 4575 **Poster Board Number:** B0230
**Presentation Time:** 11:00 AM–12:45 PM
**Synchronous BMP and Activin/TGFβ inhibition and FGF activation drives rapid generation of photoreceptor-like cells by in mouse embryonic stem cell cultures**
*Kimberly A. Wong[1, 3], Michael Trembley[4], Ichiro Hiratani[5], David M. Gilbert[6], Michael Zuber[2, 3], Andrea S. Viczian[2, 3].* [1]Neuroscience & Physiology, SUNY Upstate Medical University, Syracuse, NY; [2]Ophthalmology, SUNY Upstate Medical University, Syracuse, NY; [3]Center for Vision Research, SUNY Upstate Medical University, Syracuse, NY; [4]Pharmacology & Physiology, Aab Cardiovascular Research Institute, University of Rochester School of Medicine and Dentistry, Rochester, NY; [5]RIKEN Center for Developmental Biology, Kobe, Japan; [6]Biological Science, Florida State University, Tallahassee, FL.
**Purpose:** Photoreceptor cells derived from mouse embryonic stem (mES) cells are an attractive source for studying formation of photoreceptor cells *in vitro*. While human stem cell cultures take up to 5 months to generate photoreceptors, we have developed a culture system that can form photoreceptors in two weeks. This study was done to determine the optimal concentration of inhibitors to maximize the production of these cells.
**Methods:** Mouse D3 or primary transgenic Crx>GFP ES cells were cultured in suspension with hepatocellular carcinoma conditioned media for 5 days and transformed into pluripotent primitive ectoderm-like (EPL) organoids. Cultures were then supplemented with inhibitors, including Noggin, or dorsomorphin and SB431542, to inhibit BMP and Activin/TGFβ signaling. After differentiation, the presence of neural and retinal cell types were observed using immunofluorescence and qRT-PCR.
**Results:** Noggin, or dorsomorphin and SB431542, treatment resulted in a dose-dependent induction of markers for retinal progenitor cells, post-mitotic ganglion cells, and cone photoreceptors. After random selection of organoids at day 9, 95% of the inhibitor-treated organoids expressed markers for photoreceptors (Crx) and cone-specific opsins (M-opsin, S-opsin), and 23.8±0.7% of cells within each organoid were cone photoreceptor-like (Crx+, S-opsin+). Similar results were also observed with the crx>GFP ES cell line: after inhibitor treatment, crx>GFP promoter activation was detected as early as day 6 in 99% of organoids. Using a dose-response assay, we have optimized culture conditions to produce an average of 62±12% GFP-positive photoreceptor progenitor cells after simultaneous treatment with dorsomorphin, SB431542, and FGF2. By day 9, crx>GFP expression was sustained and Otx2 and M-opsin expression was observed in 99±1% and 74±2% of organoids, respectively.
**Conclusions:** These findings suggested that our culture system creates an environment where simultaneous BMP and Activin/TGFβ inhibition and FGF activation can bias mouse embryonic stem cells

towards photoreceptor lineages *in vitro,* which will be tested for light sensitivity in the future.
**Commercial Relationships: Kimberly A. Wong**, None; **Michael Trembley**, None; **Ichiro Hiratani**, None; **David M. Gilbert**, None; **Michael Zuber**, None; **Andrea S. Viczian**, None
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**Program Number:** 4576 **Poster Board Number:** B0231
**Presentation Time:** 11:00 AM–12:45 PM
**Non-invasive retinal imaging of fluorescent hESC-derived photoreceptor precursors in the living primate**
*Juliette E. McGregor[1], M J. Phillips[2], Sarah Walters[1, 3], Jie Zhang[1], Jennifer Strazzeri[4, 1], David DiLoreto[4, 1], Amber Walker[1], William S. Fischer[4], Qiang Yang[1], Louis DiVincenti[5], David M. Gamm[2, 6], David R. Williams[1, 3], Jennifer J. Hunter[4, 1], Merigan H. William[1, 4].*
[1]Center for Visual Science, University of Rochester, Rochester, NY; [2]McPherson Eye Research Institute, University of Wisconsin, Madison, WI; [3]Institute of Optics, University of Rochester, Rochester, NY; [4]Flaum Eye Institute, University of Rochester, Rochester, NY; [5]Division of Comparative Medicine, University of Rochester, Rochester, NY; [6]Department of Ophthalmology and Visual Sciences, University of Wisconsin, Madison, NY.
**Purpose:** The efficacy of stem cell injections into the retina to treat vision loss is conventionally evaluated post-mortem using histological methods in animal models. We explore the use of optical coherence tomography (OCT) and high resolution adaptive optics scanning light ophthalmoscopy (AOSLO) as non-invasive imaging methods to longitudinally monitor the spatial distribution, longevity and structural remodeling of photoreceptor precursor cells in the living macaque. To allow the specific identification of transplanted photoreceptor precursors *in vivo* we created a human embryonic stem cell (hESC)-derived CRX reporter line expressing the fluorescent protein tdTomato.
**Methods:** Gene targeting via homologous recombination was used to create the hESC CRX:tdTomato line, and 3D optic vesicle-like structures were generated using previously published protocols established by the Gamm lab. Photoreceptor precursor cells were dissociated and suspended in HBSS before subretinal injection into a 15-year-old male macaque *(Macaca fascicularis)* using a 41-gauge needle and a 20 psi pressure pulse. Immune suppression was provided by daily subcutaneous injection of cyclosporine (17.5 mg). Reflectance (796nm) and fluorescence (excitation 561 nm, emission 584-676 nm) AOSLO images were collected over a period of three months post-transplant. OCT (Heidelberg Spectralis) was conducted in regions expressing Tdtomato.
**Results:** Photoreceptor precursors expressed tdTomato for more than three months post transplantation, implying cell survival during that period. Precursor cell aggregates continued to remodel during that time with OCT data indicating a two-fold increase in aggregate thickness between day 52 and day 114 post-transplant. AOSLO imaging showed ring-like groupings of cells with median diameter 95 μm, similar to 'rosettes' previously seen in histological studies. Locations associated with photoreceptor precursors were hyper-reflective.
**Conclusions:** Survival, distribution and remodelling of fluorescently-labelled photoreceptor precursors injected into the subretinal space can be tracked non-invasively using AOSLO and OCT. These non-invasive methods can be used for long-term *in vivo* monitoring of stem cell transplants at a cellular spatial scale, reducing the need to sacrifice animals at a range of time points. This approach could be especially valuable applied to human therapy.
**Commercial Relationships: Juliette E. McGregor**; **M J. Phillips**, Opsis therapeutics (P), Opsis therapeutics (S), Opsis therapeutics (I); **Sarah Walters**, None; **Jie Zhang**, Canon Inc. (P), University of Rochester (P), Canon Inc. (F); **Jennifer Strazzeri**, None; **David DiLoreto**, None; **Amber Walker**, None; **William S. Fischer**, None; **Qiang Yang**, Canon Inc. (P), Montana State University (P), University of Rochester (P), Canon Inc. (F); **Louis DiVincenti**, None; **David M. Gamm**, Opsis therapeutics (P), Opsis therapeutics (S), Opsis therapeutics (I); **David R. Williams**, Canon Inc. (R), University of Rochester (P), Canon Inc. (F); **Jennifer J. Hunter**, University of Rochester (P); **Merigan H. William**, None
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**Program Number:** 4577 **Poster Board Number:** B0232
**Presentation Time:** 11:00 AM–12:45 PM
**Comparative analysis of retinal differentiation of human induced pluripotent stem cells from different somatic cell sources**
*Xiufeng Zhong[1], Guilan Li[1], Bingbing Xie[1], Guanjie Gao[1], guangjin pan[2], Jian Ge[1].* [1]State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-Sen Unversity, Guangzhou, China; [2]Key Laboratory of Regenerative Biology, Chinese Academy of Sciences, China Institute for Stem Cell Biology and Regenerative Medicine, Guangzhou Institutes of Biomedicine and Health, Guangzhou, China.
**Purpose:** The establishment of retinal differentiation platform with induced pluripotent stem cell (iPSC), holds great promise to study and treat retinal degenerations. However, the capacity of targeted differentiation of iPSCs may vary among iPSC lines from different somatic cell sources, which impact the further application. The current study is to assess the ability and efficiency of retinal differentiation among hiPSC lines.
**Methods:** hiPSCs derived from both blood cells (B-iPSCs) and urine cells (U-iPSCs) were cultured with mTeSR1. Retinal differentiation procedure was based on a published protocol with slight modification (Zhong X, Nature Communications 2014). Morphological changes were observed and imaged under inverted microscope. Immunofluorescence was done with markers specific for the early and late stages of retinal development. Efficiency of retinal differentiation at early stage was also assessed.
**Results:** In initial phase, both B-hiPSCs and U-hiPSCs could form cell aggregates under suspension culture conditions. Both B-hiPSCs and U-hiPSCs had the capacity to form neural retinal (NR) domain and retinal pigment epithelium (RPE) domain. But B-hiPSCs took less time to generate these structures than U-hiPSCs. Subsequently, the collected NR domains self-formed 3D retina cups under suspension condition. These cups consisted of thick transparent continuous NR, expressing retinal stem cell markers, such as PAX6, VSX2, LHX2 ank Ki67. The NR could be further laminated and differentiated into the all major retinal cells including RGC, PRC, amacrine cells. However, B-hiPSCs generated more retinal cups than U-hiPSCs in parallel experiments.
**Conclusions:** Human induced pluripotent stem cells reprogrammed from both blood and urine cells have the capacity to generate 3D

retinal cups in vitro. However, B-hiPSCs take less time to generate retinal cups and had higher efficiency of retinal differentiation than U-hiPSCs. Work is currently underway to explore the underlying mechanisms.

**Commercial Relationships: Xiufeng Zhong**; **Guilan Li**, None; **Bingbing Xie**, None; **Guanjie Gao**, None; **guangjin pan**, None; **Jian Ge**, None
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**Program Number:** 4578 **Poster Board Number:** B0233
**Presentation Time:** 11:00 AM–12:45 PM

**A Fluorescent *VSX2* Reporter for Neural Retina Differentiation Created in hiPSCs by CRISPR/Cas9**

*Phuong T. Lam, Christian Gutierrez, Katia Del Rio-Tsonis, Michael L. Robinson*. Biology, Miami University, Oxford, OH.

**Purpose:** Neural retina (NR) degeneration leads to vision loss, affecting millions of people worldwide. Therapies to arrest this degenerative process remain limited. Human induced pluripotent stem cell (hiPSC)-derived NR progenitors hold great potential for cell-based therapies aimed at NR regeneration. Expression of *VSX2* transcription factor specifically marks NR progenitor (NRP) cells early in eye development before becoming restricted to mature retinal biopolar neurons. Here, we describe a CRISPR/Cas9 to insert Cyan Fluorescent Protein (CFP) reporter gene into the *VSX2* locus to label living human NRP cells without disrupting the function of the endogenous *VSX2* allele

**Methods:** Homology directed repair (HDR), following CRISPR/Cas9-mediated double strand DNA breakage in hiPSCs, facilitated the replacement of the *VSX2* stop codon, in frame with viral P2A peptide fused to CFP reporter gene. This accomplishment resulted from co-electroporation a HDR DNA template with Cas9 vector encoding specific gRNA into the CB-hiPSC6.2 cell line. PCR primer sets spanning each side of HDR junctions unambiguously identified successfully targeted hiPSC from G418 resistant cells. The hiPSC-VSX2/CFP clones were differentiated to NRPs as assessed by specific expression of *VSX2* and *CFP* (blue) fluorescence. At Day 0 (D0) and D20 of NR differentiation, cells were examined for mRNA expression of VSX2-P2A-CFP, and eye field transcription factors (Pax6, RX, LHX2, SIX3, and SIX6). D20 cells were also examined for VSX2 and CFP protein by double immunofluorescence (d-IF).

**Results:** Seven of forty eight G418-resistant clones exhibited the expected PCR fragments on each side of the HDR junction as assessed by both size and sequence. One of these successfully targeted hiPSCs-VSX2/CFP clones was used to create optic cups *in vitro*. Although negative for both *VSX2* expression and CFP fluorescence at D0, at D20 of NR differentiation optic cups exhibited VSX2-P2A-CFP, Pax6, RX, LHX2, SIX3, and SIX6 mRNA expressions as well as blue fluorescence (figure 1). VSX2 and CFP proteins were also detected in these D20 optic cups by d-IF

**Conclusions:** A CRISPR-Cas9 based genome editing strategy successfully generated a VSX2 reporter cell line in hiPSCs. These cells will facilitate a simple visualization of NR progenitor during hiPSC-NR differentiation *in vitro*

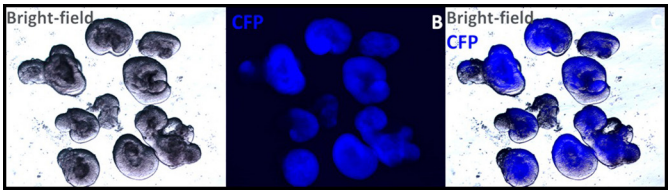



Figure 1: D30 NR-derived hiPSCs under bright-field (A), real-time CFP expression (B), and both in (C).

**Commercial Relationships: Phuong T. Lam**, None; **Christian Gutierrez**, None; **Katia Del Rio-Tsonis**, None; **Michael L. Robinson**, None
**Support:** Sigma Xi G201510151661206

**Program Number:** 4579 **Poster Board Number:** B0234
**Presentation Time:** 11:00 AM–12:45 PM

**Enrichment of mouse cone photoreceptors derived from pluripotent cells**

*Hany Abdelgawad[1, 2], Michael J. Young[1]*. [1]Department of Ophthalmology, Schepens Eye Research Institute, Massachusetts Eye and Ear, Harvard Medical School, Boston, MA; [2]Faculty of Medicine - Fayoum University, Fayoum, Egypt.

**Purpose:** Retinal degenerative disorders such as macular degeneration are characterized by irreversible changes in the photoreceptor (PR) and retinal pigment epithelium (RPE) layers. While PR precursors and progenitors give rise to rods and cones after transplantation (e.g. in rodent and porcine recipients), the alignment and number of integrated cells do not yet permit the full restoration of vision. Although published work shows it is relatively easy to generate large numbers of photoreceptor precursors from pluripotent human and mouse cells in 3D culture, the transition to differentiated cones has remained elusive.

**Methods:** In this study, we used mouse induced pluripotent stem cells (mIPSC) line labeled with s-opsin green fluorescent protein (GFP). Optic cups were generated in-vitro using a three-dimensional approach using Matrigel®. A NOTCH pathway inhibitor (DAPT) was added to the culture from day 12 to day 15 and s-opsin $GFP^+$ cells were isolated by FACS at day 21 and day 28 of culture. Subsequently, the isolated s-opsin $GFP^+$ cell populations were characterized by RT-PCR and Flow-Cytometry for expression of cone photoreceptor markers.

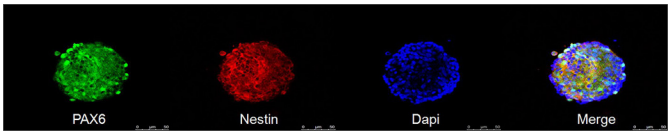
**Results:** S-opsin $GFP^+$ isolated cells expressed a number of s-opsin cone photoreceptor specific markers including OPN1SW, ARR3, GNGT2, CNGB3, PRPH2, SALL3 and DLG4.

**Conclusions:** This report provides a novel strategy for the generation of large numbers of mouse s-opsin cones that can be used for transplantation paradigms, as well as the development of high throughput screening (HTS) assays for cone development and survival. This will enable the development of tissue engineering and drug therapies to treat retinal degeneration diseases.

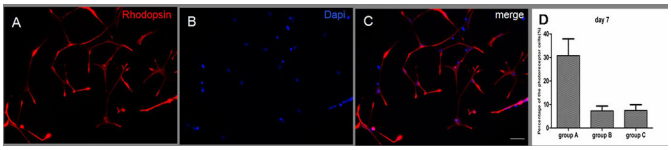
**Commercial Relationships: Hany Abdelgawad**; **Michael J. Young**, None
**Support:** Bertarelli Foundation

**Program Number:** 4580 **Poster Board Number:** B0235
**Presentation Time:** 11:00 AM–12:45 PM
**Retina organoids derived from hESCs and transplanted to immunodeficient RCS rats**
*Magdalene J. Seiler[1, 2], Bryce T. McLelland[2], Anuradha Mathur[2], Bin Lin[2], Gabriel Nistor[3], Robert B. Aramant[2], Biju Thomas[4], Hans S. Keirstead[3]*. [1]Phys. Med. & Rehabilitation, UC Irvine, Irvine, CA; [2]Sue & Bill Gross Stem Cell Research Ctr., UC Irvine, Irvine, CA; [3]AIVITA Biomedical Inc., Irvine, CA; [4]Roski Eye Inst., Ophthalmology, University of Southern California, Los Angeles, CA.
**Purpose:** The development of retina organoids derived from human embryonic stem cells (hESC) was followed after transplantation to the subretinal space of immunodeficient RCS rats.
**Methods:** CSC14 (NIH line 0284) hESCs were differentiated into retina organoids following a protocol modified after Zhong et al. 2014 (Nature Communications 5:4047) and characterized by immunohistochemistry (IHC) and qPCR. Dissected sheets of retina organoids (differentiation day 30-63) were transplanted to the subretinal space of nude RCS hosts (P44-56, 1.5-1.9 months). Ocular Coherence Tomography (OCT) monitored the development of the transplants at 2 wks to 6 mo. post-surgery. Visual function was accessed by electroretinogram (ERG) before and 2, 4, and 6 mo. after surgery. Cryostat sections through the transplants 20-113 days post-surgery (DPS) were stained with hematoxylin & eosin (H&E), or processed for immunohistochemistry (IHC) to label human donor, retinal cells and synaptic markers using light and confocal microscopy.
**Results:** IHC of retina organoids shows early lamination and development of retinal cell progenitors and later mature subtypes. qPCR analysis of transplanted retina organoids indicates an expression profile closest to that of human fetal retina and primarily comprised of various retinal progenitors (CHX10+, CRX+, NRL+, LHX2+). OCT imaging revealed the presence of rosettes containing photoreceptors, and provided data on the transplant's distance from optic nerve, volume, rosette quantification, and trophic effect on host retina. Transplanted eyes showed increased B-waves to scotopic stimulation at 2 mo. post-surgery compared to RCS controls. ERG responses were undetectable at 4-6 mo. post-surgery in RCS controls and transplants. Transplant histology revealed the development of a rosetted morphology *in vivo*, with a distinct ONL and photoreceptor outer segments located in the center of rosettes. Transplants showed evidence of migration, and extended processes into the host retina. Rosettes labeled with recoverin indicating the presence of photoreceptors, as well as cone bipolar cells. Functional testing of transplanted rats is ongoing.
**Conclusions:** Retina organoids, derived from hESCs were transplanted to the subretinal space of immunodeficient RCS rats. Organoids mature further after transplantation, develop photoreceptors with outer segments, integrate into the host retina, and can improve visual function.
**Commercial Relationships: Magdalene J. Seiler**, Ocular Transplantation LLC (P); **Bryce T. McLelland**, None; **Anuradha Mathur**, None; **Bin Lin**, None; **Gabriel Nistor**, AIVITA Biomedical (E); **Robert B. Aramant**, Ocular Transplantation LLC (P), Ocular Transpalntation LLC (E); **Biju Thomas**, None; **Hans S. Keirstead**, AIVITA Biomedical (E), AIVITA Biomedical (S)
**Support:** CIRM TR4-06648 (MS); NIH R01EY024045 (HK)

**Program Number:** 4581 **Poster Board Number:** B0236
**Presentation Time:** 11:00 AM–12:45 PM
**OTX2 promotes retinal Müller cell differentiation into photoreceptor cells and improves injured Rats' vision function**
*Yu Xiong, Xiaobo Xia*. Department of Ophthalmology, Xiangya hospital, Central South University, ChangSha, China.
**Purpose:** To develop a novel method to enhance the differentiation of rat retinal müller cells to photoreceptor cells and explore the mechanism of signaling pathway, thus providing a valuable tool for treating retinal degeneration.
**Methods:** Retinal stem cells were dedifferentiated from rat Müller glial cells, then transfected with lentivirus PGC-FU-OTX2-EGFP, empty lentivirus PGC-FU-EGFP, or no transfection randomly. In addition, the retinal stem cells were transfected with Crx siRNA or Nrl siRNA or treated with Wnt/β-catenin signaling antagonist, Dkk-1. The retinal stem cells were induced to differentiate for 3 days, then were injected into the subretinal space of adult MNU-treated rats. The population of differentiated cells and photoreceptors were assessed by immunofluorescence, the expression levels of Otx2, Crx, Nrl and β-catenin were detected using quantitative PCR and Western blotting. ERG of the retinas of rats were performed. Additionally, the apoptosis of retinal cells were eventually detected with TUNEL analysis.
**Results:** The proportion of photoreceptor cells differentiated from Otx2-tranfected stem cells was significantly higher than that of controls. Knockdown of Crx or Nrl inhibited, while Wnt promoted, the differentiation into photoreceptors cells. Otx2 promoted the expression of Crx and Nrl and activated by the Wnt/β-catenin pathway. By 2 weeks after subretinal injection, GFP-expressing transplanted cells integrated into the host retina, forming structures similar to the outer nuclear layer, expressed a photoreceptor-specific marker, manifested by a subtle increase in the a-wave amplitude using scotopic electroretinography, indicating that rod photoreceptor function was partially restored in the MNU-treated rats. Furthermore, few positive cells in the TUNEL staining and no teratoma formation was observed in the recipient retina.
**Conclusions:** OTX2 promotes the differentiation of Müller cells-derived retinal stem cells into retinal photoreceptor cells by activating Wnt signaling in vitro and in vivo.



Detected the specific marker of the neurospheres(IF)(bar=50μm)



OTX2 regulated the differentiation of the retinal stem cells.
**Commercial Relationships: Yu Xiong**; **Xiaobo Xia**, None
**Support:** National Natural Science Foundation of China, Grant/ Award Number: 81470653.

**Program Number:** 4582 **Poster Board Number:** B0237
**Presentation Time:** 11:00 AM–12:45 PM
**Human iPSC derived retinal organoids as a mean to study Retinitis Pigmentosa with CRB1 mutations**
*Kevin Achberger[1], Wadood Haq[2], Sylvia Bolz[2], Stefan Liebau[1]*. [1]Institute of Neuroanatomy, Tübingen, Germany; [2]Institute for Ophthalmic Research, Tübingen, Germany.

**Purpose:** Mutations in the CRB1 gene are amongst the leading causes for Retinitis Pigmentosa, however the exact function and localisation of the CRB1 protein in human retina is still not clarified. In our endeavour to elucidate CRB1 biology and the impact of CRB1 mutations, we use and study human induced pluripotent stem cell derived retinal organoids generated from control persons and affected patients.
**Methods:** Human iPSC cells were generated from control persons and a pair of siblings with the CRB1 mutation C948Y. Subsequently, retinal organoids were differentiated according to an adapted protocol based on Zhong et al 2014. Organoids were either fixed or analysed at different time points (d40- d190). For immunofluorescence, samples were cryo-sected and stained with respective antibodies. For electron microscopy, cells were fixed at d190, embedded in araldite and later observed under an electron microscope. Ca2+ Imaging was performed at d200 after preincubation with Fura-2 calcium dye.
**Results:** Presence of all human retinal cell as well as the inner and outer limiting membranes could be verified by immunofluorescence staining. Further, we could demonstrate the existence of an outer plexiform synapse layer with photoreceptor- bipolar cell synapses. Starting from day 40 of differentiation CRB1 could be found apical from the ZO-1 positive- outer limiting membrane in close apposition to the developing photoreceptor inner and outer segments. Furthermore, we could demonstrate the presence of connecting cilia as well as starting outer segment formation via electron microscope. Light sensitivity of organoid photoreceptors was shown via calcium imaging.
**Conclusions:** All together, we could state the morphological hallmarks and verify the functionality of the retinal organoids derived from human iPSC. We could describe CRB1 protein expression during the retinal organoid development and set the groundwork for a thorough comparison of unaffected controls with retinal organoids obtained from patients with Retinitis Pigmentosa caused by CRB1 mutations.
**Commercial Relationships: Kevin Achberger**, None; **Wadood Haq**, None; **Sylvia Bolz**, None; **Stefan Liebau**, None

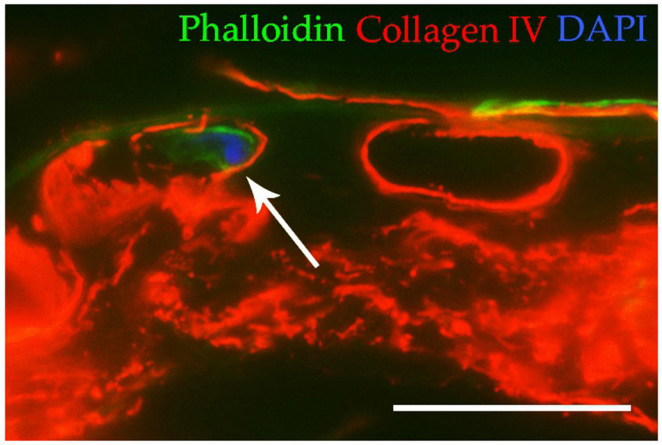
**Program Number:** 4583 **Poster Board Number:** B0238
**Presentation Time:** 11:00 AM–12:45 PM
**Misdirected migration of retinal progenitors following photoreceptor injury in zebrafish**
*Alex Yuan, Rose DiCicco, Brent A. Bell, Shiming Luo, Bela Anand-Apte, Brian D. Perkins*. Ophthalmology, Cleveland Clinic Foundation, Cleveland, OH.
**Purpose:** In zebrafish, focal laser can generate injury to the outer nuclear layer (ONL). Rapid retinal regeneration takes place with restoration of the ONL within 2-3 weeks. In 100% of lesions, cell nuclei can be found in the inner plexiform layer (IPL) up to 15 weeks following injury. In this study, we used bromodeoxyuridine (BrdU) to label proliferating cells and tracked their migration following injury.
**Methods:** An OCT-guided laser was used to ablate photoreceptors in zebrafish. One or 3 days after injury, zebrafish were labeled with 0.25 µmol/g body weight of BrdU (intraperitoneal). Eyes from injected fish were then analyzed by confocal microscopy to determine the pattern of BrdU labeling and presence of BrdU positive cells at intervals up to 14 days following injury. To determine the population of proliferating cells, we also performed immunohistochemistry using anti-PCNA antibody at similar intervals up to 14 days following injury.
**Results:** Proliferating cells labeled with PCNA on day 1 were found diffusely in all layers of the retina. Proliferating cells labeled with BrdU on day 1 and sectioned on day 3 displayed a similar diffuse pattern. A greater number of proliferating cells were labeled by BrdU and PCNA on day 3 following laser injury. On day 3, proliferating cells labeled by PCNA were found primarily in the inner nuclear layer (INL). Proliferating cells labeled by BrdU on day 3 were found primarily in the INL and ONL by day 4, with fewer in the INL and more in the ONL by day 7. Although a majority of cells labeled with BrdU either remained in the INL or migrated to the ONL, a significant portion of BrdU positive cells were also found in the IPL and ganglion cell layers (GCL). In contrast, PCNA labeling revealed the majority of proliferating cells remained in the INL and ONL but not in the IPL.
**Conclusions:** Following ONL injury, retinal progenitors in the INL proliferated then migrated predominantly to the ONL where aggregates of microglia rapidly migrated. However, some of these progenitors appeared to migrate towards the IPL and GCL. Many of the misplaced nuclei found in the IPL were labeled by BrdU injected on day 3 following injury, suggesting a migration of retinal progenitors to the IPL. These IPL cells, however were not positive for PCNA suggesting they have lost their stem cell properties and no longer proliferated. Future work to identify the expression profiles of these cells are under way.
**Commercial Relationships: Alex Yuan**, None; **Rose DiCicco**, None; **Brent A. Bell**, None; **Shiming Luo**, None; **Bela Anand-Apte**, None; **Brian D. Perkins**, None
**Support:** NIH 1K08EY023608-4, NIH IP30EY025585-01A1, Unrestricted Grant from The Research to Prevent Blindness, Inc., awarded to the Cole Eye Institute

**Program Number:** 4584 **Poster Board Number:** B0239
**Presentation Time:** 11:00 AM–12:45 PM
**Modulating p75$^{NTR}$ protects against ischemic retinopathy via increased vascular homing of mesenchymal stem cells**
*Sally L. Elshaer[1, 2], William Dave Hill[3, 2], Rajashekhar Gangaraju[4], Azza B. El-Remessy[1, 2]*. [1]Clinical and Administrative Pharmacy, University of Georgia, Augusta, GA; [2]Charlie Norwood VA Medical Center, Augusta, GA; [3]Augusta University, Augusta, GA; [4]Hamilton Eye Institute, Memphis, TN.
**Purpose:** Ischemic retinopathy is characterized by initial ischemia that progresses into neovascularization. We reported that deletion of death receptor; p75$^{NTR}$ enhanced vascular repair in oxygen-induced retinopathy model and preserved expression of angiogenic markers; SDF-1, CXCR-4 and CXCR-7 responsible for mesenchymal stem cells (MSCs) homing to vasculature in diabetic ischemic limbs. MSCs are potential candidates for neuro-regeneration in various retinal diseases, nevertheless, their role in retinal vascular repair remains poorly addressed. Our aim is to investigate the contribution of MSCs to vascular protection associated with p75$^{NTR}$ deletion in ischemic retina.
**Methods:** WT and p75$^{NTR-/-}$ mice were subjected to retinal ischemia reperfusion (I/R) injury. Murine GFP-labeled p75$^{NTR}$-positive or p75$^{NTR}$-null MSCs were injected intravitreally 2 days post-I/R and vascular homing was assessed one week later. Acellular capillaries were counted on trypsin-digested retinas 10 days post I/R. In vitro, MSCs were treated with a specific pharmacological inhibitor against p75$^{NTR}$; LM11A-31 and conditioned media was co-cultured with human retinal endothelial cells (HRECs) to examine angiogenic response.
**Results:** p75$^{NTR-/-}$ ischemic retinas showed 2-Fold increase in acellular capillaries compared to 3-Fold increase in WT as well as preserved mRNA expression of CXCR-7. p75$^{NTR}$-MSCs showed best incorporation into ischemic retinal vasculature one-week post-injection and completely protected against acellular capillaries in ischemic p75$^{NTR-/-}$ retinas. p75$^{NTR}$-null MSCs showed better incorporation into WT ischemic retinal vasculature and significantly

decreased acellular capillaries by 60% compared to ischemic WT receiving $p75^{NTR}$-MSCs. $p75^{NTR}$-null MSCs showed increased mRNA expression of CXCR-4 as well as increased mRNA and protein expression of CXCR-7. In vitro, inhibition $p75^{NTR}$ in MSCs enhanced angiogenic response of their conditional media in HRECs, where HRECs showed enhanced migration (1.3-Fold) and tube formation (2.7-Fold).
**Conclusions:** Our results showed that deletion of $p75^{NTR}$ protects against retinal ischemia by mechanisms involving improved MSCs homing to ischemic vasculature to prevent vascular degeneration. Thus, combination of MSCs injection and $p75^{NTR}$ inhibitor can serve as potential therapeutic strategy to harness vascular repair in ischemic retinopathy diseases.
**Commercial Relationships: Sally L. Elshaer**, None; **William Dave Hill**, None; **Rajashekhar Gangaraju**, None; **Azza B. El-Remessy**, None

**Program Number:** 4585 **Poster Board Number:** B0240
**Presentation Time:** 11:00 AM–12:45 PM
**Compatibility of a biodegradable retinal cell graft for the treatment of retinal degenerative blindness**
*Elliott Sohn[1, 2], Kristan S. Worthington[2], Chunhua Jiao[2], Emily E. Kaalberg[2], Stephen R. Russell[1, 2], Katherine N. Gibson-Corley[2], Robert F. Mullins[2], Edwin M. Stone[1, 2], Budd Tucker[2]*. [1]Retina Service, University of Iowa, Iowa City, IA; [2]Wynn Institute for Vision Research, Iowa City, IA.
**Purpose:** Photoreceptor cell replacement has demonstrated potential for restoring vision in retinal degenerations, but only a small fraction of transplanted cells survive and integrate into the host retina. Polymeric support scaffolds have been shown to enhance the efficacy of cell survival and integration post-transplantation. The purpose of this study was to test retinal biocompatibility of a hydrogel-based biodegradable polymer scaffold, poly (caprolactone) (PCL).
**Methods:** Methacrylate-functionalized PCL scaffolds were polymerized with Irgacure 651 using two-photon polymerization. Four month old Yucatan mini pigs had vitrectomy and hyaloid induction. A subretinal bleb was raised with BSS, followed by placement of PCL scaffold in the subretinal space, then fluid-air exchange. Contralateral pig eyes underwent control surgery. Animals were sacrificed 1 month (n=5 eyes) or 3 months (n=5 eyes) after surgery at which time ophthalmoscopy and spectral-domain OCT (SD-OCT) were performed. Pathological analyses were performed on paraffin sections of control and treated eyes. Immunostaining with CD45, CD34, CD31 antibodies was done on sections from polymer transplantation sites.
**Results:** All 10 treated eyes had complete, spontaneous retinal reattachment with implants identifiable in the subretinal space on ophthalmoscopy and SD-OCT. On SD-OCT the surface of the polymer was hyper-reflective while the body was hypo-reflective compared to the retina. No eyes had evidence of intraocular inflammation. Hematoxylin-eosin stained sections through a subset of injected eyes demonstrated subretinal location of the implanted polymer. Retinal integrity, including intact photoreceptor outer segments, was relatively well maintained. No aberrant CD45, CD34, CD31 positive cells were detected in the subretinal space around the polymer transplantation sites.
**Conclusions:** PCL polymers, which support maintenance of human iPSC-derived retinal cells, can be successfully delivered to the subretinal space and are well-tolerated by the host retina. These scaffolds may be ideal for retinal transplantation.
**Commercial Relationships: Elliott Sohn**; **Kristan S. Worthington**, None; **Chunhua Jiao**, None; **Emily E. Kaalberg**, None; **Stephen R. Russell**, None; **Katherine N. Gibson-Corley**, None; **Robert F. Mullins**, None; **Edwin M. Stone**, None; **Budd Tucker**, None
**Support:** Research to Prevent Blindness, Wynn Professorship for Regenerative Ophthalmology, Wynn Foundation

**Program Number:** 4586 **Poster Board Number:** B0241
**Presentation Time:** 11:00 AM–12:45 PM
**Preparation of Human Choroidal ECM Scaffolds to Study Cell Replacement Strategies**
*Kathleen R. Chirco[2, 1], Kristan S. Worthington[2, 1], Miles Flamme-Wiese[2, 1], Megan Riker[2, 1], Allison E. Songstad[2, 1], Malia M. Collins[2, 1], Joshua Andrade[3], Beatrix Ueberheide[3, 4], Edwin M. Stone[2, 1], Budd Tucker[2, 1], Robert F. Mullins[2, 1]*. [1]Ophthalmology & Visual Sciences, University of Iowa, Iowa City, IA; [2]Stephen A Wynn Institute for Vision Research, Iowa City, IA; [3]Proteomics Laboratory, New York University School of Medicine, New York, NY; [4]Biochemistry and Molecular Pharmacology, New York University School of Medicine, New York, NY.
**Purpose:** Endothelial cells (ECs) of the choriocapillaris are lost very early during the pathogenesis of age-related macular degeneration (AMD), and cell replacement therapy is currently the most promising option for patients with advanced AMD. Here, we sought to develop a reliable method for the production of human choroidal extracellular matrix (ECM) scaffolds, which will allow for the study of choroidal EC (CEC) replacement therapies in an environment that closely resembles the native tissue.
**Methods:** Using unfixed RPE/choroid punches from human donor eyes, four different protocols were evaluated for their ability to remove cells from the tissue. After decellularization, immunohistochemistry (IHC) was employed to compare treated to untreated punches, confirm cell removal, and assess the localization and relative abundance of the remaining ECM proteins. To further validate CEC removal, decellularized tissue ultrastructure was visualized using transmission electron microscopy (TEM), and mass spectrometry (MS) was performed to evaluate protein composition. Finally, the acellular choroid scaffold was co-cultured with RF/6A CECs or human endothelial cells to assess the ability to recellularize the tissue.
**Results:** Sequential treatment with 1% Triton X-100, 0.1% SDS, and DNase solutions is most effective at removing all native cells. Decellularized RPE/choroid tissue shows a loss of CD31 immunolabeling and endothelial cell-specific lectin labeling (UEA-I), as well as complete absence of the nuclear DAPI stain compared to native RPE/choroid. In contrast, the decellularized tissue exhibits preserved collagen IV, elastin, and laminin in both IHC and MS analyses. TEM micrographs reveal complete removal of CECs within the choriocapillaris, with retained basement membrane surrounding the vessel walls. Both RF/6A and human ECs are able to successfully migrate into the choriocapillary tubes.
**Conclusions:** Our data demonstrate the ability to remove all cells from human RPE/choroid punches while keeping the ECM largely intact. Furthermore, intact vascular walls with basal laminae are visible in the remaining ECM, suggesting that these methods are gentle enough to preserve tissue structure and allow for the optimization of cell replacement strategies.




RF/6A cells (phalloidin; green) are shown recellularizing the choroid scaffold (collagen IV; red). Cell nuclei are shown in blue. Scale bar = 50μm.

**Commercial Relationships: Kathleen R. Chirco**; **Kristan S. Worthington**, None; **Miles Flamme-Wiese**, None; **Megan Riker**, None; **Allison E. Songstad**, None; **Malia M. Collins**, None; **Joshua Andrade**, None; **Beatrix Ueberheide**, None; **Edwin M. Stone**, None; **Budd Tucker**, None; **Robert F. Mullins**, None

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