Ultrastructural studies on corneal regeneration one year after implantation of biosynthetic cell-free hydrogel implants in mini pig

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Purpose: To understand the underlying endogenous cellular mechanisms involved in the integration and regeneration of cell-free hydrogel biosynthetic artificial corneas in a mini pig model.

Methods: Mini pigs received non-penetrating implants of cell-free collagen-like peptide (CLP-PEG) or recombinant human collagen type III (RHCIII) hydrogels. After one year, the animals were euthanized and the corneas were examined using 3D serial block face scanning electron microscopy (SBF SEM). They were fixed and processed through a series of staining solutions prior to embedding in epoxy resin. Polymerized resin blocks were trimmed and transferred to a Zeiss Sigma VP FEG scanning electron microscope equipped with a Gatan 3View system, where data sets of up to 2000 images were acquired of the block surface every 50nm through automated sectioning. Selected serial image sequences were extracted and 3D reconstructions were generated using Amira software. Transmission electron microscopy (TEM) was also carried out.

Results: Both CLP-PEG and RHCIII implants were extensively remodeled with keratocyte-like cells and banded collagen. SBF SEM further revealed that the cells were arranged in stratified layers within the regenerated corneas. The anterior surfaces of both the RHCIII and CLP-PEG regenerated corneas were re-epithelialized. Whilst the RHCIII epithelium appeared similar to controls, CLP-PEG regenerated corneal epithelial cells exhibited a basal surface with numerous invaginations. Below this, exosomes were present in a layer within the remodeled anterior stroma. No large pieces of the CLP-PEG or RHCIII implant were recognizable within the central portion of the regenerated cornea by either TEM or SBF SEM. However, abundant electron-dense deposits, likely to be degraded remnants of the original implants, were observed interspersed between the collagen fibers within the remodeled tissue.

Conclusions: Both CLP-PEG and RHCIII implants were found to be extensively remodeled with keratocyte-like cells and collagen fibrils. Whilst original degraded implant material was still present within the regenerated corneas, both implant types appeared fully integrated, forming a regenerated cornea almost indistinguishable from normal host cornea. The presence of exosomes within the regenerated CLP-PEG cornea suggests active endogenous intercellular communication within the regenerated cornea.

Commercial Relationships: Philip N. Lewis; Ranjithkumar Ravichnadran, None; Jaywant Phopase, None; M. Mirazul Islam, None; Monika Ljunggren, None; Per Fagerholm, None; May Griffith, None; Keith M. Meek, None

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Reconstructed decellularized tissue for corneal regeneration

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Purpose: Although the decellularized cornea has potential to be used as a corneal substitute, decellularization procedures often reduce the function of the cornea by decreasing its transparency and disrupting the concave shape. We have developed methods of reconstructing decellularized cornea that improves corneal transparency and maintains its natural shape.

Methods: Fresh porcine corneas were harvested and treated with chemical (sodium dodecyl sulfate and Triton-X) and enzymatic (fetal bovine serum) methods. The reconstruction procedures, vitrification and riboflavin crosslinking, were carried out within a pair of curved molds that have similar curvature to the rabbit cornea. The reconstructed corneas (n=5) and native porcine corneas (n=5) were evaluated using the light transmittance test with the same thickness, Optical Coherence Tomography (OCT), pathological examination with H&E staining and transmission microscopy (TEM). The curvature of the molds and the reconstructed corneas were calculated with 2D OCT images. To quantify density and the diameter of collagen fibrils in the microstructure, ten TEM images were randomly chosen in each cornea (n=50), and areas were defined using a 1μmX1μm square. The collagen fibrils were evaluated using ImageJ software. Data is presented as mean±standard deviation (SD). The two-tailed Student’s t-test or Mann-Whitney test were used for statistical analysis depending on the size of the sample set.

Results: After the reconstruction procedures, light transmittance in the visible wave length was significantly increased. The curvature of the reconstructed cornea (7.613±0.136mm) is identical with that of the mold (7.615±0.138mm). In the assessment of macro- and micro-morphology, the reconstructed cornea showed similar structure with that of the native cornea. The reconstruction procedures significantly increased the density (107.6±23.0 to 307.0±18.0/μm², p=0.001) of collagen fibrils and significantly decreased diameter of collagen (40.7±2.5 to 31.2±3.4nm, p=0.001) of the decellularized cornea, which may correspond to the improvement in transparency.

Conclusions: We successfully reconstructed the decellularized porcine cornea with vitrification and riboflavin crosslinking using a pair of concave molds. This method produces a decellularized cornea with improved transparency and curvature for applications in corneal regeneration which may serve as substitutes for conventional human allograft transplantation.

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In vitro study of 3D tissue model of innervated corneal epithelium and stroma

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Purpose: In vitro tissue models are powerful tools to study physiological and pathological processes. Current cornea tissue models lack innervation and the dynamic conditions mimicking
in vitro parameters. Thus, we designed a silk protein biomaterial-based in vitro tissue model that includes human corneal epithelium (hCECs), stromal (hCSCs) and neurons (hNCs) cultured in a bioreactor to assess corneal ECM formation, mimics of physiological tear washing and the application of ocular pressure (IOP) to study impact on cellular phenotypes in static and dynamic environments.

Methods: The in vitro corneal tissue model was formed by co-culturing hCECs, hCSCs and hNCs in silk scaffolds to mimic corneal architecture. A bioreactor composed of an artificial anterior chamber creating ocular pressure (10-20mmHg), and an artificial tear outlet on the lid with 15-30 drops /min washing rate was used to house the 3D corneal tissue systems and sustain the culture for 2 months. Live and dead assay, immunohistochemistry, RT-PCR, and mechanical properties were investigated to assess cellular phenotype, ECM formation, tight junction (TJ) formation, and neuronal function. Data were compared between innervated and non-innervated, static and dynamic cultivated tissue systems using statistical analysis

Results: Significantly higher TJ formation and keratocytic ECM protein expression were observed in the innervated models compared to the non-innervated controls. During dynamic cultivation, the scaffold gained curvature and increased stiffness when compared to static cultivation conditions. Tear washing increased the cell layers and tight junction formation in the epithelium, while the IOP significantly increased the synthesis of ECM protein in the stroma.

Conclusions: This innervated corneal epithelium and stroma tissue model cultivated in a dynamic environment provides a useful system for studying long term interactions between neuronal innervation and corneal tissue, and the impact of IOP and tear washing on corneal tissue. Future applications include utility in drug screening and artificial cornea development for clinical translation.

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Presentation Time: 3:45 PM–5:30 PM

In vitro study: Corneal stromal stem cells cultured on cornea ECM blended silk films

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Purpose: In this in vitro study, the goal is to investigate the influence of human corneal ECM protein incorporated into silk protein films on human corneal stromal stem cell (hCSCs) functions. We will investigate the role of ECM protein species on keratocyte cellular behavior. We will focus on the isolation of endogenous cECM from human corneas, assessing the formation of corneal stroma like tissue in terms of composition and structural organization over time, as well as cellular phenotype.

Methods: Human cornea (donor cornea not suitable for transplantation) were decellularized in SDS, and then pepsin digested before lyophilization to form an ECM powder. Glycosaminoglycans and protein content in ECM powder were assessed. The cECM proteins were stabilized in the silk materials via water annealing, which affects crystallinity/mechanical properties. The collagens and proteoglycan content were quantified via mass spectroscopy and ELISA. Corneal stromal stem cells were seeded onto the modified silk films and cultured up to 6 weeks in vitro. The tissues formed were compared to plain silk and RGD-functionalized silk films, both without cECM, related to cell growth and new ECM secretion and organization. Histology, fluorescence microscopy, immunohistochemistry, qPCR and proteomic analysis were used to quantify the differences in the cCSCs cultured on the cECM blended silk films and controls. Detailed proteomics analysis will be conducted to investigate ECM formation by the cells on these silk-cECM substrates (collagen I, collagen V, collagen VI, laminin, keratan, decorin).

Results: A pepsin-based protein extraction protocol to achieve high yield of cECM components from human corneal tissues was successfully implemented. A content of 1.4% sulfated glycosaminoglycans per dry weight of corneal proteins was found and is critical for corneal tissue behavior (cGAGs comprise 2 to 5 wt% in native human cornea. hCSCs are expected to grow and proliferate on all the substrates; although improved outcomes are anticipated in the presence of the cECM in the films.

Conclusions: This study has defined a novel experimental system for examining the effect of extracellular matrix in the production of stromal matrix. We expect that future studies using this system to provide important details as to the role of the extracellular matrix in guiding corneal cell differentiation.

Commercial Relationships: Rachel Gomes, None; Siran Wang, None; Chiara E. Ghezzi, None; Whitney Stoppel, None; James L. Funderburgh, None; Kenneth Kenyon, None; David L. Kaplan, None

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Suppression of Hippo pathway in a novel proliferative corneal organ culture system

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Purpose: Shortage of available transplants may be attributed to human corneal endothelial cell (HCEC) damage during organ culture or transportation. Previously, we have shown that overexpression of YAP, a core protein in the Hippo pathway, results in proliferation in the HCEC monolayers without change in the normal phenotype. Here, we aimed to investigate the effect of YAP on HCEC proliferation or density in a proliferative corneal organ culture system.

Methods: Human or rabbit corneas were excised and cultured for 7 days in standard corneal organ culture medium MEM+2% FBS. Corneas were treated by PBS or adenovirus vector overexpressing YAP (adeno-YAP) from Day 2 on. Cell density and central corneal thickness were measured. Samples were then fixed, paraffin-embedded and examined morphologically by HE-staining and by immunofluorescent staining of YAP-1, ZO-1, Na-K-ATPase, SMA and BrdU labeling. TUNEL assay was performed to detect signs of apoptosis.

Results: Phases contrast microscopy revealed endothelial loss and signs of apoptosis after 24h of organ culture, but no endothelial cell alterations in cultures added with adenovirus-YAP. From Day 4 to Day 7 the cell density was significantly more while the central corneal thickness was less in cultures added with adenovirus-YAP. HE-staining failed to demonstrate significant differences between the two groups. Immunofluorescent staining displayed similar patterns of ZO-1, Na-K-ATPase, and SMA, but enhanced staining of YAP and BrdU labeling.

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TUNEL assay detected signs of apoptosis in the control but not the YAP group.

Conclusions: A stable organ culture system for proliferation can be established, in which overexpression of YAP promoted corneal endothelial proliferation and increased cell density. This novel organ culture protocol may be applied to eye banking, to optimize corneal grafting and to contribute to regenerative medicine.

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Presentation Time: 3:45 PM–5:30 PM

Computer Modeling for Optimized Nanoparticle-Guided Corneal Endothelial Repopulation

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Purpose: The corneal endothelium is responsible for corneal clarity; however, it poses a challenge when damaged due to its lack of regenerative potential and reducing cell population as we age. This study implements computer modeling to investigate the feasibility of strategically guiding injected nanoparticle-loaded donor human endothelial cells (HCEC) to the back of the cornea using an external magnetic field. Repopulating the corneal endothelium by injection of HCEC into the anterior chamber may serve as an alternative to surgical corneal transplants.

Method:
A computer model (Matlab) incorporating the effects of aqueous flow, stokes law, gravity, and magnetic field strength was used to investigate the necessary magnetic field strength and nanoparticle cell loading density to feasibly induce a controlled cellular movement across the anterior aqueous chamber. HCEC were cultured in human endothelial serum free media containing 10 ng/ml FGF-2, and loaded with super paramagnetic iron oxide nanoparticles (SPIONP), based on the results suggested by the computer model. Cell lineage and viability were evaluated using FACs analysis and CyQuant assays. Intracellular iron content was evaluated using Elzone particle analysis and Prussian blue staining. PCR analysis was used to evaluate expression of such HCEC markers as CD200, ACTA alpha, ATPA1, GPC4 and Zo-1 before and after loading. Lastly, SPIONP loaded HCEC were evaluated for inner cornea attachment.

Results:
The computer model indicated that SPIONP-loaded HCEC’s can be directed in a controlled manner to desired locations of the endothelium using a broad range of magnetic forces. HCEC endothelial lineage was confirmed by simultaneous expression of CD200 and glycoproteins. PCR analysis noted a significant difference in ATPA1 by day 3. SPIONP internalization was demonstrated by Prussian blue staining and Elzone particle analysis. Lastly, HCEC maintained similar viability ratios as unloaded control cells, and demonstrated the in vitro ability to attach to the endothelium layer of explant corneas.

Conclusions: HCEC readily incorporate SPIONPs. Computer modeling helps to minimize SPIONP exposure and facilitates directed cellular movement by indicating appropriate loading values. Future studies will incorporate such parameters as wound size, wound location and curvature of the cornea to further personalize this potential therapeutic treatment specific for the individual.
biological dressing for a number of ophthalmic applications. Most commercially available AM products have strict refrigeration requirements limiting the availability of the tissue. The goal of this study was to develop a sterile, “off-the-shelf” AM tissue scaffold utilizing a novel supercritical carbon dioxide (SCCO2) sterilization process in conjunction with lyophilization for use in corneal wound management.

**Methods:** AM was isolated from donated placentas, rinsed in saline, placed epithelial side up on nitrocellulose paper and stored at -80°C (native AM; N-AM). N-AM was sterilized with SCCO2, and refrozen (F-AM) or sterilized with SCCO2, lyophilized for 24 hrs and kept at room temperature (L-AM). Water content and oxygen permeability (Dk) of samples was measured following ISO standard 9913-1. The permeability of AM to antibiotics was assessed using a Franz diffusion cell apparatus. Biocompatibility was evaluated in vitro by culturing human corneal epithelial (CE) cells with membranes or in vivo by placement of membranes over the ocular surface of rabbits.

**Results:** The water content of N-AM, F-AM and L-AM was 92%, 92% and 88%, respectively. Using this information the Dk was calculated and found to be greater than 99 x 10⁻⁵ mlO₂ cm/sec cm² mmHg for all groups tested. F-AM and L-AM demonstrated increased permeability to antibiotics as compared to N-AM. CE cells attached to all groups evaluated, however, L-AM exhibited increased cell proliferation as compared to F-AM and N-AM. L-AM was secured over the ocular surface of rabbits for six days and did not induce corneal inflammation or swelling as determined by the McDonald-Schadluck scoring system.

**Conclusions:** SCCO₂ is an emerging tissue sterilization technology that provides a novel method for AM tissue graft preparation and sterilization, and better preserves the inherent biochemical and biophysical properties of the tissue. When combined with a lyophilization step, L-AM exhibits desirable properties of an ocular bandage and demonstrates improved biocompatibility compared to F-AM. Our method of sterilization, combined with lyophilization, renders a tissue graft that can be used as a bandage or a scaffold for ocular therapeutic applications without the need for costly and limiting storage requirements.

**Commercial Relationships:** Jennifer S. McDaniel; Jennifer Wehmeyer, None; Lauren Cornell, None; Anthony J. Johnson, None; David O. Zamora, None

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

**Human neural progenitor cell treatment modulates phagocytosis signaling in a rodent model for retinal degeneration**

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**Purpose:** Retinal degenerative diseases (RDDs) are characterized by the loss of photoreceptors in the retina leading to irreversible vision loss. Stem/progenitor cell transplantation provides a therapeutic promise for treating RDDs, though the modes of action of cell based therapies are largely unknown. Our previous studies have shown that a subretinal injection of human neural progenitor cells (hNPCs) into Royal College of Surgeons (RCS) rats with inherited retinal degeneration aided in visual function and photoreceptor preservation. The purpose of this study was to identify the gene expression changes in the RCS retinal tissue following treatment with hNPCs.

**Methods:** hNPCs were subretinally transplanted into RCS rats at an early stage of degeneration under immunosuppression. Visual function was examined by optokinetic response, and photoreceptor survival was determined by histology staining. Rat retinal tissue was collected for RNA-seq transcriptome analysis, and in silico analysis was performed. Gene expression was validated by qRT-PCR, and signaling analysis was examined by immunofluorescent staining.

**Results:** hNPCs subretinally injected into RCS rats at early stages of degeneration significantly preserved both photoreceptors and visual function, as compared with sham control animals. Bioinformatic analyses of RNA-seq data identified 1,263 differentially expressed genes between sham surgery RCS (RCSsham) and sham treated wild-type Long Evans (LEsham) rats, and 283 differentially expressed genes between RCSsham and hNPC-treated RCS (RCShNPCs) rats. Pathway analysis identified three affected pathways that are rescued in RCShNPCs retina. These pathways include integrin, phospholipase C, and Rho Family GTPase signaling, which all play roles in phagocytosis. Immunohistochemical analysis detected increased presence of macrophages and microglia in RCSsham, while RCShNPCs had similar amounts of macrophages and microglia as LEsham in areas with photoreceptor survival.

**Conclusions:** hNPCs offered preservation of vision after a single subretinal injection into a rodent model of retinal degeneration. Retinal tissue gene expression changes following injection of hNPCs correlate with modulation of phagocytosis signaling by macrophages and microglia. Understanding the host retinal tissue response to hNPC treatment may aid in future therapies for treating RDDs.

**Commercial Relationships:** Melissa K. Jones, None; Bin Lu, None; Mehrnoosh SaghiZadeh, None; Clive Svendsen, None; Shaomei Wang, None

**Program Number:** 5317 Poster Board Number: A0244

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

**Rescue effects of mobilized GFP-expressing bone marrow-derived stem cells in NaI/O3 induced retinal degeneration**

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**Purpose:** To investigate bone marrow-derived stem cell (BMSC) mobilization by NOX-A12, an SDF-1-inhibiting L-RNA aptamer (Spiegelmer), in combination with an increased SDF-1 gradient towards the sodium iodate (NaI) d-damaged retina. Furthermore, to quantify retinal homing and possible rescue effects of the endogenously mobilized BMSCs.

**Methods:** Experiments were performed in GFP chimera mice which had received 2x10⁶ bone marrow cells transgenic for GFP after lethal irradiation (10 Gy). Two months later retinal degeneration was induced by single i.v. injection of NaI/O3 (25 mg/kg). On day 3 BMSC mobilization was triggered by single i.v. injection of NOX-A12 (13.4 mg/kg in 5% glucose) and intraocular injection of SDF-1 (100 ng, 200 ng or 500 ng) 4 h after NOX-A12 injection. As controls i.v. injected glucose and intraocular injection of BSS were used. The number of GFP⁺ cells in the retina was quantified at day 10 by immunohistochemistry (IHC). After BMSC mobilization retinal thickness was measured in HE stained sections. Homed GFP⁺ cells were characterized and quantified in retina sections by IHC: BMSCs (Sca-1), microglia (Iba-1) and macrophages (F4/80). Differentiation of migrated BMSCs towards retinal lineage was assessed: RPE (RPE65; bestrophin), glia (GFAP), neuronal cells (βIII-tubulin). Visual function was measured at d7, 14, 21 and 28 by quantifying the optokinetic reflex.

**Results:** The highest number of GFP⁺ cells was found in mice treated with 500 ng SDF-1. Injection of SDF-1 increased the number of BMSCs in the retina. No further increase of SCs was seen with these abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to http://iovs.arvojournals.org/ to access the versions of record.
additional NOX-A12 treatment. Still, a trend towards less activated microglia was observed. The number of macrophages in the retina remained unaltered. No co-labeling of GFP+ BMSC with retin-specific markers was observed. No changes in retinal thickness were observed with NOX-A12, SDF-1 or combined treatment. However, a significant increase in visual acuity (P<0.05) was seen after NOX-A12+SDF-1 injection compared to NOX-A12+BSS. At 42 days after NaI0 injection, visual acuity in NOX-A12+SDF-1 treated animals had significantly improved compared to only SDF-1 treated animals.

**Conclusions:** Intraocular SDF-1 injection increased migration of mobilized BMSCs into the degenerated retina. BMSCs did not differentiate to retinal cells but showed a positive effect on retinal function. The rescue effect may be mediated by soluble factors.

**Commercial Relationships:** Carolyn Trepp, NOXXON Pharma AG, Berlin, Germany; Anna Kruschinski, NOXXON Pharma AG, Berlin, Germany; Axel Vater, NOXXON Pharma AG, Berlin, Germany; Volker Eznzmann, NOXXON Pharma AG, Berlin, Germany

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**Program Number:** 5319 **Poster Board Number:** A0246

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

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

**Transplantation of human induced pluripotent stem cell-derived retinal tissue in primate models of retinal degeneration with perimetric analysis**

**Hirotaka Onoe**, **Masayo Takahashi**

**Purpose:** Retinal transplantation therapy for retinitis pigmentosa is increasingly of interest. We investigated the competency of human induced pluripotent stem cell-derived retinal tissue (hiPSC-retina) following transplantation into primate model of retinal degeneration. We also tried perimetric studies with an eye of retinal degeneration model in purpose of evaluating functional restoration after retinal transplantation.

**Methods:** hiPSC-retina of differentiation day (DD) 63 was transplanted into the laser photocoagulated retina of a cynomolgus monkey, and the monkey was sacrificed for immunohistological analysis at graft age of DD282 following in vivo imaging. A rhesus monkey was trained to perform perimetric analysis, and visual field defect was investigated after focal laser photocoagulation.

**Results:** OCT imaging demonstrated the presence of graft retina-like sheets in regions where the host ONL was substantially degenerated. Photoreceptors in the graft were observed to form rosette-like structures. FA revealed no evidence of rejection. All the rosettes were positive for recoverin, rhodopsin, and cone opsins, indicating terminal photoreceptor maturation. No proliferating (Ki67-positive) cells were observed in rosettes. Photoreceptor cells, co-expressing human markers and recoverin, were found to be in contact with host bipolar dendrites. We next successfully detected focal visual field loss in the corresponding area after laser photocoagulation, which was never observed before injury. Transplantation of hiPSC-retina was safely done in the photocoagulated area.

**Conclusions:** We demonstrated that the graft could survive, mature, and possibly integrate with host bipolar cells in the eye of the monkey model. With successful detection of visual field defect in the photoreceptor degenerating retina and transplantation within the degeneration site, we intend to monitor light perception of the graft area.

**Commercial Relationships:** Hiroshi Shirai, None; Michiko Mandai, None; Masaharu Kinoshita, None; Hirotaka Onoe, None; Keizo Matsushita, None; Atsushi Kuwahara, None; Suguru Yamasaki, Hiroko Terasaki, Yoshiki Sasai, Masayo Takahashi, None; Retinal Regeneration, Riken center for developmental biology, Kobe, Japan; Ophthalmology, Nagoya University, Nagoya, Japan; Hiroaki University, Hiroaki, Japan; Functional Probe Research Laboratory, Riken center for developmental biology, Kobe, Japan; Regenerative and Cellular Medicine Office, Sumitomo Dainippon Pharma Co., Ltd., Kobe, Japan; Neurogenesis and Organogenesis Group, Riken center for developmental biology, Kobe, Japan.

**Support:** Research Center Network for Realization of Regenerative Medicine (AMED)
**Analysis of cytoplasmic transfer consistent with donor-host cell fusion following photoreceptor transplantation**

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**Purpose:** Photoreceptor transplantation could be a future treatment for patients with retinal degeneration, however controversy exists over whether transplanted donor cells integrate or fuse with the host retina. Cell fusion – the merging of two separate lipid bilayer plasma membranes – results in the transfer of cytoplasmic contents, including organelles and proteins between fused cells, and occurs in development, homeostasis, disease and regeneration. We tested the hypothesis that cytoplasmic transfer occurs between donor photoreceptor precursors and mature host photoreceptors using an experimental model of heterologous photoreceptor transplantation.

**Methods:** To study the occurrence of cytoplasmic transfer between donor and host photoreceptor cells, we transplanted fluorescently-activated cell sorted Nrl::GFP donor photoreceptor precursors into the subretinal space of adult host mice without retinal degeneration in which DsRed was expressed ubiquitously. We analyzed the co-localization of green fluorescent protein (GFP) and DsRed fluorescence 3 weeks following transplantation. We also computed the Manders overlap coefficient (MOC) to measure the co-distribution GFP and DsRed in perinuclear photoreceptor cytoplasm in the host outer nuclear layer (ONL).

**Results:** We detected extensive cytoplasmic colocalization of DsRed and GFP in cells located in the host ONL. We found that 93.8 ± 4.1% (mean ± SEM, N = 3 eyes) of morphologically normal GFP+ photoreceptor cells in the host retina colocalised DsRed. The median MOC value was 0.9.

**Conclusions:** The appearance of well-formed fluorescence-labeled donor photoreceptor cells in the host retina following transplantation is most commonly due to cytoplasmic transfer between donor and host retinal cells, instead of migration of donor cells into the host outer nuclear layer. These findings are consistent with donor-host cell fusion. The clinical consequences of photoreceptor fusion are currently unknown, however this mechanism may also be useful therapeutically.

**Commercial Relationships:** Mandeep S. Singh, None; Jasmin Balmer, None; Alun R. Barnard, None; Robert Maclaren, None

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**Human Retinal Engineering using 3D Two-Photon Polymerization and Chitosan Hydrogels**


**Purpose:** Recent advances in induced pluripotent stem cell (iPSC) technology have paved the way for the production of patient-specific photoreceptor precursor cells that are ideal for photoreceptor cell replacement therapy and treatment of retinal degenerative diseases. The use of polymer support scaffolds is critical for cellular survival and integration, but attempts to materialize this concept have ultimately been unsuccessful for two reasons: 1) mechanical mis-matching between material and tissue and 2) improper cell packing. We hypothesize that chitosan hydrogels, which have tunable compressive moduli, can act as suitable substrates for human iPSC-derived photoreceptor cells. We also hypothesize that two-photon polymerization can be used to create precise structures that facilitate dense packing and proper orientation of photoreceptor precursor cells.

**Methods:** Methacrylate-functionalized chitosan (MeCTS) was polymerized at various concentrations and UV light exposure times. The compressive modulus was measured using dynamic mechanical analysis in static mode. Complex prototype structures with varying pore sizes were created using two-photon polymerization of commercially available photoresists. Photoreceptor precursor cells were dissociated from 3D developing eye cups and plated on the hydrogel surface or prototype structure. Cellular survival, identity, morphology and directionality were characterized up to four weeks post-plating using rt-PCR, Western blotting, and immunocytochemistry.

**Results:** Increasing MeCTS concentration or light exposure time increased compressive modulus of MeCTS hydrogels. These materials supported the attachment and development of photoreceptor precursor cells in vitro. When seeded onto structured materials, photoreceptor precursor cells nested in the vertical pores of the structure, and aligned in a manner similar to naturally occurring photoreceptor cells.

**Conclusions:** These results lay the foundation for an autologous stem cell-based strategy for restoring vision to patients affected with retinal degenerative diseases. The developed constructs mimic the in vivo retinal environment in terms of stiffness and structure. Importantly, this knowledge will be fundamental to the development of effective cell-based grafts for sub-retinal transplantation.

**Commercial Relationships:** Kristan S. Worthington, None; Luke A. Wiley, None; Bailey B. Banach, None; Allison E. Songstad, None; Emily E. Kaalberg, None; Robert F. Mullins, None; Edwin M. Stone, None; Budd A. Tucker, None

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**Characterization of hiPSC-RPE on a prosthetic Bruch’s Membrane**

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**Purpose:** The Bruch’s membrane is a multilayered collagenous structure, positioned at the basal side of the retinal pigment epithelium (RPE) cells, immediately apical to the choroidal vasculature in the eye. Disruption of the integral intra-cellular communication and physiologic interaction between these cell layers results in the thickening of this membrane in both genetic and age related macular degenerative diseases. It’s positioning in the eye makes it an obligate component of any model investigating the pathophysiology of the RPE/choroid complex ex vivo. The goal of this study was to evaluate the utility of a Bombyx mori silk fibroin (BMSF) membrane to serve as a prosthetic Bruch’s membrane.

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Methods: Human inducible pluripotent stem cells (hiPSC)-derived RPE or human fetal RPE (hRPE), were cultured either directly on BMSF membrane or extracellular matrix (ECM)-coated BMSF membrane. After 60-90 days in culture, morphologic, gene/protein expression and functional characteristics of hiPSC-RPE and hRPE on BMSF membrane were compared to hiPSC-RPE and hRPE grown on ECM coated plastic/permeable support transwells that have previously been shown to display important characteristics of in vivo adult human RPE.

Results: Both hiPSC and hRPE grew on BMSF membranes in culture with varied efficiency dependent on the matrix coating of the membrane. Furthermore, hiPSC-RPE and hRPE grown on ECM-coated BMSF membrane supported RPE growth and pigmentation while BMSF alone displayed poor adhesion. Furthermore, hiPSC-RPE cultured on matrix-coated BMSF displayed numerous physical, gene/protein expression and functional characteristics akin of in vivo adult human RPE cultures.

Conclusions: The BMSF membrane is sufficient to support the long-term culture of RPE from both fetal and hiPSC origins and will provide a suitable Bruch’s membrane-like platform for in vitro disease modeling and in vivo cell-transplantation studies.

Commercial Relationships: Ruchira Singh, None; Audra Shadforth, None; Chad Galloway, None; Ali Hashim, None; Leslie MacDonald, Shuko Suzuki, None; Damien G. Harkin, None

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Program Number: 5323 Poster Board Number: A0250

Presentation Time: 3:45 PM–5:30 PM

The effects of scaffold rigidity on retinal pigment epithelial cells
Corina White, Ronke Olabisi. Biomedical Engineering, Rutgers, The State University of New Jersey, Piscataway, NJ.

Purpose: Although transplantation of retinal pigment epithelial (RPE) cells has shown promise for the treatment of retinal degenerative diseases, this therapeutic approach is not without challenges. Two major challenges that must be addressed are RPE cell migration and dedifferentiation and inflammatory response. In other areas of tissue engineering such as bone and neural engineering, it is well established that scaffold rigidity has significant effects on cells. The aim of this work is to understand how the rigidity of a scaffold, a relatively unexplored design aspect in retinal tissue engineering, affects RPE cells, particularly the pathways associated with the aforementioned challenges.

Methods: Scaffolds were fabricated with poly(ethylene glycol) diacrylate(PEGDA) using UV photopolymerization. The scaffolds were designed to have different elastic moduli, higher modulus correlates to higher rigidity, through the use of different molecular weight PEGDA. ARPE-19 cells were cultured on these scaffolds. Cells cultured on scaffolds and TCPS were analyzed using fluorescent microscopy, metabolic activity assay, and real time PCR.

Results: Fluorescent images revealed that cells demonstrated qualitatively different adhesion patterns on scaffolds of varying moduli. In addition, when normalized to day 1 metabolic activity, cells on high modulus scaffolds increased metabolic activity to a significantly higher level compared to other groups on day 14 (p<0.05). The gene expression of four genes associated with inflammation and RPE dedifferentiation were analyzed. On day 7, the fold change expression of IL-6 and IL-8 were significantly different on the two scaffolds (p<0.05).

Conclusions: Scaffold rigidity is an important design parameter in several areas of tissue engineering but remains relatively unexplored in retinal tissue engineering. This study demonstrates that scaffold rigidity affects cell adhesion, activity, and expression. Though more exploration is needed, this begins to lay a foundation for optimizing scaffold rigidity to promote long term success of RPE scaffold implants.

ARPE-19 cells cultured on TCPS (A), high (B), and low (C) modulus scaffolds stained for nuclei (blue) and dead (red) cells.

(A) Cellular metabolic activity of ARPE-19 cells on Days 7 & 14 on different substrates. (B) Gene expression of cells cultured on scaffolds of varying rigidity represented as fold change relative to TCPS. *p<0.05, Student’s T, #p<0.05, ANOVA

Commercial Relationships: Corina White, None; Ronke Olabisi, None

Program Number: 5324 Poster Board Number: A0251

Presentation Time: 3:45 PM–5:30 PM

A biodegradable ultra-thin membrane for retinal tissue engineering: preliminary study using ARPE-19 cells
Augustinus Laude¹, Edgar Y. Tan², Shuai Wang², Wai Yee Yeong².
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Purpose: In the development of a retina tissue construct to act as a tissue scaffold for cells for implantation into the eye, we describe the fabrication of an ultra-thin membrane. We postulate that a thinner membrane will provide improved degradation rate with reduced degradation by-product in the retina post implantation. Also, added porosity would allow the membrane to more mimic Bruch’s membrane in facilitating transport across.

Methods: The membrane was prepared by casting a biodegradable mixture of chloroform, polyethylene glycol (PEG) and polycaprolactone (PCL) onto a petri dish containing PEG solution (see Figure 1). This method of fabricating using a combination of phase separation and particulate leaching gave us the ability to develop nano-sized pores on the membrane, casting on PEG solution enables us to develop an ultra-thin product due to the spreading on the surface of the solution. Thickness of the product was measured using an optical profiler and pore size and distribution was determined using Scanning Electron Microscope (SEM). ARPE-19 cells (American Type Culture Collection, n=3) were used and
incubated for 7 days. Live/dead staining was used to determine retina pigment epithelium (RPE) cells proliferation.

**Results:** Using an optical image profiler, the thickness of the membrane was shown to be approximately 3μm, similar to Bruch’s membrane. A SEM was also done to determine that the pore size is approximately 0.377μm (±0.031). The contact angle of the membrane was reduced by 5° compared to pure hot compressed PCL film, showing that the membrane is slightly hydrophilic which may induce better cell proliferation. This is likely due to the effect of PEG on the surface of the membrane. ARPE-19 cells were determined to be almost completely viable on the surface of the membrane after the use of live/dead staining.

**Conclusions:** Using a simple Langmuir-Blodgett technique combined with particulate leaching, an ultra-thin biodegradable, porous membrane with an average pore size of 300nm can be fabricated. An ARPE-19 cell line has also been shown to survive on the surface of the membrane with minimal cell death and limited cytotoxic activity being observed. Further studies are needed to determine the effect of the cell-scaffold interaction and to determine the degradation rate of the scaffold.

![Diagram of a bioprinting process](image)

**Commercial Relationships:** Augustinus Laude; Edgar Y. Tan, None; Shuai Wang, None; Wai Yee Yeong, None

**Support:** NHG ARG Grant 14009.

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**Program Number:** 5325 Poster Board Number: A0252

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

**Tissue Engineered Human Blood-Retinal Barrier-on-a-Chip**

**Jun Jeon**, **Nathan Hotaling**, **Marjon Zamani**, **Roba Dejene**, **Donald Ingber**

**Biosystems & Biomaterials Division, National Institute of Standards & Technology, Gaithersburg, MD; Wys Institute for Biologically Inspired Engineering, Harvard University, Boston, MA; Unit on Ocular Stem Cell and Translational Research, National Eye Institute, National Institutes of Health, Bethesda, MD; Section on Epithelial & Retinal Physiology & Disease, National Eye Institute, National Institutes of Health, Bethesda, MD.

**Purpose:** Recent advancements in the field of microfluidics have provided the means to observe tissue and organ-level behaviors in a controlled biomimetic microenvironment. In this study, we recapitulated the human blood-retinal barrier (BRB) in a microfluidic platform using induced pluripotent stem cell (iPSC)-derived retinal pigment epithelium (RPE) and endothelial cells (ECs) and compared the functionality of our model to that of a standard Transwell coculture system.

**Methods:** The poly(dimethylsiloxane) (PDMS) based microfluidic chip used in the experiment was composed of two vertically-stacked microchannels separated by a porous polyethylene terephthalate membrane. Developmentally guided differentiation protocols were used to generate RPE and ECs from iPSCs. The following assays were performed to characterize the functionality of the co-culture system in the chip: permeability to FITC-dextran, vascular endothelial growth factor (VEGF) and pigment epithelium-derived factor (PEDF) secretion, RPE pigmentation, and fluid transport across top and bottom channels.

**Results:** Donor-matched sets of iPSC-derived RPE cells and ECs were successfully generated and co-cultured in our microfluidic device. The top channel was seeded with RPE cells, and the bottom channel was seeded with ECs. The cells were maintained using tissue-specific culture medium continuously perfused by a peristaltic pump, and different flow rates were given to each cell type to more closely mimic the microenvironment of the BRB. The RPE and EC monolayers demonstrated barrier resistance, secreted cytokines in a polarized fashion, and transported water from top channel towards the bottom channel.

**Conclusions:** Using microfluidic channels to co-culture RPE cells and ECs, we showed that our chip platform is able to model the human BRB as well as a standard Transwell culturing system. In addition, the unique functions offered by the chip, specifically its ability to quantify in-situ the rate of fluid transport across the BRB model, helps pave the way for developing a model of different ocular diseases on the chip, which can serve as an efficient drug discovery platform in the future.

**Commercial Relationships:** Jun Jeon, None; Nathan Hotaling, None; Marjon Zamani, None; Roba Dejene, None; Donald Ingber, Kapil Bharti, None

**Support:** NHG ARG Grant 14009.

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**Program Number:** 5326 Poster Board Number: A0253

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

**Bioprinting of blood-retinal barrier using human iPSC derived cells for modeling human choroidal neovascularization**

**Min Jae Song, Russ Quinn, Roba Dejene, Kapil Bharti**

**National Eye Institute, National Institute of Health, Bethesda, MD.

**Purpose:** Advanced stage of age-related macular degeneration (AMD) has two main sub-types, “wet” and “dry” forms. In wet AMD choriocapillaris proliferate and breach the blood-retinal barrier (BRB) formed by RPE tight junctions, and leak fluid in the eye leading to blindness. In dry form of AMD, BRB is compromised because of RPE cell death leading to a direct exposure of the back of the eye to the immune system. Currently, there is no human model to study BRB pathology in AMD. We are combining bioprinting with induced pluripotent stem (iPS) cell technology to develop a 3D in vitro disease model of human BRB.

**Methods:** We optimized “bioink” (cell concentration and hydrogel) formulation for several cell types required for the choroid. A collagen-derived gel is used for encapsulation of endothelial cells, choroidal fibroblasts, and ocular pericytes for bioprinting with additional hydrogels to provide microenvironment conducive for microvascular network formation. We bioprinted a 3D engineered vascularized tissue with a defined geometry on degrading and non-degrading scaffolds.

**Results:** We have successfully generated 3D microvascular network using choroid fibroblasts, retinal pericytes, and iPSC-derived/primary retinal endothelial cells (Fig. 1). Diameter of printed vessels varies from 10 to 30 μm. Defined geometry of printed vascular network allows quantification of angiogenesis, which represents “wet” AMD like disease processes. We observed that angiogenesis was significantly promoted by VEGF treatment, a key proangiogenic factor in pathological conditions.

**Conclusions:** We successfully developed a choroid-like model and demonstrated angiogenic capability of this bioprinted choroid. However, stability of printed microvasculature needs to be enhanced.

**Commercial Relationships:**
to maintain vascular structure for a longer period (>6 weeks). RPE monolayer will be generated on the top of the printed choroid. Thus, this model will allow us to discover RPE and endothelial interaction and underlying mechanisms in BRB pathology in diseases such as “dry” and “wet” forms of AMD.

Confocal image of cross section of bioprinted choroid. Green, red, and blue indicate endothelial cells, pericytes, and nuclei, respectively.

**Commercial Relationships:** Min Jae Song; Russ Quinn, None; Roba Dejene, None; Kapil Bharti, None

**Support:** NIH intramural fund

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**Program Number:** 5327 **Poster Board Number:** A0254

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

**Co-Culture Stimulated Differentiation of Mesenchymal Stem Cells to Trabecular Meshwork Cells**

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**Purpose:** Due to reduced trabecular meshwork (TM) cellularity in glaucoma, stem cells offer potential therapeutic benefits if properly differentiated and engrafted into TM. Towards this goal, Zhou et al. (ARVO, 2015) studied differentiation of adult adipose-derived mesenchymal stem cells (adMSCs) to a TM lineage by co-culture. Here we further characterize adMSC to TM differentiation by co-culture using genetic, protein-level and functional assays.

**Methods:** Human adMSCs (Lonza; n=2 lines) and primary human TM cells (Stamer lab; n = 3 lines) were examined. We used 3 co-culture approaches with decreasing degrees of adMSC-TM cell interaction: direct co-culture (DCC) after tagging cells to allow later sorting, Transwell co-culture (TWCC), and media swap co-culture (MSCC). Pure TM and adMSC cultures were positive and negative controls. After 1, 2 or 3 wks of co-culture, cells were characterized 3 ways: (i) qRT-PCR; (ii) myocilin induction after 100 nM Dexamethasone (DEX) for 1 wk, assessed by labelling/flow cytometry; and (iii) cell contraction, assayed by area change of collagen I gels 24 hr after cell loading.

**Results:** adMSCs in DCC showed increased/decreased levels of characteristic TM/MSC message by qRT-PCR (Fig 1A). Similarly, DEX-treated adMSCs from DCC showed increased myocilin expression vs. control MSCs, although myocilin levels were less than in primary TM cultures (positive controls, Fig 1B). By qRT-PCR, adMSCs from TWCC and MSCC showed RNA differences vs. control MSCs, with MSCC exhibiting more TM-like characteristics than TWCC (Fig 2A). However, adMSCs from TWCC were less contractile (similar to TM cells) while adMSCs from MSCC were similar to MSCs (Fig 2B).

**Conclusions:** Three co-culture paradigms show that adMSCs can differentiate towards a TM-like phenotype, in agreement with Zhou et al, with differentiation extent depending on assay technique. Further optimization of co-culture conditions is needed to increase differentiation towards a full TM phenotype.
Responses of L5 pyramidal neurons of V1 to electrical stimulation

Shelley I. Fried1, Seung Woo Lee2. 1Neurosurgery, Massachusetts General Hospital, Boston, MA; 2Research, Boston VA Healthcare System, Boston, MA.

Purpose: Direct electrical stimulation of primary visual cortex (V1) has been proposed as a means to restore vision to the blind. Despite some success in eliciting visual perception in human subjects as well...
as in non-human primates, a well-reasoned stimulation strategy has not been developed. For example, it is not known whether certain layers of V1 are more sensitive to electric stimulation than others nor are the responses of specific neuronal types to stimulation well understood. Here we measured the responses of L5 pyramidal neurons in vitro to stimulation from electrodes positioned at different cortical depths.

**Methods:** Cell-attached patch clamp was used to record spikes from L5 pyramidal neurons of V1 in an in vitro mouse brain slice preparation. Single conical-shaped platinum/iridium stimulating electrodes with impedance of 1 MΩ were positioned such that the electrode tip was initially centered directly above the soma of a targeted cell. Stimulus waveforms were cathodic-first biphasic current pulses with a pulse duration of 200 μs and no interphase intervals. Stimulation was delivered at a frequency of 200 Hz for 0.1 s (20 total pulses). The amplitude of stimulation ranged from 0 to 30 μA. Measurements were repeated as the electrode tip was translated in 100 μm steps along the apical dendrite of targeted neurons as well as along the axon.

**Results:** The strongest responses (highest spike counts) were elicited when the electrode tip was positioned close to the soma (layer 5 – 6); latencies for these spikes were 0.2 - 0.4 ms. Average thresholds were 14.09 μA (STDEV: 4.83 μA). Fewer spikes were elicited when the electrode was positioned over the distal apical dendrites (layer 1 – 4), even at amplitude levels of 30 μA (the maximum that could be delivered in our system). Interestingly, spikes arising from stimulation over the apical dendrite were bigger and sharper and had longer latencies (1-2 ms) than those that arose from stimulation over the axon (layers 5 – 6).

**Conclusions:** Our results suggest that L5 pyramidal neurons of V1 are maximally sensitive to stimulation over deeper cortical layers (5 – 6). Moreover, the results also suggest that electrode position can alter the spike shape. Therefore, electrode depth is not only important for threshold optimization, but also, the depth of penetration may also contribute to distinct visual percepts.

**Commercial Relationships:** Shelley I. Fried, None; Seung Woo Lee, None

**Support:** NIH/NEI: 1R01 EY023651; VA/RR&D: 1I01RX000350-01A1

**Program Number:** 5329 **Poster Board Number:** A0256

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

**Pulse trains to percepts: Developing quantitative models of the percepts produced by sight recovery technologies**

**Ione Fine**, Geoffrey M. Boynton1, Arup Roy2, Robert J. Greenberg2.

1Psychology, University of Washington, Seattle, WA; 2Second Sight Medical Products, Inc., Sylmar, CA.

**Purpose:** An extraordinary variety of sight recovery therapies are either about to begin clinical trials, have begun clinical trials, or are currently being implanted in patients. For sight recovery technologies which use artificial stimulation of the retina to elicit percepts (optogenetics, small molecule photoswitches, and electrical prostheses) the interplay between the stimulating technology and the underlying neurophysiology is thought to result in significant distortions of the perceptual experience. However, we do not have quantitative models that can predict the perceptual experience produced by these implants. Here we describe a quantitative model that can predict patient percepts for an epiretinal implant, based on a series of psychophysical studies examining the brightness, size and shape of patient percepts as a function of the pattern of electrical stimulation.

**Methods:** Our spatio-temporal model of the effects of electrical stimulation begins with transforming the pulse train on each electrode into a spatiotemporal representation of current on the retina, based on the height of the array from the retinal surface and a model of the spread of current from a metal disc in a semi-infinite medium. Retinal current is then passed through a perceptual sensitivity model that predicts neuronal activity over time and space based on a model that (1) describes sensitivity as a cascade of nonlinear transformations of the spatiotemporal pattern of stimulation, (2) assumes simultaneous stimulation of both ON- and OFF-pathways, and (3) includes axonal stimulation that falls off as a function of distance from the ganglion cell body.

**Results:** This model can successfully predict the percepts produced by epiretinal stimulation over both time and space in current human patients.

**Conclusions:** This model is also capable of generating predictions about the likely perceptual quality of future implants. These predictions can (1) provide the basis for better stimulation protocols, (2) provide insights into what aspects of the implant are likely to be limiting perceptual performance, and (3) provide patients and reimbursement agencies with clearer insights into what sort of perceptual improvements these implants are likely to provide in the real world.

A simulation of the Seattle space needle as viewed with an epiretinal prosthetic device.

**Commercial Relationships:** Ione Fine, Second Sight Medical Products, Inc. (P), Second Sight Medical Products, Inc (C); Geoffrey M. Boynton, None; Arup Roy, Second Sight Medical Products, Inc., Second Sight Medical Products, Inc. (I), Second Sight Medical Products, Inc. (P); Robert J. Greenberg, Second Sight Medical Products, Inc. (P), Second Sight Medical Products, Inc (I), Second Sight Medical Products, Inc. (I), Pixium Vision (I), Second
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