ARVO 2016 Annual Meeting Abstracts

336 Corneal Wound Repair and Regenerative Medicine
Tuesday, May 03, 2016 11:00 AM–12:45 PM
Tahoma 3, TCC  Paper Session
Program #/Board # Range: 3475–3481
Organizing Section: Cornea

Program Number: 3475
Presentation Time: 11:00 AM–11:15 AM
Adipose-derived Mesenchymal Stem Cells Therapy for Cornea Regeneration: A Multi-photon Intravital Study in a Mouse Model
Ladan Espandar¹, Jonathan Mandell¹, Tomas Blanco¹. ¹University of Pittsburgh, Pittsburgh, PA; ²Duke University, Durham, NC.
Purpose: Adipose-derived stem cells (ADSCs) have gained increasing attention as a source of regenerative therapy because of their abundance, easy accessibility and capacity for self-renewal and differentiation; however, their fate, dynamics and differentiation of engrafted ADSCs are not well understood. Insight into in vivo stem cell biology during corneal repair could help to optimize therapeutic strategies for corneal diseases. Multiphoton intravital microscopy (MP-IVM) allows for noninvasive, real-time, and serial quantitative imaging in living animals. We explored the location of engrafted GFP-labeled ADSCs in mouse cornea after subconjunctival and intraperitoneal (IP) injection of cells in a total corneal epithelium debridement model by MP-IVM.
Methods: Subcutaneous white adipose tissue was collected from enhanced green fluorescence protein (eGFP) transgenic mice (C57Bl/6-Tg(UBC-GFP)) and ADSCs were isolated and expanded. Eight C57Bl/6 male mice were used in the study (4 in each group). After application of ethanol (100%), the epithelium was mechanically scraped from the whole corneal surface in the right eye of each mouse. Then, in group A, GFP-mADSCs (1x10⁶ in 50 μL) were injected subconjunctivally in both eyes (N=4). In group B, after debridement in right eye, 1x10⁶ cells/μL were injected IP. (N=4) MP-IVM follow up and slit-lamp examination were performed for a period of 4 weeks.
Results: Subconjunctivally-injected GFP-mADSCs were found in the epithelium and anterior stroma of the injured eye by MP-IVM. No cells were found in uninjured eyes. Conversely GFP-mADSCs were found in epithelium, anterior and posterior stroma of injured cornea after IP injection. No cells were found in the contralateral uninjured eye. After 4 weeks, mice were euthanized and the results were confirmed by histology.
Conclusions: ADSCs injected either subconjunctivally or IP engrafted within the cornea in response to an injury and aid to restore the damage tissue. This study opens a new promising field for the management of the ocular surface diseases by using stem cell therapy. Further studies with longer follow-ups are needed to dissect the exact functional and molecular characteristics of mADSCs in corneal wound healing process that will help to optimize possible therapeutic application of mesenchymal stem cells in corneal diseases.

Commercial Relationships: Ladan Espandar, None; Jonathan Mandell, None; Tomas Blanco, None
Support: NIH P30-EY08098

Program Number: 3476
Presentation Time: 11:15 AM–11:30 AM
Mesenchymal stem cells restore corneal transparency via secretion of hepatocyte growth factor
Sharad Mittal, Afsaneh Amouzegar, Masahiro Omoto, Sunil Chauhan. Schepens Eye Research Institute, Harvard Medical School, Boston, MA.

Purpose: Mesenchymal stem cells (MSCs) have been shown to promote repair in corneal injury and inflammation. The purpose of this study was to investigate the underlying mechanisms by which MSCs inhibit stromal fibrosis and restore transparency in corneal injury.
Methods: Mesenchymal stem cells were generated from bone marrow of wild-type C57BL/6 mice, and characterized by flow cytometry for the expression of CD29+SCA1+CD45–CD34–. Corneal injury was induced by mechanical removal of the corneal epithelium and anterior stroma. Real-time PCR was performed on injured corneas as well as on resting and IL1β-stimulated MSCs to quantify anti-inflammatory and growth factors, including interleukin-10, TNFα-stimulated gene/protein 6 (TSG6), transforming growth factor beta (TGFβ) and hepatocyte growth factor (HGF). HGF in MSCs was knocked down using HGF-specific siRNA. Control or HGF-silenced MSCs were intravenously injected into mice 1h after corneal injury. Corneal epithelial injury and transparency were evaluated using corneal fluorescein staining and biomicroscopy. Immunohistochemistry was performed to examine the effect of HGF on TGFβ-induced α-smooth muscle actin (αSMA; a marker of fibrosis) expression in a corneal fibroblast cell line (MK/T1).
Results: Real-time PCR analysis showed that IL1β-stimulated MSCs expressed significantly higher levels of HGF compared to unstimulated resting MSCs (p=0.0035). Following injury, corneas of MSC-treated mice showed a 2-fold increase in the expression of HGF compared to MSC-untreated injured cornea (p=0.01). siRNA-mediated silencing of HGF expression in MSCs substantially abrogated their wound repair function. Quantification of corneal opacity scores demonstrated that HGF-silencing of MSCs led to a significant 2-fold reduction in their capacity to prevent the development of stromal fibrosis compared to wild type MSCs (p=0.016). Immunohistochemistry data demonstrated that HGF completely suppressed TGFβ-induced α-SMA expression in corneal fibroblasts (p=0.0001)
Conclusions: These findings indicate that mesenchymal stem cells exert their anti-fibrotic function primarily via secretion of hepatocyte growth factor.

Commercial Relationships: Sharad Mittal, Afsaneh Amouzegar, None; Masahiro Omoto, None; Sunil Chauhan, Schepens Eye Research Institute (P)
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Program Number: 3477
Presentation Time: 11:30 AM–11:45 AM
Extracellular vesicles derived from human mesenchymal stem cells promote corneal wound repair by increasing epithelial cell proliferation and reducing neovascularisation in a rat corneal alkali burn model
Thomas Ritter¹, Cerine Laf², Oliver Tracey³, Grace A. O’Malley⁴, Serika Naicker¹, Hossein Elbadawy¹, Aideen E. Ryan¹, Gerry Fahy¹, Martin J. Leahy², Cerine Lal³, Oliver Treacy¹, Grace A. O’Malley³, Tomás Blanco³, Martin J. Leahy², Jonathan Mandell¹, Sunil Chauhan¹. Schepens Eye Research Institute, Harvard Medical School, Boston, MA.

Purpose: To investigate if topicaly applied mesenchymal stem cell (MSC)-derived extracellular vesicles (EVs) promote corneal wound repair in a rat alkali burn model
Methods: EVs were isolated from media conditioned by human bone marrow-derived MSCs (n=4) using a series of centrifugation, filtration and ultracentrifugation steps followed by transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA;
NanoSight) quality control. Pro-healing effects of hMSC-derived EVs (hMSC-EV) were examined in vitro by cell migration and scratch wound healing assays using human corneal epithelial (HCE) and endothelial (HCEC) cell lines. Human cornea wound healing rate was also determined ex vivo using fluorescein penetration test. Rat corneal alkali burn injury was induced using 1M NaOH and injury was treated with or without 30mg of hMSC-EVs. Efficacy of treatment was monitored by biomicroscopy and optical coherence tomography (OCT) followed by extensive tissue analysis at the end of the observation period.

**Results:** Successful isolation of EVs was confirmed by immune-gold TEM showing expression of CD63 and TSG101 on their surface. NTA showed the average size of EVs ranged between 50-130nm. Significant increases in HCE and HCEC cell migration in the presence of MSC-EVs were observed compared to PBS control and EV-depleted MSC conditioned media. Moreover, a significant increase in scratch wound closure of cells treated with hMSC-EVs was observed. In vivo, analysis of images taken at day 0, 1, 3 and 7 of fluorescein stained corneal injury showed significantly enhanced healing of the corneal surface at day 1 and 3 when treated with hMSC-EVs compared to PBS-control. OCT analysis revealed that hMSC-EVs reduced inflammation and maintained the physiological structure of the eye. Importantly, a reduction in corneal damage and inflammatory infiltrating cells and an increase in proliferating epithelial cells (Ki67-positive) was observed following hMSC-EV treatment. Finally, human corneas treated with hMSC-EVs healed faster than corresponding PBS-control by 12.1%±29 and 6.1%±3.3 at 24 and 48 hours respectively.

**Conclusions:** Our results show that topical hMSC-EV application is a novel promising cell-free therapy with high potential for translation in ocular surface disease treatment.

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**Program Number:** 3478

**Presentation Time:** 11:45 AM–12:00 PM

**The Effect of Bone Marrow Mesenchymal Stem Cell Secretome on Corneal Epithelial Wound Healing**

Medi Eslani1, Asadolah Movahedan1, Ilham Putra1, Judy Hamouie1, Asadolah Movahedan1, Ilham Putra1, Judy Hamouie1, Asha Tadepalli1, Xiang Shen2, Neda Azharkhamsheh3, Elham Ghahari4, Peiman Hematti5, Ali R. Djalllian6, None

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**Program Number:** 3479

**Presentation Time:** 12:00 PM–12:15 PM

**Somatic Gene Therapy of Mucopolysaccharidosis with CRISPR/Cas9 Genome Editing**

Winston W. Kao1, Tarsis Ferreira1, Fei Dong1, Yueh-Chiang Hu2, Cas9 Genome Editing

Grace A. O’Malley1, None; Martin J. Leahy1, None; Mindy K. Call1, Vivien J. Coulson-Thomas1, Jianhua Zhang1, Taylor Rice2, Ophthalmo, University of Cincinnati, Cincinnati, OH; Developmental biology, Cincinnati Children’s Hospital medical Center, Cincinnati, OH.

**Purpose:** When molecular cloning was first introduced in the 1970’s, it created an extraordinary expectation of gene therapy for curing congenital and acquired diseases. However, the use of gene therapy for treating diseases in human and animal models remains daunting. The newly developed genome editing technique CRISPR (clustered regularly interspaced short palindromic repeats) offers a novel possibility of gene therapy in curing diseases caused by congenital genetic mutation. In present studies, attempts were made to examine the possibility of using CRISPR to correct genetic mutation in postnatal Gusb mice (an autosomal point mutation of β-glucuronidase, β that manifests symptoms of mucopolysaccharidosis type VII, also known as Sly syndrome).

**Methods:** sgRNA specifically targeting the Gusb mutant allele and the correcting donor DNA were designed and tested in Gusb fibroblasts derived from the homozygous mutant mice. The validated sgRNA and donor DNA, and SpCas9 endonuclease were packaged into AAV-DJ virus. Both AAV-DJ-SpCas9 and AAV-DJ-sgRNA-Donor were co-injected directly into cornea stroma and/or systemically into the body via tail vein infusion. The experimental mice were weighed and examined weekly by HRTII in vivo confocal microscopy.

**Results:** To test the effectiveness of CRISPR/Cas9 to correct the genetic mutation, Gusb fibroblasts were transfected with: 1) a pX458 plasmid (Addgene) containing the U6-promoter driven sgRNA

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Dimitrios Karamichos1, Pouriska Kivanany2, Tina B. McKay1, Matthew Petroll2, None; Fei Dong, None; Yueh-Chiang Hu, None; Mindy K. Call, None; Vivien J. Coulson-Thomas, None; Jianhua Zhang, None; Taylor Rice, None
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Quercetin inhibits corneal scar formation in vivo

Dimostarios Karamichos1, Pouriska Kivanany2, Tina B. McKay1, Matthew Petroll2, 1Ophthalmology, Dean McGee Eye Institute/OUHSC, Oklahoma city, OK; 2Veterinary Medicine & Surgery, University of Missouri, Columbia, MO; 3Veterinary Medicine & Surgery, Biomedical Research Foundation, Missouri, Columbia, MO; 4Veterinary Medicine & Surgery, University of Missouri Ruth M. Kraeuchi Ophthalmology Endowment Fund, partially from NIH/NEI R01EY17294 and University of Missouri Veterans Affairs 1I01BX00035701

Purpose: Quercetin was previously reported identification of a novel flavonoid, Quercetin, as a significant inhibitor of corneal fibrosis in vitro. Our current study tests the efficacy of Quercetin on corneal scarring in both mice and rabbit models.

Methods: Mice: Wild-type C57BL/6j mice aged 8-12 weeks were anesthetized with ketamine/xylazine injected intraocularly, and stromal scars were generated by epithelial and stromal debridement of 2x2mm using an Alger brush. Topical application of Quercetin at concentrations of 50, 5, 1, and 0.5mM or vehicle was administered following injury. Corneal scarring was assessed on day 21 using western blot and H&E. Rabbits: Six New Zealand White rabbits underwent lamellar keratotomy (LK) surgery, followed by treatment with either Quercetin (5 mM, 3 rabbits) or vehicle (3 rabbits) twice daily for 3 days (1 drop per dose). Stromal backscattering (haze) was assessed from 7 to 21 days using in vivo confocal microscopy.

Results: Mice: A single dose of 5mM Quercetin significantly reduced scar formation compared to vehicle treated controls. Slight scar formation was observed at lower Quercetin levels (0.5mM and 1mM) but still significantly less when compared to controls. Quercetin treatment significantly reduced expression of fibrotic markers, α-smooth muscle actin and Collagen III. Rabbits: At 21 days after LK, stromal backscattering in control and Quercetin treated rabbits was 13,577 + 935 and 8501 + 3415 haze units, respectively (P value for two-tailed t-test = 0.068, Power = 0.472). No adverse effects of topical application of Quercetin were observed on mice or rabbits.

Conclusions: Topical application of a single low dose of Quercetin ameliorates stromal scar development in vivo. These results suggest that Quercetin may be a potent therapeutic for inhibiting fibrosis in the cornea. Further preclinical studies are needed to optimize drug dose and verify safety profile.

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Program Number: 3480
Presentation Time: 12:15 PM–12:30 PM

ROCK Inhibitor HA1077: Potently Inhibits Corneal Fibrosis and Neovascularization

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1Harry S. Truman Veterans Hospital, Columbia, MO; 2Veterinary Medicine & Surgery, Biomedical Sciences, Veterinary Pathology, and Mason Eye Institute, University of Missouri, Columbia, MO; 3Veterinary Medicine & Surgery, University of Missouri, Columbia, MO; 4Mason Eye Institute, University of Missouri, Columbia, MO; 5Veterinary Medicine & Surgery and Veterinary Pathology, University of Missouri, Columbia, MO.

Purpose: Rho associated Kinase (ROCK) pathways regulate cellular proliferation, migration, adhesion, wound healing, fibrosis, and angiogenesis in vivo. We tested the postulate that topical ROCK inhibitor HA1077 application to the eye will attenuate corneal fibrosis and neovascularization in vivo in a rabbit disease model by preventing exuberant wound healing.

Methods: Twelve New Zealand White rabbits were used under IACUC protocol. Corneal fibrosis and neovascularization (CNV) were produced by a single topical alkalai (1N NaOH) application for 1min to the central cornea. Following corneal wounding, animals were divided into two groups: Group-1 served as controls and received 50µL Balanced Salt Solution (BSS) topically twice daily for 3 days. Group-2 served as treatment cohort and received 50µL HA1077 (3mM) topically twice daily for 3 days. Contralateral untreated eyes served as negative controls. Serial slit- and stereomicroscopy evaluated for the presence and degree of ocular inflammation, corneal edema, and corneal opacity. Intraocular pressures were recorded with an applanation tonometer. Corneas were harvested on day-14 with H&E and immunofluorescence staining employed to characterize levels of fibrosis, CNV, inflammation, and apoptosis.

Results: Biomicroscopy detected a significant decrease (~2.8 fold; p<0.01) in corneal fibrosis and CNV in eyes treated with HA1077 compared to BSS-treateed controls. Further, HA1077-treated corneas showed significant decreases in fibrosis markers (smooth muscle actin, fibronectin, and F-actin; 55-60%; P<0.0001), and did not exhibit significantly increased numbers of CD11b+ (2-11%) or TUNEL+ (0-5%) cells compared to BSS-treated controls during immunofluorescence analyses. A substantial decrease in CNV was also noted in HA1077-treated eyes as compared to BSS-treated controls (quantification pending). Clinical eye examinations and histological evaluation did not reveal evidence of acute toxicity from HA1077 topical application.

Conclusions: ROCK inhibitor HA1077 is a viable option for treating corneal fibrosis and neovascularization resulting from chemical injuries. Additional in vivo analysis is warranted.

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