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INTERNATIONAL OCULAR SURFACE SOCIETY

11TH ANNUAL MEETING

APRIL 26, 2008

9:00 am ~ 7:00 pm

OCULAR STEM CELL

Room ~ Atlantic 1-2
Fort Lauderdale Grande Hotel & Yacht Club
Fort Lauderdale, Florida, USA

WELCOME REMARKS

9:00 **Kazuo Tsubota, MD**

PAPER PRESENTATIONS SESSION I

Basic Science Presentation Guideline

8 min Presentation
4 min Discussion

Moderators: Winston W-Y Kao, PhD and Shigeto Shimmura, MD

9:05 **Use of mesenchymal stem cells in treating corneal diseases: restoration of corneal transparency in Lum$^{-/-}$ mice**
Winston W.-Y. Kao, PhD

9:19 **Side population stem cell molecular signature reveals role of dual specificity phosphatases and G1 to S cell cycle control proteins in stem cell slow cycling**
J. Mario Wolosin, PhD

9:33 **Gene transfer to primary corneal epithelial cells in vitro**
Mark Rosenblatt, MD, PhD

9:47 **Long-term culture of murine corneal epithelial progenitor cells from a single globe**
Hideyuki Miyashita

10:01 **The role of N-cadherin in the limbal stem cell niche**
Shigeto Shimmura, MD

10:15 **Neurotrophic factors and receptors in human limbal stem cell niche**
De-Quan Li, MD

10:29 **Role of FGF-7 in ocular surface squamous neoplasia**
Chia-Yang Liu, PhD

10:45 **Refreshment Break (15 minutes)**

AWARD LECTURE

11:00 **Inseparable relationships between stem cells and the niche: lessons from *Drosophila***
Ting Xie, PhD
Associate Investigator, Stowers Institute for Medical Research, Kansas City, Missouri

12:00 **Lunch Break at the poolside terrace**

PAPER PRESENTATIONS SESSION II

Clinical Science Presentation Guideline

7 min Presentation
3 min Discussion

Moderator: John Sutphin, MD

13:30 **Comparison of the corneal epithelial wound healing process among flap-on/ flap-off LASIK and epithelium on/epithelium off penetrating keratoplasty**
Wei-Li Chen, MD, PhD

13:42 **Amniotic membrane for the treatment of acute Stevens-Johnson syndrome**
Darren G. Gregory, MD

13:54 **Temporary sutureless amniotic membrane patch for acute alkaline burns**
Ahmad Kheirkhah, MD

14:07 **Sutureless amniotic membrane transplantation for partial limbal stem cell deficiency**
Ahmad Kheirkhah, MD

14:18 **Minimal conjunctival limbal autograft for total limbal stem cell deficiency**
Ahmad Kheirkhah, MD

14:30 **Outcome of ipsilateral ex-vivo cultivated limbal epithelial transplantation transplantation (CLET) for partial limbal stem cell deficiency**
Virender S. Sangwan, MS

14:42 **Objective outcome measures in ocular surface reconstruction using cultured limbal epithelial transplantation**
Alex J Shortt, MD MRCOphth

14:54 **Penetrating keratoplasty after ex-vivo cultured oral mucosal epithelial transplantation in total limbal stem cell deficiency**
Abraham Solomon, MD

15:00 **Refreshment Break (15 minutes)**

PAPER PRESENTATIONS SESSION III

Clinical Science Presentation Guideline
7 min Presentation
3 min Discussion

Moderator: Murat Dogru, MD, PhD

15:15 **Ten-years follow-up (1998-2008) of autologous cultured limbal stem cell transplantation**
Paolo Rama, MD

15:27 **Penetrating keratoplasty after oral mucosa transplantation for total limbal stem cell deficiency**
Lingyi Liang, MD

15:38 **Lid margin reconstruction using oral mucosa membrane and amniotic membrane in chronic Stevens-Johnson syndrome**
Lingyi Liang, MD

15:50 ***In Vivo* confocal microscopy observations and ocular findings in Mulvihill-Smith progeria-like syndrome**
Murat Dogru, MD, PhD

16:02 **Activation and migration of limbal melanocytes onto corneal surface after ocular surface reconstruction—an in vivo confocal microscopic case report**
Wei-Li Chen, MD, PhD

16:14 **Goblet cell transplantation using nasal mucosa in cicatricial ocular surface disease**
Jae Hoon Kim, MD

16:26 **The effect of application of mesenchymal stem cells on the corneal wound healing in a chemical burn model**
Mee Kum Kim, MD

GENERAL DISCUSSION – 30 minutes

CLOSING REMARKS

17:15 **Scheffer CG Tseng, MD, PhD**

RECEPTION AT POOLSIDE TERRACE

17:30 ~ 19:00

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ABSTRACTS

AWARD LECTURE

Inseparable relationships between stem cells and the niche: lessons from *Drosophila*

Ting Xie, PhD
Stowers Institute for Medical Research, Kansas City, MO 64110 USA

Stem cells in adult tissues have the capacity to self-renew and generate differentiated progeny that replenish lost or damaged cells. The *Drosophila* ovary is an effective system for studying relationships between the niche and stem cells because the stem cells and their associated niches are well defined and versatile genetic tools for dissecting gene functions are readily available. My lab focuses on two types of stem cells in the *Drosophila* ovary: germline stem cells (GSCs) for generating oocytes and somatic stem cells (SSCs) for producing somatic follicular epithelial cells. Using a combination of genetic and cell biological approaches, we have studied multiple signaling transduction pathways for their roles in controlling stem cell self-renewal and differentiation, such as BMP, Wnt and JAK-STAT. In addition, we have revealed essential roles of cadherin-mediated cell adhesion in anchoring GSCs and SSCs to their niches, and have defined essential functions of ATP-dependent chromatin remodeling factors and the microRNA pathway in controlling GSC and SSC self-renewal. Recently, we have shown that Notch signaling is required for GSC niche formation and maintenance. In this meeting, I will report our recent progress in understanding of adult stem cell regulation in the *Drosophila* ovary.

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PAPER PRESENTATION SESSION I

Use of mesenchymal stem cells in treating corneal diseases: restoration of corneal transparency in Lum$^{-/-}$ mice

Hongshan Liu[1], Chia-yang Liu[1], James V. Jester[2], Martin Sieber[3], John Chang[3], ***Winston W.-Y. Kao[1]***
[1]Ophthalmology, University of Cincinnati, Ohio; [2]The Eye Institute, University of California at Irvine, Irvine, California; [3]Bionet Corp., Taiwan

Abstract: Lumican knockout mice exhibit thin and cloudy corneas due to disorganized stromal collagen lamellae. Here, we examined the possibility whether human umbilical cord mesenchymal stem cells (MSC) transplanted to corneas of *Kera*$^{-/-}$ and *Lum*$^{-/-}$ mice could differentiate and synthesize keratocan, a characteristic marker of corneal keratocytes, and lumican that has a pivotal role in collagen fibrillogenesis. Thus, the presence of such proteoglycans may restore an organized collagen lamellae and increase corneal transparency in *Lum*$^{-/-}$ mice. MSC and hematopoietic stem cells (HSC) obtained from human umbilical cord were labeled by DIO *in vitro*, and then intrastromally injected into corneas of *Kera*$^{-/-}$ and *Lum*$^{-/-}$ mice. Morphological changes of human umbilical cord-derived cells were noted in both MSC and HSC that assumed dendritic morphology in one week after intrastromal injection by a ZEISS stereomicroscope. The number of transplanted HSC reduced by 80.4% (n=8) after five weeks of HSC injection in comparison to that of one week after injection of HSC; whereas 43.5% (n=10) of transplanted MSC remained. Immunostaining with anti-CD45, CD90, and F4/80 antibodies indicated that many leukocytes, T-cells, and macrophages of recipient origin appeared in corneal stroma of *Kera*$^{-/-}$ mice after the injection of HSC, suggesting graft rejection caused by HSC transplantation; in contrast few inflammatory cells were detected following MSC transplantation. Immunostaining confirmed that MSC and HSC were negative for both keratocan and lumican before the transplantation. Immunostaining and western blot revealed that keratocan and lumican were present in the extracellular matrix of cornea injected with MSC, but not that of HSC. Corneal haze and thickness were determined with Heidelberg Retina Tomograph II with corneal module (HRTII). In *Lum*$^{-/-}$ mice, corneal haze of treated corneas significantly decreased in comparison to that of un-treated corneas at 8 to 12 weeks after the injection of MSC. Correspondingly, corneal stromal thickness significantly increased after 12 weeks of MSC injection as compared to un-treated corneas. The results indicate that MSC transplanted into the mouse cornea can differentiate and assume a keratocyte phenotype and express keratocan and lumican. These findings suggest that MSC transplantation is a potential treatment strategy for certain genetic and/or acquired corneal diseases.

Side population stem cell molecular signature reveals role of dual specificity phosphatases and G1 to S cell cycle control proteins in stem cell slow cycling

J. Mario Wolosin, *MAM Akinci, Peter S. Reinach[1], Z. Wang[1], A. Barash*
Ophthalmology, Mount Sinai School of Medicine, New York, NY; [1]Biological Sciences, SUNY State College of Optometry, New York, NY

Purpose: Side population (SP) cells isolated from limbus and conjunctiva derive from the *in vivo* slow cycling population (Budak et al., J. Cell Sci. 2005), a distinguishing stem cell feature. While SP cells display multiple stem cells features *in vitro*, they exhibit a poor ability to initiate clonal growth over feeder cells. The purpose of this study was to identify cell-signaling pathways that may underpin this phenotype.
Methods: The adherent cells from 12 hr cultures of freshly isolated human and rabbit conjunctival cells were cytometrically sorted into SP (~ 0.5 % of adhering cells) and non-SP (G1) cohorts. Extracted RNA was used to establish an SP/nonSP differential gene expression profile using microarrays. Applying robust filtering criteria we created a differential gene list containing all transcripts that are over-expressed or under-expressed in the SP. Data mining tools were used to identify differentially affected cell activities and pathways. Western blots of sorted conjunctival cells and immunostaining of total overnight cultures were used for validation at the protein and functional levels.
Results: Application of the SP differentially expressed gene list to the MAP kinase cascades using GenMapp revealed very large over-expressions of the several dual specificity phosphatases (DUSPs) that selectively dephosphorylate the ERK1/2 (DUSP4, 5 and 6), p38 (DUSP1) or JNK (DUSP1 and 4) EGF-activated MAP kinases. At the gene level the total MAPKs/MAPK phosphatase expression was 7-fold lower in the SP. Consistent with this ratio, Western blots showed that in SP cells ERK and p38 remain essentially unphosphorylated in response to EGF stimulation. The predicted outcome is blocking the EGF induction of proliferation and migration. Conversely, phosphatase inhibition with vanadate enhanced conjunctival and limbal cell clonogenic index. Similar analysis of the cell cycle pathway (Kegg) revealed 3-fold under-expression of the cyclins that promote S-phase protein synthesis and concomitant over-expressions of inhibitors (p15, p19, p27 and p 57) of these kinases.
Conclusions: The semi-quiescent phenotype of ocular surface SP stem cells can be traced to intrinsic changes in the expression of DUSP and G1 to S control proteins. Manipulation of these expressions may prove critical to improve epithelial ocular surface stem cell expansion *in vitro*.

Gene transfer to primary corneal epithelial cells in vitro

Mark Rosenblatt, *Charles Kim, Lauro Oliveira*
Department of Ophthalmology, Dyson Vision Research Institute, Weill Cornell Medical College

Purpose: To evaluate the transfer of heterologous genes to corneal epithelial cells using a lentiviral vector in vitro.
Methods: Corneal epithelial cell suspensions were obtained from rabbit corneoscleral tissue following double enzymatic digestion. Cells were plated at a density of $1\times10^3/cm^2$ and allowed to grow for 5 days to 70-80% confluency, whereupon they were transduced with 5 dilutions of a GFP-expressing lentiviral vector. Gene transfer was monitored using fluorescence microscopy and fluorescence activated cell sorting (FACS). At three days post-transduction, one set of P_0 cells was dual sorted by FACS for GFP expression and Hoechst dye exclusion, while a second set of cells was serially passaged to P_2 with GFP expression levels monitored at each passage.
Results: GFP-expressing lentiviral vectors (10^5 cfu/mL) are able to effectively transduce rabbit corneal epithelial cell cultures in vitro. GFP-positive cells with normal epithelial cell morphology and growth patterns were visualized with live cell imaging at four days post-transduction. In addition, GFP-positive side population cells were isolated with double sorting by FACS. The percentage of side population cells that were successfully transduced is similar to that of putatively more mature corneal epithelial cells. The percentage of cells transduced at P_0 is markedly dose-dependent, approaching 9% at the highest concentrations of lentivirus used in the study. A purified population of GFP-expressing cells obtained from FACS was readily expanded to confluency at P_1. Following serial passages from P_0 to P_2, transduced epithelial cells maintained a quantitatively similar GFP-positive phenotype, although the morphology of these cells matured during this time.
Conclusions: Lentiviral vectors can transfer heterologous genes to corneal epithelial cells in a stable and effective manner. Transferred genes are stably expressed through at least three passages. Furthermore, these vectors transduce both mature corneal epithelial cells and putative stem cells, without predilection for one population over the other.

Long-term culture of murine corneal epithelial progenitor cells from a single globe

Hideyuki Miyashita[1], *Xiaoli Ma*[1,2], *Tetsuya Kawakita*[1], *Miyuki Kubota*[1], *Satoshi Yoshida*[1], *Kazuo Tsubota*[1,3] *and Shigeto Shimmura*[1,3]
[1]Department of Ophthalmology; Keio University School of Medicine, Tokyo, Japan
[2]Department of Ophthalmology; China Medical University, Shenyang, China
[3]Deparment of Ophthalmology; Tokyo Dental College, Ichikawa, Japan

Purpose: Genetically modified mice are a powerful tool to study the molecular mechanisms involved the maintenance and differentiation of ocular stem cell. However, culture of murine corneal epithelial cells (MCEs) has proven difficult, making the use of such mice for in vitro studies almost impossible. The purpose of this study is to establish a method of long-term MCE culture from a single eye globe.
Methods: Corneal limbal explants of C57BL6/J mice were cultured in serum-free low-Ca2+ medium supplemented with EGF and cholera toxin (CTx). Epithelial cells were subcultured at 1:3 split until P4, and then at a lower density after P4. The expression of p63, keratin (K) 19, K12 and involucrin was investigated by RT-PCR analysis, immunocytochemistry and Western blotting analysis. Differentiation potential and stratification ability were also analyzed.
Results: Thirty of 32 corneal explants (93.8%) were successfully subcultured to P1. Cultures without CTx did not proliferate past P2 (n=12), but 55% of cultures supplemented with CTx achieved P4 (n=20). The cells showed cobblestone appearance, expressed p63, K19 and involucrin, but were negative for K12. Cells expressed K12 mRNA in differentiation medium, and formed stratified epithelium by air-lifting.
Conclusions: Using this procedure, MCEs from a single eye globe can be cultured long-term and retain the potential to differentiate and stratify. This procedure can be a powerful tool for study molecular mechanism by using genetically modified mouse with limited availability.

The role of N-cadherin in the limbal stem cell niche

Shigeto Shimmura[1,2], *Kazunari Higa*[2], *Hideyuki Miyashita*[1], *Tetsuya Kawakita*[1], *Naoko Kato*[1], *Yoko Ogawa*[1], *Jun Shimazaki*[2] *and Kazuo Tsubota*[1]
[1]Department of Ophthalmology; Keio University School of Medicine, Tokyo, Japan
[2]Deparment of Ophthalmology; Tokyo Dental College, Ichikawa, Japan

Abstract: N-cadherin (N-cad) is a key component of the bone marrow stem cell niche. In order to determine the importance of N-cad in the basal limbal epithelium, we performed histological and functional analysis using an in vitro cultivated epithelial sheet model. We found that N-cad +, K15+ corneal progenitor cells were associated with aquaporin-1 (Aqp-1) expressing stromal cells in a Ca^{2+} dependent fashion. Aqp-1+ cells were stromal cells in the limbal stroma, and not melanocytes, vascular endothelial cells or lymphatic endothelial cells. In colonies of limbal epithelial cells using 3T3 feeder cells, N-cad expression was found along the edge of the colony in direct contact with feeder cells. Peripheral colony cells had a significantly higher secondary colony forming efficiency compared to central cells. Furthermore, colonies using 3T3 feeder cells transfected with N-cad anti-sense miRNA were significantly smaller than mock transfected cells. We then used a duplex-feeder model of the limbal stem cells niche using two layers of feeder cells to reproduce the limbal phenotype. Only N-cad+ 3T3 cells produced epithelial sheets with basal K15/ suprabasal K12 expressing cells observed in limbal tissue. Our data suggests that N-cad is a functionally important molecule in the limbal stem cell niche.

Neurotrophic Factors and Receptors in Human Limbal Stem Cell Niche

De-Quan Li, *Hong Qi, Fang Bian, Eliseu Yung Chuang, Stephen C. Pflugfelder*
Ocular Surface Center, Cullen Eye Institute, Department of Ophthalmology,
Baylor College of Medicine, Houston, Texas, USA

Abstract: Neurotrophic factors play a vital role in survival and self-renewal of neuron and non-neuron stem cells. This study was to explore patterned expression and potential role of neurotrophic factors and their receptors at the human limbal epithelial stem cell niche. The immunoreactivity of NGF and GDNF, as well as their corresponding receptors TrkA and GFRα-1, were exclusively and sparsely localized in the basal layer of limbal epithelium, where stem cells reside. They were co-localized with stem cell associated markers ABCG2, p63, and EGFR, but not Keratin 3. The expression of these 4 neurotrophic mRNAs and proteins was significantly higher by limbal than corneal epithelium. In limbal epithelial culture, these 4 molecules co-expressed with ABCG2, p63 and EGFR. The TrkA and GFRα-1 positive subpopulations were enriched for certain properties (including ABCG2 and p63 expression, and slow cycling BrdU label-retaining cells) of putative limbal epithelial stem cells. These findings revealed that neurotrophic factors NGF, GDNF and receptors TrkA and GFRα-1 expressed by basal limbal epithelial cells may serve as new markers for human corneal epithelial stem/progenitor cells, and play vital role in maintaining the “stemness” of the limbal stem cells in their niche.

Role of FGF-7 in ocular surface squamous neoplasia

Chia-Yang Liu[1], *Taiichiro Chikama*[1,2], *Johanna T.A. Meij*[1], *Yasuhito Hayashi*[1,3], *I-Jong Wang*[4] , *Liu Yang*[2], *Teruo Nishida*[2], *and Winston W.Y. Kao*[1]

1. Department of Ophthalmology, University of Cincinnati Medical Center
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3. Department of Ophthalmology, Ehime University School of Medicine
4. Department of Ophthalmology, National Taiwan University Hospital

Abstract: We hypothesized that human ocular surface squamous neoplasia (OSSN) may result from continuous growth stimulation of corneal epithelial progenitor cells. In the present study, we determined the effects of excess FGF-7 on proliferation and differentiation of corneal epithelium in a novel *Krt12-rtTA/tet-O-FGF-7* double transgenic mouse model. When adult double transgenic mice were induced to over-express FGF-7 by doxycycline (Dox), they manifested epithelial hyperplasia with increases in phospho-pERK1/2, nuclear beta-catenin and BrdU labeled cells, an altered expression pattern of keratin 14 (K14), a normal expression pattern of K12, and normal absence of K10. The hyperplasia in adult cornea was fully reversed two weeks after removal of Dox chow. In contrast, double transgenic embryos that were exposed to Dox, beginning from embryonic day 0.5 to postnatal day 21, developed papillomatous tumors in the cornea, resembling human OSSN, and ectopic gland-like structure in the limbus, accompanied by down-regulation of K12, and up-regulation of K14, Pax6 and p63. The epithelial anomalies observed in young experimental mice did not fully resolve after the termination of Dox induction. Taken together, *Krt12-rtTA/tet-O-FGF-7* mice may be an animal model for human OSSN.

PAPER PRESENTATION SESSION II

Comparison of the corneal epithelial wound healing process among flap-on/flap-off LASIK and epithelium on/epithelium off penetrating keratoplasty

Wei-Li Chen, Fung-Rong Hu
Department of Ophthalmology, National Taiwan University Hospital

Purpose: To compare the corneal epithelial wound healing process among “flap-on” / “flap- off” Epi-LASIK and “epithelial-on” / “epithelium-off” penetrating keratoplasty (PK).

Methods: Forty-six eyes of 27 patients underwent “flap-on” Epi-LASIK, 28 eyes of 15 patients underwent “flap-off” Epi-LASIK, 10 eyes of 10 patients underwent “epithelium-off “PK” and 8 eyes of 8 patients underwent “epithelium-on” PK were compared. Slit-lamp biomicroscopy and in vivo confocal microscopy were recorded before, weekly in the first month and at 3 and 6 months after surgery. By in vivo confocal microscopy, cellular morphology of selected images of the corneal basal/apical surface epithelium was evaluated. Internal light reflectivity on Z scan profile was used to evaluate postoperative stromal reaction.

Results: By in vivo confocal microscopy, different healing processes of corneal epithelial layer among the 4 groups were found. Cells in most epithelial flaps of “flap-on” LASIK were unhealthy on early post-operative days and were rapidly replaced by new growing cells. Cells in most corneal epithelial layers of “epithelium-on” PK also underwent severe pathological changes on early post-operative days. The transient amplifying cells (TACs) on the basal epithelial layer were supposed to undergo cell death in all 4 kinds of surgeries.

Conclusion: Different corneal epithelial wound healing process was found in all 4 groups by in vivo confocal microscopy. In vivo confocal microscopy may provide a good tool to observe corneal epithelial wound healing process in various corneal surgeries.

Amniotic membrane for the treatment of acute Stevens-Johnson syndrome

Darren G. Gregory
University of Colorado School of Medicine

Purpose: Assess amniotic membrane transplantation (AMT) as a treatment of acute Stevens-Johnson syndrome (SJS).

Methods: Six consecutive patients with acute SJS and severe ocular inflammation were treated with AMT of the lid margins, palpebral conjunctiva and ocular surface. Final visual acuity, dry eye symptoms and cicatricial complications were assessed.

Results: Followup ranged from 3-16 months. All had >20/30 visual acuity. All had minimal or no dry eye symptoms. 4 had mild scarring of the inferior tarsal conjunctiva (1 had cicatricial entropion and trichiasis). 3 had cicatricial occlusion of the inferior lacrimal puncta. No other significant sequelae occurred.

Conclusion: AMT is effective in preventing the serious ocular sequelae of acute SJS.

Temporary sutureless amniotic membrane patch for acute alkaline burns

Ahmad Kheirkhah[1], Daniel A Johnson[2], Deval R Paranjpe[3], Victoria Casas[1], VK Raju[1], Scheffer CG Tseng[1]
[1]Ocular Surface Center, Miami, FL, [2]University of Texas Health Science Center at San Antonio, TX, [3]Allegheny General Hospital, Pittsburgh, PA

Purpose: To evaluate the clinical outcome of a new sutureless approach of temporary amniotic membrane patch (ProKera™) in eyes with acute burns.
Methods: Retrospective review of 5 eyes presenting with Grades I to III of acute alkaline burn receiving ProKera™ insertion within 8 days of injury.
Results: These eyes presented with total (2 cases) or extensive (60-75%, 3 cases) corneal epithelial defects with limbal (120-360°) and conjunctival (30-60%) epithelial defects. ProKera™ was inserted within 3.7 ± 3.1 days after burn and repeated 1-3 times for 3 cases. Conjunctival defects re-epithelialized in 8.2 ± 5 (range, 5-17) days, while limbal/corneal defects healed in 13.6 ± 8.3 (range, 5-25) days. The latter completed with circumferential closure of limbal defects followed by centripetal healing of corneal defects. In 3 eyes, early peripheral corneal neovascularization was followed by marked regression upon healing. During 16.8 ± 10.8 months of follow-up, all eyes retained stable surface with improved corneal clarity without limbal deficiency or symblepharon.
Conclusion: This sutureless application of an amniotic membrane patch allows for early delivery of its biologic actions, which may help preserve remaining limbal stem cells for rapid expansion and prevent late cicatricial complications in mild and moderate acute alkaline burns.

Sutureless amniotic membrane transplantation for partial limbal stem cell deficiency

Ahmad Kheirkhah, *Victoria Casas, VK Raju, Scheffer CG Tseng*
Ocular Surface Center, Miami, FL

Purpose: To evaluate the results of sutureless amniotic membrane (AM) transplantation using fibrin glue for reconstructing corneal surfaces with partial limbal stem cell deficiency (LSCD).
Methods: Eleven eyes of 9 patients that had LSCD with 120º to almost 360º of limbal involvement underwent superficial keratectomy to remove the conjunctivalized pannus followed by AM transplantation using fibrin glue. Additional sutureless AM patch (ProKera™) was used in 7 patients, and mitomycin C was applied on the cornea in 4 eyes and during fornix reconstruction in 7 eyes. The surgery was repeated in 3 eyes for residual pannus.
Results: During a mean follow-up of 14.2 ± 7.7 months (range, 6-26 months), all eyes maintained a smooth and stable corneal epithelial surface without recurrent erosion or persistent epithelial defect, and showed less stromal cloudiness and vascularization. Best corrected visual acuity improved in 9 eyes (81.8%). Corneal epithelialization proceeded by epithelial growth over AM (n=4), accompanied by dissolution of AM (n=4) or a combination of both (n=3). No complication was noted regarding initial or repeated uses of fibrin glue.
Conclusion: AM transplantation using fibrin glue appears to be a safe and effective method of restoring a stable corneal epithelium for cases with partial LSCD. This approach avoids the need of transplanting limbal epithelial stem cells.

Minimal conjunctival limbal autograft for total limbal stem cell deficiency

Ahmad Kheirkhah, *VK Raju, Scheffer CG Tseng*
Ocular Surface Center, Miami, FL

Purpose: To report the results of a single 60-degree conjunctival limbal autograft (CLAU) combined with amniotic membrane (AM) transplantation for an eye with total limbal stem cell deficiency (LSCD).
Methods: One eye of a patient with chronic total LSCD and symblepharon due to chemical burn was subjected to symblepharon lysis and removal of pannus from corneal surface, AM transplantation to cover the conjunctival and corneal surface as a permanent graft, a single 60-degree CLAU to the superior limbal area, and insertion of ProKera™ as a temporary AM patch to cover CLAU.
Results: After surgery, corneal epithelialization over AM was evident adjacent to CLAU on day 6, progressed to pass the horizontal midline by day 11, and was completed by day 18. During a follow-up of 1 year, the corneal surface remained stable and smooth and the stroma considerably regained clarity with regression of mid-stromal vascularization. The best corrected visual acuity improved from 20/400 to 20/50. The conjunctival inflammation completely resolved and the fornices were deep.
Conclusion: A single 60-degree CLAU combined with AM transplantation as both a permanent graft and a temporary patch can restore the entire corneal surface in an eye with total LSCD due to a chemical burn.

Outcome of ipsilateral ex-vivo cultivated limbal epithelial transplantation transplantation (CLET) for partial limbal stem cell deficiency

Virender S. Sangwan, *Rishi Swarup, Geeta K. Vemuganti, Anees Fatima*
LV Prasad Eye Institute, Kallam Anji Reddy Campus, Hyderabad, India

Aim: To study the outcome of ipsilateral CLET for partial or focal limbal stem cell deficiency (LSCD).
Methods: Records of 17 patients who underwent ipsilateral CLET between July 2001 and September 2005 were reviewed for demographics, etiology, outcome and complications.
Results: Most common etiology was chemical injury (9 cases) . Mean follow-up was 14.5 months. Mean reduction in conjunctivalisation was 1.27 quadrants. 6 patients had stable ocular surface with <1 quadrant of peripheral conjunctivalisation. 7 patients had partial success with peripheral recurrence of conjunctivalisation. 2 patients had failed CLET.
Conclusion: Ipsilateral CLET is a worthy management option in partial LSCD.

Objective outcome measures in ocular surface reconstruction using cultured limbal epithelial transplantation

Alex J Shortt, *Peng T Khaw, John K Dart, Stephen J Tuft, Julie T Daniels*
University College London Institute of Ophthalmology and Moorfields Eye Hospital London

Purpose: To develop and employ objective outcome measures to determine the outcome of ex-vivo cultured limbal epithelial cell (LEC) transplantation.
Methods: Ten eyes of 10 patients with profound LESC deficiency arising from chemical injury (4 eyes), aniridia (3 eyes), ectodermal dysplasia (1 eye), Reiger's anomaly with pax6 haplo-insufficiency (1 eye), and unknown etiology (1 eye) were treated with cultured limbal epithelial transplantation. Allogeneic LECs (7 eyes) or autologous LECs (3 eyes) were cultured on human amniotic membrane and transplanted onto the recipient eye after superficial keratectomy. A set of objective outcome measures were compared pre and at 6 months post treatment. These measures were; clinical parameters of LESC deficiency (loss of epithelial transparency, superficial corneal vascularization, epithelial irregularity and epithelial breakdown), visual acuity, impression cytology and cytokeratin expression profiles, and in-vivo confocal corneal confocal microscopy. Success was defined as an improvement in the defined clinical parameters of LESC deficiency, an improvement in visual acuity, restoration of a more normal corneal phenotype on impression cytology, and the appearance of a regular hexagonal basal layer of cells on corneal confocal microscopy.
Results: The success rate using this technique was 60% (autografts 33%, allografts 71%). All patients with a successful outcome experienced an improvement in visual acuity of ≥2 lines Snellen acuity. Preoperatively CK3+ and CK19+ cells cells accounted for 12±2.4% (mean±SEM) and 80±2.15% of cells respectively whereas postoperatively these accounted for 69±6.43% ($p<0.0001$) and 30±6.34% ($p<0.0001$) of cells respectively. Goblet cells accounted for 8±1.19% of cells preoperatively and 1±0.35% of cells postoperatively ($p<0.0001$).
Conclusions: This data demonstrates that impression cytology with cytokeratin profiling and in-vivo confocal microscopy are very useful in objectively determine outcome in ocular surface reconstruction. These outcome measures confirm that cultured LEC transplantation is an efficacious technique for treating LESC deficiency. The widespread use of such standardized and objective outcome measures may facilitate a comparison between the different culture methods and techniques in use.

Penetrating keratoplasty after ex-vivo cultured oral mucosal epithelial transplantation in total limbal stem cell deficiency

Abraham Solomon, *Genia Maftzir, Faik Orucov, Omar Hammad, and Joseph Frucht-Pery*
Department of Ophthalmology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Purpose: To report the results of penetrating keratoplasty (PKP) after ex-vivo cultured oral epithelial mucosal transplantation (COMET) in patients with total limbal stem cell deficiency (LSCD).
Methods: COMET was performed in 6 eyes of 6 patients with bilateral LSCD resulting from bilateral chemical burns (4 eyes) and aniridia (2 eyes). Autologus oral epithelium was cultured from oral mucosal biopsies on denuded amniotic membrane (AM) for two weeks, using MMC treated 3T3 fibroblasts, and air-lifting for one week. Ex-vivo cultured oral epithelial cells on AM were transplanted on the ocular surface of these patients following extensive removal of the fibrovascular tissue from the cornea and perilimbal areas. In two of the 6 patients, PKP was performed two years after COMET.
Results: Variable epithelial defects with partial dissolution of the AM were noted during the first 2 weeks after COMET, with complete corneal epithelialization after 4-6 weeks. The epithelium appeared smooth but opaque, and neovascularization was evident in the periphery of the cornea. After PKP, the donor cornea epithelialized

within a few days. The transplanted cornea remained clear with a stable corneal epithelium 6 months after PKP. The best corrected visual acuity improved from hand motions to 20/200 in one patient, and from hand motions to finger counting in the second patient. Histology of the removed corneal buttons showed variable stratification of the corneal epithelium, with no signs of the AM.
Conclusions: Ex-vivo oral epithelial cell transplantation cultured on the amniotic membrane followed by penetrating keratoplasty appears to be an effective method for the management of patients with bilateral total limbal stem cell deficiency.

PAPER PRESENTATION SESSION III

Ten-Years follow-up (1998-2008) of autologous cultured limbal stem cell transplantation

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Purpose: To report on long-term survival of autologous cultured limbal stem cells after transplantation in limbal stem cell deficiency.
Methods: One hundred and twenty five procedures in 112 patients have been done between July 1998 and April 2008. Seventy-one patients (76 transplants) with a follow-up longer than two years were included. Stem cells were obtained from a 2X2 mm limbal biopsy and cultivated on a fibrin substrate. All patients had moderate or severe limbal stem cell deficiency due to chemical burns in most of the cases (96,8%). Diagnosis of limbal stem cell deficiency was based on cause and clinical signs.
Results: Success was reported in 48/71 patients (67,60%) after a single procedure and in 53/71 (74,64%) after a second procedure. Ten (14,08%) after one procedure and 9/71 (12,67%) had partial success with improvement of signs and symptoms but mixed phenotype population (K3/K19) after impression cytology with mild signs of conjunctival migration. Therteeen patients (18,30%) after one procedure and 9/71 (12,67%) were considered failed, with three early failures (within three months) and six late failures (within one year). We did not report any failure after one year. None of the repeated procedures failed.
Conclusions: Cultured limbal stem cell transplantation is a new procedure that holds out fresh possibilities for the treatment of limbal stem cell deficiency. This procedure has a very low risk for the donor eye and allows repeating the biopsy in case of need. The cells can be stored frozen thus permitting to schedule the surgery and banking. It allows also treating bilateral diseases when there is a spared limbal area, even small. The results are stable after one year and in case of failure the procedure can be successfully repeated, even more than once. The handiness and ease of long-distance transportation of the fibrin-cultured epithelial sheets suggest that this method can now be widely applied.

Penetrating keratoplasty after oral mucosa transplantation for total limbal stem cell deficiency

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Purpose: To report a unique form of reepithelialization after penetrating keratopathy (PKP) in a patient who had prior oral mucosa membrane transplantation for total limbal stem cell deficiency (LSCD).
Methods: The remaining left eye of a patient was diagnosed as ocular demodecosis, failed corneal graft, and neurotrophic keratopathy because of chromic chemical burn. He received oral mucosa membrane transplantation 14 months ago to the limbal region for total LSCD. Tee tree oil (TTO) lid scrub and TTO ointment were used to clear the preexisting Demodex infestation in his lashes. After the Demodex count dropped to zero and the fluorescein clearance test of the tear reached to a normal level, PKP combined with amniotic membrane transplantation and lateral tarsorrhaphy were performed.
Results: The amniotic membrane was dissolved 6 days after surgery. At that time the graft was clear with an intact epithelium. The best corrected visual acuity was improved from hand movement 3 feet preoperatively to finger counting 3 feet postoperatively. From the 8th day postoperatively, a cluster of semi transparent epithelial cells were noted on the border of the graft and then moving circumferentially and centripetally without epithelial defect or neovascularization. At the latest follow up of 45 days postoperatively, the graft was almost replaced by such semi transparent epithelium. The stroma of graft remained clear and the vision acuity further improved to 20/200.
Conclusions: The transplanted oral mucosa cells exist at least more than one year after OMT. The donor epithelial cells are replaced by the recipient oral mucosa epithelial cells resulting in a semi transparent epithelium. This finding provides the sound basis for taking this new approach for total LSCD.

Lid margin reconstruction using oral mucosa membrane and amniotic membrane in chronic Stevens-Johnson syndrome

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Purpose: Lid margin pathologies such as keratinization, distichiasis, trichiasis and scarring are highly correlated with sight-threatening corneal complications (Di Pascuale et al, 2005). We would like to report a surgical procedure using oral mucosa and amniotic membrane to reconstruct the lid margin in a case of chronic Steven-Johnson syndrome (SJS).
Methods: A surgical video will be demonstrated.
Results: Before surgery, the patient had incomplete blinking and incomplete closure, irregular, overriding and keratinized lid margin, extropion, trichiasis, districhiasis, and severe dry eye of both eyes. All these problems resulted in symptomatic lid spasm, photophobia, diffusive ocular surface inflammation and superficial punctate keratopathy with corneal neovascularization. These irritative symptoms resolved dramatically from the first postoperation day with reduced inflammation, better lid blink and closure, and a continuous tear meniscus. The superficial punctate keratopaphy was healed from the third days postoperatively.
Conclusions: Persistent ocular irritative symptoms caused by the cicatricial complications of eye lids in chronic SJS can be resolved by transplantation of oral mucosa membrane and amniotic membrane. This procedure is vital to restore patient's comfort before corneal surface reconstruction.

In vivo confocal microscopy observations and ocular findings in Mulvihill-Smith progeria-like syndrome

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Purpose: To investigate the confocal microscopic and ocular findings in a rare case with progeroid syndrome.
Case Presentation: A 27-year-old female patient disclosed a variety of dysmorphic features, including progeroid aspect, short stature, microcephaly, hair loss, multiple pigmented nevi, nail dystrophy, as well as sensorineural hearing loss, immunodeficiency, diabetes mellitus, multiple hyper extensible subluxated joints. Ophthalmological findings included bilateral band keratopathy, cataract, and retinal macular degeneration. Confocal microscopy of the limbal region demonstrated blunting or absence of palisades of Vogt as well as extensive inflammatory infiltrates consisting of dendritic cells, extensive fibrosis and anterior corneal stromal scarring when compared with normal controls. Blood examination revealed marked decrease in the level of Mn-SOD (150ng/ml), Cu,Zn-SOD (7.97ng/ml).
Conclusion: Confocal microscopy effectively disclosed features of limbal stem cell deficiency including loss of palisades of Vogt, limbal epithelial and stromal fibrosis as well as stromal vascularization.

Activation and migration of limbal melanocytes onto corneal surface after ocular surface reconstruction – an in vivo confocal microscopic case report

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Purpose: Recently, melanocytes reside in the limbal basal layer have been proposed to be the candidate niche cells of limbal stem cells. Here we report a case who received ocular surface reconstruction by amniotic membrane transplantation (AMT). Activation and migration of limbal melanocyes onto the central cornea and transplanted amniotic membrane was proved by in vivo confocal microscopy.
Materials and Methods: A case of cicatricial SJS received AMT to release severe symblepharon on his right eye. The AM covered the whole bulbar conjunctiva. Epithelialization of bulbal amniotic membrane completed within 2 weeks without any complications. However, dense pigmentation on the AM and corneal surface combined with whorl like epitheliopathy was found 2 weeks after the operation. In vivo confocal microscopy revealed dendritiform cells with strange reflectivity in the corneal basal epithelial layer. Migrated melanocytes were diagnosed.
Results: The whorl like epitheliopathy recovered, and the migrated melanocytes on the corneal surface disappeared within 6 months after surgery. The migrated melanocytes on amniotic membrane also decreased. The final best corrected visual acuity of this patient was 20/20. No major complication was found.
Conclusion: Activation and migration of limbal melanocytes onto the corneal surface can be identified by in vivo confocal microscopy in patients receiving ocular surface reconstruction. The role of these migrated cells is interesting and needed for further clarification.

Goblet cell transplantation using nasal mucosa in cicatrical ocular surface disease

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Purpose: To investigate the possibility of replacing the metaplastic ocular surface with nasal mucosa which contains a lot of goblet cells and enriched vascularization. To compare the results of transplantation of oral mucosa and nasal mucosa in cicatricial ocular surface disease.
Methods: Three patients with severe recurrent pterygium of the eyes, and 6 patients with chemical burns to the eyes which were characterized by devastated cicatrical metaplasia, symblepharon, fibrous vascular proliferation, corneal opacity, and limbal deficiency were included in this study. The excision of pterygium, symblepharolysis, and the removal of fibrous vascular tissue was performed. After that, nasal mucosa autografts (or oral mucosa autografts) with amniotic membrane transplantations and limbal autografts were performed on bare sclera or the whole cornea over the perilimbal sclera.
Results: There was no recurrence of pterygium and symblepharon in all 9 patients. Well-formed conjunctival fornix and stable ocular surface were maintained. There was abundant vascularization to support the limbal grafts and increased mucin secretion which is needed for the ocular surface maintenance, while there were poor epithelization and ischemic changes on some oral mucosal autografts.
Conclusions: The goblet cell transplantation with enriched vascularized nasal mucosa was found to be a very effective method of treatment for cicatrical metaplastic ocular surface disease. The nasal mucosa also found to be more effective than the oral mucosa for ocular surface reconstruction, especially on the conjunctival surfaces.

The effect of application of mesenchymal stem cells on the corneal wound healing an a chemical burn model

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Purpose: To investigate the anti-inflammatory and anti-angiogenic effects of mesenchymal stem cells (MSC) in the chemically burned corneas.
Methods: We mechanically removed the corneal epithelium of rats after 100% alcohol instillation. The rats were then randomized into four groups: fresh media, conditioned media derived from the MSC culture (MSC-CM) or MSC were applied topically to the damaged corneas for two hours immediately after the injury. MSC-CM was applied either once or three times per day for three consecutive days. Corneal surface was evaluated every week. After three weeks, the corneas were stained with the hematoxylin-eosin and the expression of interleukin (IL)-2, interferon (IFN)-γ, IL-6, IL-10, transforming growth factor (TGF)-β1, thrombospondin-1 (TSP-1), matrix metalloproteinase-2 (MMP-2) and vascular endothelial growth factor (VEGF) were analyzed. $CD4^+$ cells were assessed in the corneas.
Results: We found that both MSC and three-time applied MSC-CM (1) reduced corneal inflammation and neovascularization, (2) decreased IL-2 and IFN-γ, while increased IL-10 and TGF- β1 as well as IL-6, (3) reduced the infiltration of $CD4^+$ cells, and (4) upregulated the expression of TSP-1, while downregulated that of MMP-2. Interestingly, whereas three-time application of MSC-CM was partially effective to suppress corneal inflammation, transplantation of MSC achieved a better outcome.
Conclusion: It suggests that the anti-inflammatory and anti-angiogenic action of MSC in the chemically burned corneas might be mediated in part through paracrine pathways involving soluble factors such as IL-10, TGF-β1, IL-6 and TSP-1.