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9TH OCULAR SURFACE AND TEAR WORKSHOP

APRIL 29, 2006

"Stem Cell Niche"
&
"Ocular Surface Reconstruction"

FORT LAUDERDALE GRANDE HOTEL & YACHT CLUB

FORT LAUDERDALE, FLORIDA, USA

PROGRAM

International Ocular Surface Society

10:00 WELCOME REMARKS

SESSION I: BASIC RESEARCH PRESENTATIONS
10:10 ~ 12:00, Moderator: Scheffer C.G. Tseng, MD, PhD

10:10 **Isolation and demonstration of limbal epithelial progenitor cells deep in the limbal stroma**
Yasutaka Hayashida, MD, PhD

10:20 **Isolation and enrichment of human limbal epithelial progenitor cells by rapid adhesion on collagen I substrate**
Yasutaka Hayashida, MD, PhD

10:30 **Culture and characterization of human oral mucosal epithelium**
Hua He, PhD

10:40 **Defining the limbal stem cell niche: role of basement membrane components and adhesion molecules**
Friederich Kruse, MD

10:50 **Apoptosis of murine corneal and limbal epithelial cells is induced by dissolution**
Yukihiro Matsumoto, MD

11:00 **Reversal of myofibroblasts by amniotic membrane stromal extract**
Wei Li, MD

11:10 **Activation of the Wnt pathway in corneal and limbal epithelial cell gene expression**
Roger Beuerman, PhD

11:20 **Hyaluronan-collagen matrices promote epithelial-to-mesenchymal transition in rabbit limbal epithelial cells**
Ying-Ting Chen, MD

11:30 **Melanocytes in the basal limbal epithelium**
Shigeto Shimmura, MD

12:00 ~ 2:00 LUNCH BREAK (NOT INCLUDED)

SESSION II: GUEST LECTURE AND CLINICAL PRESENTATIONS
2:00 ~ 3:30, Moderator: Murat Dogru, MD

GUEST LECTURE
2:00 **When the drops don't work; managing severe anterior segment inflammation**
John Dart, MA, DM, FRCOphth

PRESENTATIONS
2:30 **Amniotic membrane serving as a stem cell niche**
Martin Grueterich, MD

2:40 **Stage wise surgical approach without stem cell transplantation for chronic chemical burns**
Ahmad Kheirkhah, MD

2:50 **Ocular surface and tear functions in graft versus host disease**
Samantha Ward, OD

3:00 **Management of superior limbic keratoconjunctivitis in chronic ocular GVHD with amniotic membrane patch graft**
Stella K. Kim, MD

3:10 **Transplantation of ex-vivo expanded autologous oral epithelium on amniotic membrane in total stem cell deficiency – preliminary results**
Abraham Solomon, MD

3:30 ~ 4:00 REFRESHMENT BREAK

SESSION III: FREE PAPER PRESENTATIONS
4:00 ~ 5:30, Moderator: Kazuo Tsubota, MD

4:00 **Implications of smoking on ocular surface and tear functions**
Murat Dogru. MD

4:10 **Clinical use of ProKera for treating acute chemical burns**
Victoria Casas. MD

4:20 **New surgical management for superior conjunctivochalasis**
Gabriela Blanco, MD

4:30 Successful tear lipid layer treatment for severe dry eye patients by low-dose lipid application on the full-length lid margin
Eiki Goto, MD

Presidential Lecture
4:45 Free radical theory and dry eye
Kazuo Tsubota, MD

6:00 ~ 8:00 COCKTAIL RECEPTION

SESSION I

Isolation and Demonstration of Limbal Epithelial Progenitor Cells Deep in the Limbal Stroma

Yasutaka Hayashida, MD, PhD
Associates: Wei Li, MD, Hua He, PhD, Ying-Ting Chen, MD, Yukihiro Matsumoto, MD, Scheffer C.G. Tseng, MD, PhD

Purpose: To demonstrate that limbal epithelial stem cells might be situated much deeper into the limbal stroma so that they cannot be isolated in total by conventional dispase digestion. *Methods:* Human limbal corneal epithelial sheets were isolated by 10 mg/ml dispase II in KSFM at 37 ℃ for 2h from the corneoscleral ring after corneal transplantation, trypsinized, and cultured on plastic with or without 3T3 fibroblast feeder layers in SHEM medium. The remaining limbal stroma was surgically dissected and digested by 2 mg/ml collagenase A solution in serum-free KSFM medium at 37 ℃ for 16h to isolate embedded cells, which were then cultured on plastic with or without 3T3 fibroblast feeder layers in KSFM. Clonal growth was monitored daily under a phase contrast microscope and the epithelial phenotype was verified by immunostaining to pancytokeratins using limbal cryosections as a control. *Results:* Dispase-isolated limbal epithelial sheets generated monolayers of large squamous epithelial cells on plastic in SHEM, but small epithelial colonies on 3T3 feeder layers in either SHEM at day 6, and reached confluence at day 8. In contrast, collagenase-isolated stromal cells contained clusters of pancytokeratin-positive small epithelial cells when seeded on plastic without 3T3 feeder layers. They could generate large epithelial colonies consisting of small epithelial cells on 3T3 feeder layers in KSFM as early as day 2, and reached confluence at day 5. *Conclusions:* These findings indicate that limbal epithelial progenitor cells lie deeper in the limbal stroma than expected, and these cells cannot be isolated in total by conventional dispase digestion, and further suggest that modifications are needed to achieve effective ex vivo expansion.

Isolation and Enrichment of Human Limbal Epithelial Progenitor Cells by Rapid Adhesion on Collagen I Substrate

Yasutaka Hayashida, MD, PhD
Associates: Wei Li, MD, Hua He, PhD, Ying-Ting Chen, MD, Yukihiro Matsumoto, MD, Scheffer C.G. Tseng, MD, PhD

Purpose: To demonstrate that limbal epithelial progenitor cells can be isolated preferentially bas on their rapid adhesion. *Methods:* Human limbal epithelial sheets were isolated from the cadave corneoscleral ring after dispase II in KSFM at 4 ℃ overnight. Epithelial sheets were trypsinized, and seeded on plastic coated with type I collagen in serum-free KSFM medium. Epithelial cells were divided into two groups: one .was cultured and subcultured by removing non-adherent cells after 12 min seeding (R+/R+), while the other was subcultured without removing non-adherent c (R-/R-). Clonal growth was monitored daily under a phase contrast microscope and the epithelial phenotype was verified by immunostaining to cytokeratin 12.
Results: Dispase-isolated limbal epithelial sheets generated large colonies of small epithelial cell on collagen-coated plastic surfaces in KSFM, and reached confluence at day 7 in P0, and grew w from P1 to P4 passage. Cytokeratin 12 positive cells became less and less in R+/R+ group.
Conclusions: Additional studies are underway to fine tune the culturing system to see if limbal epithelial progenitor cells can be isolated and enriched based on rapid adhesion adhesion.

Culture and Characterization of Human Oral Mucosal Epithelium

Hua He, PhD
Associates: Wei Li, MD, Scheffer C.G.Tseng, MD, PhD

To seek alternative stem cell source other than the cornea for treatment of total corneal stem-cell deficiency in both eyes, we decided first to optimize the cell culture method of human oral muco epithelium and characterize the cultivated epithelial cells. Our results showed the isolated human epithelial cells can be expanded into an epithelial sheet on denuded amniotic membrane (dAM) with 3T3 feeder layer in SHEM medium by 2-3 weeks. The most cultivated epithelial cells were alive when reaching confluence by Live & Dead assay. These cells expressed keratin 3 but not keratin 12. Some cells were also p63 positive indicating some progenitor cells of oral mucosal epithelium had been maintained and expanded. The further experiment to transplant the cultivate oral mucosal epithelial sheet to human cornea is in progress.

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Defining the limbal stem cell niche: Role of basement membrane components and adhesion molecules

Friedrich E. Kruse, MD

Purpose: To characterize the role of basement membrane components and cell-matrix adhesion molecules in the generation of a microenvironmental niche for human corneal epithelial stem cells. *Methods*: Corneal sections from 20 donors (age 61-91 years) were stained by indirect immunofluorescence using a broad panel of antibodies against basement membrane components and cell-matrix adhesion molecules. In double labeling experiments, antibodies against putative stem cell and differentiation markers, e.g. p63, ABCG-2, connexin 43 (Cx43), were used in addition. *Results:* As compared to the corneal and conjunctival basement membranes, the limbal basement membrane showed increased immunoreactivity for the basement membrane components agrin, BM40/SPARC, laminin (alpha1, alpha2 and ß1 chains), collagen type IV (alpha1 and alpha2 chains), nidogen-1 and -2, collagen type XVI, versican, and tenascin-C, whereas reduced immunoreactivity was observed for collagen type IV (alpha3 and alpha4 chains), collagen type V, fibulin-2, fibrillin-1, and clusterin. Patchy staining patterns of agrin, BM40/SPARC, tenascin-C, and versican appeared to co-localize with p63/ABCG-2-positive and Cx43-negative cell clusters in the limbal basal epithelium. Stem cell-containing basal cell clusters at the limbus disclosed preferential immunoreactivity for dystroglycan, integrins alpha3 and ß1, and syndecan-1, but showed distinct gaps for integrins alpha6 and ß4, which are involved in hemidesmosome formation. *Conclusions:* Basement membrane heterogeneity and specific cellular adherence mechanisms may be involved in providing a unique microenvironment for corneal stem cells at the limbus. The characteristics of this microenvironment could serve as tools for their selective enrichment and in vitro expansion.

Apoptosis of murine corneal and limbal epithelial cells is induced by dissolution of basement membrane by dispase digestion

Yukihiro Matsumoto, MD
Associates: Wei Li, MD, Scheffer C.G. Tseng, MD, PhD

Purpose: To determine whether the enzymatic digestion of the basement membrane (BM) by dispase may trigger apoptosis of the basal epithelial cell. *Methods:* Sixteen mouse eyeballs were enucleated and divided into two groups. Control Group (n=6) that were studied either immediately (n=2) or stored in a serum-free KSFM medium at 4C /18h (n=2) or at 37C/1h (n=2). Dispase Digestion Group (n=10) that were incubated in 10 mg/ml dispase in KSFM at 4C for 2h, 8h, 18h (n=2 each) or at 37C for 1h and 2h (n=2 each). Following incubation, eyes were cryosectioned for HE and TUNEL staining. *Results:* All eyes in Control Group showed intact morphology, and occasional TUNEL-positive superficial epithelial cells following 4C/18h storage. There were TUNEL-positive epithelial cells in the detached limbus following 1h and 2h dispase digestion at 37C. Following dispase digestion at 4C, a marked increase of TUNEL positive epithelial cells was noted at 8h and 18h treatments while partial and total epithelial detachment was observed respectively. *Conclusion:* Simple digestion of epithelial BM by dispase is sufficient to induce murine corneal and limbal epithelial apoptosis.

Reversal of Myofibroblasts by Amniotic Membrane Stromal Extract

Wei Li
Associates: Hua He, PhD, Ying-Ting Chen, MD, Scheffer C.G. Tseng, MD, PhD

Purpose: Amniotic membrane (AM) prevents myofibroblast differentiation of keratocytes and maintains keratocyte phenotype through inhibition of TGF-β signaling pathway. It remains unclear whether AM can reverse differentiated myofibroblasts to fibroblasts. *Methods:* AM mesenchymal cells (AMMCs) harvested from AM stroma were cultured in DMEM with 10%FBS, AM stromal extract (ASE) was added to AMMCs culture at different passage. Differentiation of AMMCs was monitored by morphology and α-SMA expression. *Results:* About 70% AMMCs spontaneously differentiated into myofibroblasts in primary culture in DMEM/10%FBS, and almost all AMMCs differentiated in secondary passage (P2). ASE inhibited α-SMA expression of P0 AMMCs, moreover, caused P2 and P3 AMMCs to lose their characteristic myofibroblast shape and α-SMA expression. P2 AMMCs cultured on AM stromal matrix surface also changed their shape to dendritic or elongated coupled with decrease of expression of α-SMA. *Conclusion:* ASE as well as AM stromal matrix can reverse differentiated myofibroblast to fibroblast phenotype.

Activation of the Wnt pathway in corneal and limbal epithelial cell gene expression

Roger W. Beuerman, PhD
Associates: Jing Li, PhD, Donald Tan, MD

Purpose: The Wnt pathway is involved in the control of proliferation of stem cells and in the limbal stem cell niche Wnt may confer certain critical properties for the response to tissue damage. *Methods:* Human limbal epithelial cells were isolated from human donor and HCEC cell purchased from Cascade Biologics. LiCl (20mM) or 6-bromoindirubin-3'-oxime (BIO) (5μM) was added to the culture medium to activate the Wnt pathway. Real time PCR analysis of cytokertins (CK) 3, 4, 12, 19, as well as p63 and ABCG2 gene expression was carried out using P2-P4 cells 4 and 8hrs after stimulation. *Results:* Human limbal and corneal epithelial cells express multiple Wnt genes, the receptors (Frizzled), Wnt pathway associated proteins including glycogen synthase kinase-3 β, β-catenin, adenomatous polyposis coli (APC), multiple secreted Frizzled-related peptides (sFRPs),T-cell factor 1 and 4. Nuclear translocation of β-catenin was observed by immunofluorescent staining shortly after adding LiCl or BIO into the culture medium. In both primary cultured limbal epithelial cells and HCEC cells, increased p63 expression was observed 4hrs after BIO or LiCl stimulation. The expression of Ck3 is increased in P1 limbal epithelial cells. However, no change of cytokeratin gene expression was observed in HCEC cells. Prolonged incubation in the presence of LiCl or BIO (over 24hrs) lead to cell death in both cell types. *Conclusions:* Our study suggested that human limbal and corneal epithelial cells are subject to the regulation of Wnt pathway and that it may influence the morphogenetic profile of the cells.

Hyaluronan-Collagen Matrices Promote Epithelial-to-Mesenchymal Transition in Rabbit Limbal Epithelial Cells

Ying-Ting Chen, MD
Associates: Chia-Wei Huang, Sung-Huei Tseng, Lynn LH Huang, Scheffer CG Tseng,

Purpose: Corneal epithelial cells stem from basal limbal epithelium. Limbal stem cells (LSC) have been demonstrated to have high plasticity and it is related to the niche environment. As hyaluronan was proven to facilitate fast recovery of hematopoesis by participating in hematopoietic niche assembly. In this study, we aimed to study the influence of hyaluronan-collagen matrices on the plasticity of LSC. *Methods*: Rabbit limbal explants were cultivated in 3 conditions: Group A, platic dish; Group B, collagen type I-coated dish; Group C, Hyaluronan-collagen type I coated dish. Immunofluoresceince study using anti-K3, anti-vimentin, anti-β-catenin antibodies was performed to determine the phenotypes of cultivated cells derived from limbal explants. BrdU was used to compare cell proliferation rate between different groups. Time lapse microscopy was employed to determine the cell migration rate. *Results:* Cells from group A showed typical squamoid epithelial morphology with positive staining of cytoplasmic K3 and intercellular β-catenin. Cells from group B showed oval to spindle-like morphology, with positive staining of K3 and intercellular β-catenin. Cells from group C demonstrated spindle to dendritic morphology, with negative staining to weak/sporadically positive staining to K3, translocation of intercellular β-catenin into cytoplasmic counterpart, and positive staining to vimentin. BrdU study showed a decreased proliferation rate for cells grown in group B and C, in comparison to group A. Migration rate was observed to be the highest in group B, followed by Group C, and finally with Group A being the lowest. *Conclusions*: Extracellular matrix may play an important role in the differentiation of limbal stem cells. Hyaluronan-collagen matrices induce an epithelial-to-mesenchymal transition and increases cell migration ability. Whether EMT induced by these matrices can be used as a strategy to prevent LSC from committing into terminal epithelial differentiation will merit further studies.

Melanocytes in the basal limbal epithelium

Shigeto Shimmura, MD
Associates: Kazunari Higa, PhD, Tetsuya Kawakita, MD, Jun Shimazaki, MD, Kazuo Tsubota, MD

The corneal limbus contains pigmented cells lining the pallisades of Vogt. The pigments are mostly found within basal epithelial cells that interact with melanin forming melanocytes. Melanocytes can be identified in the limbal epithelium as dendritic cells that extend cellular processes to surrounding basal epithelial cells. Interestingly, pigments within each cell form "melanin caps" that face the ocular surface, the direction from which cells are exposed to environmental light and UV radiation. We have found that melanocytes expressing the specific antigen, MART1, are observed in the basal limbal epithelium at a density of approximately 1 melanocyte for every 10 K19 positive epithelial cells. MART+ melanocytes are also present in non-pigmented eyes as well. Melanocytes may play a functional role in the limbal stem cell niche.

SESSION II

Amniotic membrane serving as a stem cell niche?

Martin Grueterich, MD
Associates: E. Espana, MD, S.C. Tseng, MD, PhD

Cryopreserved human amniotic membrane (hAM) has been successfully used to expand and transplant human limbal epithelium. Furthermore hAM has been used as a substrate to culture oral mucosa for ocular surface reconstruction. In this presentation a summary of the current understanding of the stem cell niche in relation to hAM properties is given.

Stage Wise Surgical Approach without Stem Cell Transplantation for Chronic Chemical Burns

Ahmad Kheirkrah, MD
Associates: Victoria Casas, MD, Scheffer C.G. Tseng, MD, PhD

Purpose: To determine if amniotic membrane transplantation (AMT) with fibrin glue can restore corneas with nearly total limbal stem cell deficiency. *Methods*: Pannus removal followed by AMT and fibrin glue was performed in one corneal quadrant and repeated in several sessions, each two to three months apart. *Results:* Normal corneal surface was successfully recovered by this new surgical technique without stem cell transplantation in 3 eyes. Fluorescein staining showed early limbal epithelial expansion before corneal epithelialization. *Conclusion*: Fibrin glue facilitates AMT and stabilizes AM for regeneration presumably by providing a healthy milieu for in vivo expansion of residual stem cells.

Ocular Surface and Tear Functions in Graft versus Host Disease

Samantha Ward, OD
Associates: Wang Y, Ogawa Y, Okada N, Igarashi A, Goto E, Kawakita T, Dogru M, Tsubota K

Purpose: To investigate the ocular surface and tear function alterations in severe dry eye patients with graft versus host disease (GVHD). *Methods:* Sixteen of 8 GVHD patients and 14 eyes of 7 healthy control non smokers (3 males, 4 females) were included in this study. None of the subjects had a history of Sjogren's syndrome, collagen diseases, other systemic or ocular diseases, history of ocular surgery, contact lens or drug use. All subjects underwent measurements of tear film lipid layer interferometry, tear evaporimetry, tear film break up time (BUT), Schirmer test-I, ocular surface fluorescein staining, conjunctival impression and brush cytology. *Results:* Tear quantity and stability time was significantly shorter in GVHD. Tear lipid layer showed significant slowing in spread over the tear film. There was a significant increase in tear evaporation rate. Conjunctival impression cytology revealed significant loss of goblet cells and advanced squamous metaplasia in GVHD. Brush cytology showed significant conjunctival neutrophil infiltration. *Conclusion:* Tear film and the ocular surface epitheliae showed distinctive quantitative and qualitative disturbances in GVHD related to ocular surface inflammation.

Management of superior limbic keratoconjunctivitis in chronic ocular GVHD with amniotic membrane patch graft

Stella K. Kim, MD
Associate: Nimit Shah, MD

Purpose: To describe a case of superior limbic keratoconjunctivitis (SLK) in the setting of chronic ocular graft vs. host disease (GVHD) successfully treated with amniotic membrane patch graft. *Method:* A retrospective case review with a review of chronic ocular GVHD and superior limbic keratoconjunctivitis management. *Result:* 44-year old woman with history of mycosis fungoides (T cell lymphoma) status post total body radiation and allogeneic peripheral hematopoietic stem cell transplantation (HSCT) in April 2003, presented with a history of skin GVHD and chronic ocular GVHD with SLK features. Her sentinel symptoms (severe irritation/pain/photophobia) were 8-10 out of 10; Ocular Surface Disease index (OSDI) of 42, and clinical features included Schirmer's score of 0 mm without anesthetic, superior bulbar keratoconjunctivitis with positive Rose Bengal staining and filamentary keratitis bilaterally. She failed medical management of ocular GVHD, including lubrication, punctal occlusion, topical steroid, acetylcystaine, cyclosporine, and systemic immonosuppression, including FK506 and photopheresis. Silver nitrate application to treat SLK was deferred as it would cause worse chronic ocular GVHD. The patient underwent a complete excision of SLK lesion from the left superior bulbar conjunctiva with amniotic membrane patch graft in March 2005. Post-operative medications included topical antibiotic and steroid with lubrication. At 1 week follow-up, she had "95% improvement" of her symptoms, rating sentinel symptoms of 1-2 out of 10, with healed conjunctival epithelium. Histopathology of the specimen revealed dilated "snake like" vessels, hyperkeratosis, parakeratosis and scanthosis, consistent with SLK, with features of apoptosis in the epithelium consistent with ocular GVHD. She requested to have the second eye managed in the similar fashion; and the second eye had similar post operative course.

11-months follow-up revealed improvement of her sentinel symptoms 0-1/10 bilaterally, OSDI of 19, resolution of superior conjunctival injection with no Rose Bengal staining and minimal artificial tears 2-3 times a day (compared to preop use of artificial tears every 5 minutes). SLK in chronic GVHD is a newly described entity (ARVO 2005) and can be addressed with medical management and bandage contact lens. Amniotic membrane has not been described previously for the management of SLK. *Conclusion:* A successful treatment of refractory SLK associated with chronic ocular GVHD with excision/amniotic membrane patch graft is described. Given the non-immunonologic, non-angiogenic properties of amniotic membrane, it may be considered in the management of refractory cases of SLK associated with chronic ocular GVHD.

Transplantation of ex-vivo expanded autologous oral epithelium on amniotic membrane in total stem cell deficiency – preliminary results

Abraham Solomon, M.D.,
Associates: Anat Sharon, M.D., Joseph Frucht-Pery, M.D., Genia Mafzir, BSc, Mordechai Sela, M.D

Purpose: to present the preliminary results and the technical problems of transplantation of ex-vivo expanded autologous oral epithelium on amniotic membrane in total stem cell deficiency. *Methods:* Three patients with total stem cell deficiency after a chemical burn (2 patients) and aniridia (one patient) had ocular surface reconstruction using epithelial cells from the oral mucosa. Small biopsies (3x3 mm) of oral mucosa were harvested by a Maxillo-Facial surgeon from the lower buccal mucosa below the dental line under local anesthesia. Epithelial cells were separated from the tissue, and seeded on the basement membrane side of denuded amniotic membrane. Mitomycin C treated 3T3 fibroblasts served as a feeder layer. Cultures were submerged in SHEM medium for 2 weeks, and them air-lifted for 1-2 weeks. After a total of 3-4 weeks, the ex-vivo expanded oral epithelium on AM was transplanted in the corresponding patients, after extensive removal of superficial fibro-vascular tissue from the cornea, and removal of the sub-conjunctival scar tissue. Parallel cultures of oral epithelium on plastic and on AM were used to assess the characteristics of the oral epithelium. *Results:* The transplanted epithelial surfaces were intact for the first 2-3 days. This was followed with variable large epithelial defects in the center of the corneas, which eventually epithelialized within the following 2-3 weeks. In one patient, partial recurrence of fibrovascualr tissue was evident in the lower third of the cornea. Parallel cultures of the oral epithelium on AM demonstrated stratified epithelium, which was positive for CK3 and negative for CK19. Some portions of the AM were denuded of oral epithelium in vitro. *Conclusions:* cultured oral epithelium on AM presents a promising option for resurfacing of the cornea with total stem cell deficiency. However, the long term survival of the transplanted oral epithelium may be limited, and the true nature of the remaining epithelium on the ocular surface is not clear.

SESSION III

Implications of Smoking on Ocular Surface and Tear Functions

Murat Dogru, MD
Associates: Sasaki Y, Imamura Y, Okada N, Igarashi A, Goto E, Kawakita T, Sato E, Ward S, Saito I, Tsubota K

Purpose: Smoking, a well-known risk factor for aging, has been reported to induce inflammation in several tissues and shortening of telomere length in human body. We aimed to investigate the effects of smoking on ocular surface health and tear functions in this prospective controlled clinical trial and report our preliminary findings. *Methods:* Fourteen of 10 heavy smokers (5 males, 2 females; age range: (36-47 years) who smoked 20 cigarettes per day for 20 years and 14 eyes of 7 healthy control non smokers (3 males, 4 females; age range: 38-43 years) were included in this study. None of the subjects had a history of Sjogren's syndrome, collagen diseases, other systemic or ocular diseases, history of ocular surgery, contact lens or drug use. All subjects underwent measurements of hemoglobin CO concentration, tear film lipid layer interferometry, tear evaporimetry, tear film break up time (BUT), Schirmer test-I, ocular surface fluorescein staining, conjunctival impression and brush cytology. Samples from brush cytology were also evaluated for MUC5AC mRNA expression in Real Time PCR. *Results:* The mean Hb CO level was significantly higher in smokers compared to nonsmokers. Tear film break up time was also significantly shorter in smokers. Tear lipid layer showed significant slowing in spread over the tear film. Tear quantity was significantly reduced in smokers with a concomitant significant increase in tear evaporation rate. Conjunctival impression cytology revealed significant loss of goblet cells and advanced squamous metaplasia in smokers with a decrease in MUC5AC mRNA expression. Brush cytology showed significant conjunctival neutrophil infiltration in smokers. *Conclusion:* Tear film and the ocular surface epitheliae showed distinctive quantitative and qualitative disturbances in heavy smokers, which we attributed to the marked ocular surface inflammation associated with chemicals in cigarette smoke.

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Clinical Use of ProKera for Treating Acute Chemical Burns

Victoria Casas, MD
Associates: Ahmad Kheirkrah, MD, Scheffer C.G. Tseng, MD, PhD

We will present a case who suffered from acute chemical burn to one eye. She presented to us on Day 10 after burn involving total corneal and limbal surfaces and superior and inferior conjunctival surfaces. We will present the sequential clinical photos documenting how healing was facilitated by ProKera, a sutureless amniotic membrane Symblepharon ring. There was a preferential migration of limbally derived epithelial cells circumferentially to the surrounding limbus first before centrifugal migration to fill in the corneal surface in the area covered by ProKera. However, in the area not covered by ProKera, slow conjunctival healing occurred and led to granulation tissue. This finding supports the unique property of amniotic membrane in delivering an environment geared up for regeneration rather than repair.

New Surgical Management for Superior Conjunctivochalasis

Gabriela Blanco, MD
Associates: Victoria Casas, MD, Ahmad Kheirkrah, MD,
Scheffer C.G. Tseng, MD, PhD

Purpose: To demonstrate superior limbic keratoconjunctivitis (SLK) is associated with superior conjunctivochalasis (Cch), which can be treated by Tenon reinforcement with cryopreserved amniotic membrane. *Methods*: Maneuvers under slit lamp examination and the operating room are introduced before surgery. *Results*: Redundant conjunctival folds and the lack of Tenon adhesion to the sclera are found in SLK. Tenon replacement by amniotic membrane without conjunctival recession or resection is effective in restoring patient's comfort. *Conclusion*: SLK is associated with superior Cch, which is worsened by lid blinking. This new surgical approach is directed to strengthening adhesion without causing scarring in the conjunctiva.

Successful tear lipid layer treatment for severe dry eye patients by low-dose lipid application on the full-length lid margin

Eiki Goto, MD
Associates: Murat Dogru, MD, Yukihiro Matsumoto, MD, Minako Kaido, MD, Kazuo Tsubota, MD

Purpose: To test tear lipid layer treatment by low-dose lipid application on the full-length lid margin for the treatment of severe dry eye patients refractory to conventional treatments. *Design:* Interventional case report. *Case Report:* Four eyes of 2 severe dry eye patients with chronic Stevens-Johnson syndrome with dark tear interference images, which did not respond to conventional treatments, were included. To supply lipid using a commercially available non-preserved topical medication having both polar and non-polar lipid base, ofloxacin eye ointment (Tarivid®, Santen, Japan) was selected. Low-dose ofloxacin ointment application on the full-length lid margin was carried out 3 times a day for 2 weeks. After the treatment, the symptom of ocular dryness improved, lipid layer thickness increased, tear-film break-up time was prolonged, and corneal fluorescein staining decreased. *Conclusion:* For severe dry eye patients with chronic Stevens-Johnson syndrome, tear lipid layer treatment by low-dose ointment applied on the full-length lid margin was shown to be an effective approach. The applied lipid helped to form an increased lipid layer thickness, which contributed to tear stability, leading to the improvement of the symptom.

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