7th International Conference on the

Tear Film & Ocular Surface: Basic Science and Clinical Relevance

Taormina 2013

tfos

Sponsored by Alcon. Also sponsored by Allergan, Bausch & Lomb, Santen, SIFI, TearLab, Vistakon, Horus, Novaliq, TearScience, NicOx, SOOFT, Otsuka, AVR, Iris Pharma, Senju, Menicon, Eleven Biotherapeutics and EyeGate.

TFOS Governing Board

David A. Sullivan, President (USA)
Kazuo Tsubota, President-Elect (Japan)
Suzanne M. J. Fleiszig, Vice-President (USA)
Sarah F. Hamm-Alvarez, Treasurer (USA)
Michael A. Lemp, Secretary (USA)
Pablo Argüeso, Councilor (USA)
Penny Asbell, Councilor (USA)
Stefano Bonini, Councilor (Italy)
Erich Knop, Councilor (Germany)
Thomas J. Millar, Councilor (Australia)
Kelly K. Nichols, Councilor (USA)
Friedrich P. Paulsen, Councilor (Germany)
Christine Purslow, Councilor (UK)
Eduardo M. Rocha, Councilor (Brazil)

TFOS Corporate Sponsors

Akorn
Alcon
Allergan
Bausch+Lomb
CIBA VISION
Contact Lens Spectrum
CooperVision
Eleven Biotherapeutics
EyeGate
Horus Pharmaceutical
IRIS Pharma
Johnson & Johnson
Laboratoires Théa
Menicon
Merck
NicOx
Novaliq
Oculus
Optima Pharmazeutische
Otsuka
Santen
Senju
SIFI
SOOFT Italia
TearLab
TearScience
TRB Chemedica
Vistakon

TFOS Medical & Scientific Advisory Board

Jason J. Nichols, Chair (USA)
Esen Akpek (USA)
Eduardo C. Alfonso (USA)
Dilek D. Altinors (Turkey)
Pasquale Aragona (Italy)
Dimitri Azar (USA)
Stefano Barabino (Italy)
Christophe Baudouin (France)
Carolyn Begley (USA)
Jose Benitez del Castillo (Spain)
Barbara Caffery (Canada)
Virginia L. Calder Knutson (UK)
Shukti Chakravarti (USA)
Robin L. Chalmers (USA)
James Chodosh (USA)
Jennifer Craig Dean (New Zealand)
Reza Dana (USA)
Denise De Freitas (Brazil)
Murat Dogru (Japan)
Henry F. Edelhauser (USA)
M. Elizabeth Fini (USA)
Desmond Fonn (Canada)
Gary N. Foulks (USA)
Dan Gamache (USA)
Gerd Geerling (Germany)
Peter Gierow (Sweden)
Mike Gilmore (USA)
Ilene K. Gipson (USA)
Ben J. Glasgow (USA)
Eiki Goto (Japan)
Kari Green-Church (USA)
Darren Gregory (USA)
May Griffith (Canada)
Michel Guillon (UK)
Linda D. Hazlett (USA)
Jutta Horwath-Winter (Austria)
Lyndon Jones (Canada)
Winston Kao (USA)
Tetsuya Kawakita (Japan)
Jae Chan Kim (Korea)
Shigeru Kinoshita (Japan)
Nadja Knop (Germany)
Donald R. Korb (USA)
Gordon Laurie (USA)
Andrea Leonardi (Italy)
Todd Margolis (USA)
James P. McCulley (USA)
Alison McDermott (USA)
Austin K. Mircheff (USA)
Juan Murube del Castillo (Spain)
J. Daniel Nelson (USA)
Teruo Nishida (Japan)
Gary D. Novack (USA)
Terrence O'Brien (USA)
Yoko Ogawa (Japan)
Santa Jeremy Ono (USA)
Eric Papas (Australia)
Jerry R. Paugh (USA)
Ian Pearce (UK)
Victor Perez (USA)
James W. Putney (USA)
Maurizio Rolando (Italy)
Mark Rosenblatt (USA)
Robert A. Sack (USA)
Debra A. Schaumberg (USA)
Jun Shimazaki (Japan)
Shigeto Shimmura (Japan)
Fiona J. Stapleton (Australia)
John Sutphin (USA)
Donald Tan (Singapore)
Timo Tervo (Finland)
Ikuko Toda (Japan)
Alan Tomlinson (UK)
Louis Tong (Singapore)
Ray Tsai (Taiwan)
John L. Ubels (USA)
Hitoshi Watanabe (Japan)
Kerstin Wickstrom (Sweden)
Mark D. P. Willcox (Australia)
Norihiko Yokoi (Japan)
Manfred Zierhut (Germany)

7th International Conference on the Tear Film & Ocular Surface: Basic Science and Clinical Relevance

Conference Program & Abstract Book

Taormina, Sicily, Italy

September 18-21, 2013

Title Sponsor:

Alcon

TFOS Conference Director

David A. Sullivan (USA)

TFOS Conference Organization & Management

Amy Gallant Sullivan (USA)

TFOS Conference Coordination

Rose M. Sullivan (USA) Linda Herrington (USA) Solange Voss (USA)

TFOS Conference Corporate Sponsors

Alcon (USA) – Title Sponsor

Allergan – Silver Sponsor
Bausch+Lomb – Silver Sponsor
Santen – Silver Sponsor
Akorn
Eleven Biotherapeutics
EyeGate
Horus Pharmaceutical
IRIS Pharma
Menicon
SIFI – Bronze Sponsor
TearLab – Bronze Sponsor
Vistakon – Bronze Sponsor
NicOx
Novaliq
Otsuka
Senju
SOOFT Italia
TearScience

TFOS Conference Scientific Program Committee

James Aquavella (USA)
Stefano Bonini (Italy)
Anthony Bron (UK)
James Chodosh (USA)
Darlene Dartt (USA)
Murat Dogru (Japan)
Mihaela Gadjeva (USA)
Juana Gallar (Spain)
May Griffith (Sweden)
Sarah Hamm-Alvarez (USA)
Pedram Hamrah (USA)
Motoko Kawashima (Japan)
Donald Korb (USA)
Gordon Laurie (USA)
Florence Malet (France)
Alison McDermott (USA)
Balaraj Menon (USA)
Thomas Millar (Australia)
Christine Purslow (UK)
Shigeto Shimmura (Japan)
David A. Sullivan (USA)
Fiona Stapleton (Australia)
Mark Willcox (Australia)
Jianjiang Xu (China)

TFOS Young Investigator Committee

Monica Alves (Brazil)
Mindy Call (USA)
Cintia S. De Paiva (USA)
Ana Isabel Guzman-Aranguez (Spain)
Osama Ibrahim (Japan)
Georgios Kontadakis (Greece)
Kaevalin Lekhanont (Thailand)
Flavio Mantelli (Italy)
Maria Markoulli (Australia)
Rachel L. Redfern Rump (USA)
Stefan Schrader (Germany)
Benjamin D. Sullivan (USA)
Louis H.T. Tong (Singapore)
Tais Hitomi Wakamatsu (Brazil)

Preface

A significant, international research effort is being directed towards understanding the composition, function and regulation of the preocular tear film. This effort is motivated by the recognition that the tear film plays a critical role in maintaining corneal and conjunctival integrity, protecting against microbial challenge and preserving visual acuity. In addition, research is stimulated by the knowledge that tear film deficiency, which occurs in countless individuals throughout the world, may lead to ocular surface desiccation, corneal ulceration, an increased incidence of infectious disease, and potentially pronounced visual disability.

To promote further progress in this field of vision research, the 7th International Conference on the Tear Film & Ocular Surface: Basic Science and Clinical Relevance will be held at the Palazzo dei Congressi in Taormina, Sicily, Italy, from September 18 to 21, 2013. This Conference, which is sponsored by TFOS (www.TearFilm.org), is designed to assess the current knowledge and 'state of the art' research on the structure and function of tear film-producing tissues, tears and the ocular surface in both health and disease. The goal of this Conference is to promote an international exchange of information that will be of value to basic scientists involved in eye research, to clinicians in the eye care community, and to pharmaceutical and diagnostic companies with an interest in tear film or ocular surface disorders.

To help achieve this objective, numerous scientists, clinicians and industry representatives from 41 countries, including Algeria, Argentina, Australia, Austria, Belgium, Brazil, Bulgaria, Canada, China, Croatia, Czech Republic, Denmark, Finland, France, Georgia, Germany, Greece, Hungary, India, Italy, Japan, Netherlands, New Zealand, Nigeria, Norway, Poland, Portugal, Romania, Russia, Singapore, South Korea, Spain, Sweden, Switzerland, Taiwan, Thailand, Turkey, United Kingdom, United States, Uruguay and Venezuela have registered as participants in this Conference.

This book contains the scientific program, as well as the abstracts of the keynote, oral and poster presentations, of this TFOS Conference.

David A. Sullivan
President & Founder

Acknowledgments

TFOS expresses its appreciation to Sabrina Zappia and CITYnet (www.citynetonline.it), Julie Karimi and JAKA Congressi (www.jaka.it), Haydée Marangoni and h.design (www.hdesign.biz), the Comune di Taormina, Palazzo dei Congressi, San Domenico Palace Hotel, La Botte, La Giara, and the Q Lounge Bar for their help with this Conference.

Recognition

TFOS congratulates the following individuals, who were the recipients of the Conference Young Investigator Awards: Shruti Aggarwal (USA), Terry Coursey (USA), Juan Ding (USA), Giulio Ferrari (Italy), Jing Hua (USA), Kyu-Yeon Hwang (South Korea), Wendy R. Kam (USA), Zhen Meng (USA), Sang-Mok Lee (USA), Geraint Parfitt (USA), Rose Reins (USA), Xio Wei Tan (Singapore), Kokoro Sano (Japan) and Martin Schicht (Germany).

Thursday, September 19, 2013

Welcome

8:00 *Giuseppe Castronovo (Italy)*

Opening Remarks

8:05 *Shigeru Kinoshita (Japan)*

5th Claes H. Dohlman Conference Address

Chairperson – Shigeru Kinoshita (Japan)

8:10 The Importance of Translational Research in Medicine and Ophthalmology. Dimitri Azar. University of Illinois College of Medicine, Chicago, IL, USA

Session I

Translating Tear Film & Ocular Surface Protein Discoveries into Treatments

Chairpersons - Jose Benitez del Castillo (Spain), Gordon W. Laurie (USA), Martin Schicht (Germany)

8:35 **Keynote Address:** Proteoglycan 4 (PRG4, lubricin) as the Natural Ocular Surface Boundary Lubricant: Translating an Idea into a Cure. Tannin A Schmidt. University of Calgary, Calgary, Canada

9:00 **Keynote Address**: Trefoil Family Peptide 3. Friedrich Paulsen. Department of Anatomy, Friedrich Alexander University Erlangen Nuremberg, Erlangen, Germany

9:25 **Keynote Address:** Protein polymer mediated delivery of lacritin therapeutics. J. Andrew MacKay1, Wan Wang1, Gordon W. Laurie2, Sarah Hamm-Alvarez1. 1University of Southern California School of Pharmacy, Los Angeles CA; 2University of Virginia School of Medicine, Charlottesville, VA US

9:50 Changes To The Tear Film Proteome In Keratoconus. Siva Balasubramanian1, David Pye2, Mark Willcox2. Deakin School of Medicine, 1 University of New South Wales, Australia 2

10:05 **Poster Session I (with Coffee & Tea)**

Cutting-Edge Surgery: Induction and Treatment of Ocular Surface Disease

Chairpersons - James V. Aquavella (USA), Shigeto Shimmura (Japan) , Jianjiang Xu (China)

10:55 **Keynote Address**: Surgery-Induced Dry Eye Disease (i.e.. Refractive, Cataract, PK, Plastic). José AP Gomes. Cornea/External Disease Service and Advanced Ocular Surface Center, Department of Ophthalmology, Federal University of São Paulo, Brazil

11:20 **Keynote Address**: New Innovations In The Surgical Treatment Of Ocular Surface Disease. Jodhbir S. Mehta. Cornea and External Eye Disease Service, Singapore National Eye Centre, Singapore

11:45 **Keynote Address:** Use and Misuse of Amniotic Membrane Transplantation in Ophthalmology. Sophie Deng. Jules Stein Eye Institute, University of California, Los Angeles CA USA

12:10 Quantitative Comparative Proteomics Of Human Tear Fluid And Labial Saliva Provides Physiological/ Biochemical Evidence Supporting Salivary Gland Transplantation For Dry Eye Treatment. Remco Crefcoeur1, Peter Raus1,2,3, Maithili Krishnan1, Martijn Pinkse1, Leen Slaets1,4, Gordon Laurie5, Niels Hellings1,4, Peter Verhaert1,4. 1Department of Biotechnology, Delft University of Technology, Delft, Netherlands; 2 Miró Center for Ophtalmology, Mol, Belgium; 3Faculty of Medicine and Pharmacy, Free University of Brussels; 4Biomedical Research Center, Hasselt University, Hasselt, Belgium; 5Laboratory for Cell Biology, University of Virginia, VA, USA

12:25 **Poster Viewing & Lunch**

Poster Discussion I

Chairpersons - Shruti Aggarwal (USA), Darlene A Dartt (USA), Jun Shimazaki (Japan)

13:55 RAB3D And RAB27 Play Distinct Roles In Regulating Tear Protein Secretion From Lacrimal Gland Acinar Cells. Zhen Meng1, Maria Edman-Woolcott1, Yu Zhou2, Ebrahim Zandi2, Sarah Hamm-Alvarez1. School of Pharmacy1, Keck School of Medicine2, University of Southern California, Los Angeles, CA, USA

14:00 Tracking The Progression Of Tear Break-Up. Carolyn Begley1, Adam Winkeler1, Richard Braun2. Indiana University1 University of Delaware2 USA

14:05 Comparison Of Reading And Blink Rates In Dry Eye And Normal Subjects During Visual Function And Reading Tasks. George Ousler1, Keith Lane1, Endri Angjeli1, Colleen Heckley1, John Rodriguez1. Ora, Inc., Andover, MA, USA

14:10 Long-Term Results Of The Treatment By 3% Diquafosol Ophthalmic Solution In Dry-Eye Patients With Sjögren's Syndrome. Yukiko Sonomura1,2, Norihiko Yokoi2, Aoi Komuro2,3, Hiroaki Kato2, and Shigeru Kinoshita2. 1Department of Ophthalmology, Kyoto Yamashiro General Medical Center, Kyoto, Japan; 2Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan; 3Komuro Eye Clinic, Kyoto, Japan

New & Emerging Clinical Endpoints for Ocular Surface Disease

Chairpersons - Anthony J. Bron (UK), Kyu-Yeon Hwang (Korea), Christine Purslow (UK)

14:15 **Keynote Address**: A State Of The Art Analysis Of Osmolarity As A Diagnostic Measure For Dry Eye Disease. Benjamin D. Sullivan. TearLab Corp., San Diego CA, USA

14:40 **Keynote Address:** Towards Quantitative Imaging Of The Tear Film Dynamics And Understanding Its Clinical Relevance. Jannick P. Rolland. University of Rochester, Rochester, NY, USA

15:05 **Keynote Address**: Salivary Biomarkers for Disease Detection. David Wong. University of California Los Angeles Dental Research Institute, Los Angeles CA, USA

15:30 The Basis Of Staining Of The Ocular Surface By Topical Dyes. Anthony J. Bron1, Pablo Arguëso2, Murat Irkec3, Frank V. Bright4. Nuffield Laboratory of Ophthalmology, Nuffield Department of Clinical Neurosciences, University of Oxford, UK, 1Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, MA, USA, 2Department of Ophthalmology, Hacettepe University School of Medicine, Ankara, Turkey, 3Department of Chemistry, The State University of New York, Buffalo, New York, USA4

15:45 **Poster Session I (with Coffee & Tea)**

Macro to Micro: Imaging Approaches for Understanding the Ocular Surface

Chairpersons – Murat Dogru (Japan), Pedram Hamrah (USA), Sang-Mok Lee (USA)

16:35 **Keynote Address:** Imaging Inflammatory Processes *In Vivo*. Paul Kubes. University of Calgary, Calgary, Alberta, Canada

17:00 **Keynote Address**: Imaging The Ocular Surface *In Vivo* In Health And Disease. Christophe Baudouin. Quninze-Vingts Hospital AP-HP Univ Paris, Ophthalmology, Paris, France

17:25 *In Vivo* High Resolution MRI Study in a Corneal Alkali Burn Model. Fabio Bignami1, Giulio Ferrari1, Chiara Giacomini1, Eleonora Capitolo2, Linda Chaabane2, Paolo Rama1. Cornea and Ocular Surface Unit - Eye Repair Lab, 1Institute of Experimental Neurology - Division of Neuroscience, 2San Raffaele Scientific Institute, Milan, Italy.

17:40 Corneal Nerves May Mediate Corneal Dendritic Cell Trafficking—An Intravital Multiphoton Microscopy Study. Pedram Hamrah1,2, Takefumi Yamaguchi1,2, Kai Hu1,2, Deshea Harris2. 1Cornea Service, and Schepens Eye Research Institute, Massachusetts Eye & Ear Infirmary, Department of Ophthalmology and 2Harvard Medical School, Boston, MA

Poster Session I

Chairpersons - Shruti Aggarwal (USA), Darlene A Dartt (USA), Jun Shimazaki (Japan)

1 Prompt Versus Delayed Application Of Amniotic Membrane In A Patient With Stevens-Johnson Syndrome. Kimberly C. Sippel, Jessica B. Ciralsky. [1]Department of Ophthalmology, Weill Cornell Medical College[2], New York, NY USA

2 Effect of Tear Film Instability on High Order Aberrations of the Corneal Surface after Laser Subepithelial Keratomileusis. Kyung Chul Yoon, Hyun Ho Jung, Han Jin Oh Department of Ophthalmology, Chonnam National University Medical School and Hospital, Gwangju, Korea

3 The Effect Of Surgical Technique On The Performance Of Cell-Free Collagen Type III Corneal Implants. Rodolfo A. Elizondo1, Monika Kozak Ljunggren1, David Olsen2, Kimberley Merrett1,3, Chyan-Jang Lee1,3, Göran Salerud4, James Polarek2, Per Fagerholm1, May Griffith1,3. Integrative Regenerative Medicine Centre & Dept. of Clinical and Experimental Medicine, Linköping University, Sweden1; FibroGen Inc., San Francisco, CA, USA2; Ottawa Hospital Research Institute, Ottawa, Ontario, Canada3; Integrative Regenerative Medicine Centre & Dept. of Biomedical Engineering, Linköping University, Sweden4

4 Toward A Tissue And Gene Therapy Of Herpes Related Corneal Blindness. Eric E. Gabison1, 2, Marc Labetoulle4, Marine Gailledrat5, Jose A. Sahel3, Benoit Chapellier 3. 1Cornea Department, Fondation A. de Rothschild, Paris, France. 2Ophthalmology Department, Hôpital Bichat, Paris, France. 3Institut de la vision, Paris, France. 4Ophthalmology Department, Hôpital du Kremlin Bicêtre, Paris, France. 5Cellectis, Paris, France.

5 Histological Changes in Eye-Banked Human Corneas after Single and Repeated Cross-Linking Treatment with Riboflavin and UV-A. Ithar M. Beshtawi1, Chantal Hillarby1, Clare O'Donnell1,2, Arun Brahma3, Fiona Carley3, Hema Radhakrishnan1. 1The University of Manchester, 2Optegra, 3Manchester Royal Eye Hospital, Manchester, UK

6 Ocular Surface Temperature Changes after Cataract Surgery. Giannaccare G, Versura P, Strobbe E, Cellini M, Bravetti GO, Campos EC Ophthalmology Unit, DIMES, Alma Mater Studiorum University of Bologna, S. Orsola-Malpighi Teaching Hospital, Bologna – Italy

7 A prospective randomized comparative study on the efficacy and safety of three different surgical procedures for conjunctivochalasis. Seika Shimazaki-Den1, Daisuke Tomida1, Miki Iwasaki2, Murat Dogru1, Jun Shimazaki1. Tokyo Dental College Ichikawa General Hospital1, Chiba, Japan; Ryogoku Eye Clinic2, Tokyo, Japan

8 Effect of Phototoxicity on the Ocular Surface and Tear Film by an Operating Microscope. Hyung Bin Hwang, M.D., M.S., Hyun Seung Kim, M.D., Ph.D. Department of Ophthalmology, St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea

9 Effect of Botulinum Toxin Type A Injection for Lateral Canthal Rhytides on Tear Film Stability and Tear Production. Min-Chieh Ho1, Wei-Cherng Hsu1,2, Yi-Ting Hsieh1,2. Department of Ophthalmology, Buddhist Tzu Chi General Hospital, Taipei Branch, New Taipei, Taiwan1 School of Medicine, Tzu Chi University, Hualien, Taiwan2

10 The Effect of Botulinum Toxin Type A: A Treatment for Blepharospasmic Patients on Tear Film Property. Yuka Hosotani, Yumiko Nakamura, Osamu Mimura. Department of Ophthalmology, Hyogo College of Medicine, JAPAN

11 Ocular Surface Alterations In Blepharospasm Patients Treated With Botulinum Toxin-A Injections. Murat Irkec1, Sibel Kocabeyoglu1, Hande Taylan Sekeroglu1, Mehmet C. Mocan1, Ali Sefik Sanac1. Hacettepe University School of Medicine,1, Ankara, Turkey

12 Dry Eye Disease and Tear Osmolarity Following Botulinum Toxin Injection in Patients with Blepharospas. A Denoyer1-3, C Virevialle1,2, G Rabut1,2, A Labbé1-4, and C Baudouin1-4. Quinze-Vingts National Ophthalmology Hospital1, Paris, France; 2Clinical Center for Investigation, INSERM CIC 503, Paris, France; 3Institut de la Vision, University Paris 6, INSERM UMRS968, CNRS U7210, Paris, France; 4University of Versailles, Versailles, France.

13 The Effect of Contact Lens on Tear Osmolarity Depends on the Tear Osmolarity Itself. Alessandro Fossetti1,2, Francesco Cozza1, Carlo Falleni1,4, Alessandro Farini1,3 , Jacopo Siroki1. Istituto Regionale Studi Ottici e Optometrici (IRSOO)1, Vinci, Italy, Università degli Studi di Firenze2, Italy, INO -CNR, Firenze3, Italy, Università degli Studi di Torino4, Italy

14 Tear Volume, Osmolarity And Optical Quality In Silicone Hydrogel Contact Lens Wearers. Giancarlo Montani1, Sudhir Patel2. Università del Salento Italy1, NHS National Services, Edinburgh, Scotland, UK2

15 Tear Volume And Osmolarity In Keratoconus Patients Fitted With Rigid Gas Permeable Lenses. Giancarlo Montani,1 Sudhir Patel,2 Università del Salento Italy1, NHS National Services, Edinburgh, Scotland, UK2

16 Intrasubject Tear Osmolarity And Tear Meniscus Height Changes With Two Types Of Eye Drops. Giancarlo Montani1, Michele Carullo2. Università del Salento1, Ambulatorio di 3° livello Ospedale di Conversano ASL/BA2, Italy

17 Dry Eye Animal Models: Tear Osmolarity And Ocular Surface Dysfunction. Davi L Marques1, Monica Alves,MD PhD1, 2, Carolina Maria ModuloMSc1, Adriana Andrade Batista Murashima1, Lilian Eslaine Costa Mendes da SilvaMSc1, Eduardo Melani RochaMD PhD1. 1Faculty of Medicine of Ribeirão Preto, University of São Paulo; 2Pontific Catholic University of Campinas, Brazil

18 Comparison Of Ocular Surface Disease Index And Tear Osmolarity As A Marker Of Ocular Surface Dysfunction In VDT Workers. Pasquale Aragona MD, PhD1, Rosaria Spinella MD, PhD1, Laura Rania MD, PhD1, Elisa Postorino MD, PhD1 and Concettina Fenga MD2. 1Department of Experimental Medical-Surgical Sciences, Section of Ophthalmology, University of Messina, Messina, Italy. 2Department of Social and Environmental Health, Section of Occupational Health, University of Messina, Messina, Ital.

19 Computed Tear Film And Osmolarity Dynamics On An Eye-Shaped Domain. Longfei Li1, R.J. Braun1, W. D. Henshaw2, J.T. Banks2, and P.E. King-Smith3. 1Department of Mathematical Sciences, University of Delaware, Newark, DE 19716-2553 USA. 2Lawrence Livermore National Laboratory, Box 808, L-550, Livermore, CA 94551-0808 USA. 3College of Optometry, The Ohio State University, Columbus, OH 43210-1280 USA.

20 Mathematical Modeling Of Tear Break-Up And Fluorescent Intensity. R.J. Braun1, N. Gewecke1, C.G. Begley2, P.E. King-Smith3, J.I. Siddique4. 1Dept of Mathematical Sciences, University of Delaware, Newark, DE 19716; 2School of Optometry, Indiana University, Bloomington, IN; 3College of Optometry, The Ohio State University, Columbus, OH 43210; 4Dept of Mathematics, Pennsylvania State University, York, PA

21 Computational Models Of Tear Film Breakup. R.J. Braun1, and P.E. King-Smith2, and J.J. Nichols3. 1Department of Mathematical Sciences, University of Delaware, Newark, DE 19716-2553 USA. 2College of Optometry, The Ohio State University, Columbus, OH 43210-1280 USA. 3College of Optometry, University of Houston, Houston, TX 77204 USA.

22 The Effect Of The Blink Cycle On The Multilamellar Model Of The Tear Film Lipid Layer. P. Ewen King-Smith1, Richard J. Braun2, Kathleen S. Reuter1, Daniel Powell1, Heather L. Chandler1, Melissa D. Bailey. 1College of Optometry, Ohio State University, Columbus, OH, 2Mathematical Sciences Dept., University of Delaware, Newark, DE U.S.A

23 **Discussion**: Tracking The Progression Of Tear Break-Up. Carolyn Begley1, Adam Winkeler1, Richard Braun2. Indiana University1 University of Delaware2 USA

24 The Tear Film as a Fluid Shell. Anthony J. Bron1, Norihiko Yokoi2, Georgi A. Georgiev3. Nuffield Laboratory of Ophthalmology, University of Oxford, UK, 1Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan2, Department of Biochemistry, University of Sofia, Sofia, Bulgaria3

25 The Effect of Noninvasive Tear Film Break-Up Time Measurement on Tear Meniscus Height. Shinya Watanabe1, Shizuka Koh1, Hitoshi Watanabe1,2, Chikako Ikeda1,Yoshihiro Takai1, Motokazu Tsujikawa1, Naoyuki Maeda1, Kohji Nishida1. 1Department of Ophthalmology, Osaka University Graduate School of Medicine, Osaka, Japan, 2Kansai Rosai Hospital, Amagasaki, Japan

26 Increased Tear Film Break-Up Time as a Sensitive Indicator in the Diagnosis of Early Dry Eye in Type 2 Diabetics. Rajarathna Thangavel1, Manjula Jain2, Rajesh Jain1, Pamela D'Souza1. Department of Ophthalmology1, Department of Pathology2, Lady Hardinge Medical College, New Delhi, India

27 Evaluation Of Age-Related And Gender-Related Changes In Noninvasive Tear Break-Up Time With A Newly Developed Keratograph. Jiaxu Hong1, Zuguo Liu2, Xinghuai Sun1, Jianjiang Xu1. 1Department of Ophthalmology and Visual Science, Eye, Ear, Nose, and Throat, Hospital, School of Shanghai Medicine, Fudan University, 83 Fenyang Road, Shanghai 200031, China; 2Eye Institute of Xiamen University, Fujian 361005, China

28 Noninvasive Observation of Tear Film Break-Up with Newly Developed Keratograph. Shizuka Koh1, Chikako Ikeda1, Yoshihiro Takai1, Shinya Watanabe1, Hitoshi Watanabe1,2, Motokazu Tsujikawa1, Naoyuki Maeda1, Kohji Nishida1. 1Department of Ophthalmology, Osaka University Graduate School of Medicine, Osaka, Japan, 2Kansai Rosai Hospital, Amagasaki, Japan

29 The Evaluation of Maximal Opening Time-Break Up Time Index in Contact Lens Wearers Exposed to Adverse Environment Conditions. Akiko Endo1, Murat Dogru1, Naoki Toriyama1, Kozue Kasai1,Daisuke Tomida1,Takefumi Yamaguchi1,Kazunari Higa1, Seika Den Shimazaki1, Yoshiyuki Satake1, Jun Shimazaki1. 1Tokyo Dental College Ichikawa General Hospital, Department of Ophthalmology, Ichikawa, Chiba, Japan

30 Beyond Non Invasive Break Up Time: A Novel Method To Quantify Contact Lens On Eye Wetting Kinetics Over The Full Interblink Period. Michel Guillon1,2, Cécile Maissa1,2. 1OTG Research & Consultancy, London, UK; 2School of Life and Health Science, Aston University, Birmingham, UK

31 Influences of Radiotherapy on Meibomian Glands and Tear Film. Takahiro Hiraoka, Yasuaki Ito, Tetsuro Oshika. Department of Ophthalmology, Faculty of Medicine, University of Tsukuba; Ibaraki, Japan

32 Study Of Meibomian Gland Distribution Of The Lower Lid In Children. Dominique Bremond-Gignac1,2, Heiko Pult3,4, Frédéric Chiambaretta5. 1Ophthalmology, St Victor Center, University Hospital of Amiens, UPJV, Amiens, France. 2CNRS IRIS UMR8194, Paris V University, France. 3Optometry 1 Vision research, Weinheim, Germany. 4Cardiff University, School of Optometry & Vision Sciencs, Cardiff, UK. 5Ophthalmology, University Hospital of Clermont-Ferrand, France

33 The Repeatability Of Clinical Measurements Of Dry Eye And Meibomian Gland Dysfunction. Maria Markoulli2 Brien Holden1,2,3 Eric Papas1,2,3 1Brien Holden Vision Institute, Sydney, Australia, 2School of Optometry & Vision Science, University of New South Wales, Sydney, Australia, Sydney, Australia, 2School of Optometry & Vision Science, University of New South Wales, Sydney, Australia, 3Vision Cooperative Research Centre, Sydney, Australia

34 Meibomian Gland Dysfunction (MGD) Prevalence: Sensitivity to Diagnostic Criteria. Nisha Yeotikar1, Hua Zhu1,2, Negar Babaei1, Daniel Tilia1, Eric Papas1,2,3. Brien Holden Vision Institute1, School of Optometry and Vision Science, University of New South Wales2, Vision CRC3, Sydney, Australia

35 Development And Use Of A Novel Inexpensive Meibomian Gland Transillumination Device. E. Ian Pearce, Michelle Snowball, Anna Green, Melissa McKinnon, Elaine F. Wilson. Vision Sciences, Glasgow Caledonian University, Glasgow, Scotland

36 Infra-Red Imaging of Meibomian Glands & Evaluation of the Lipid Layer in Sjogren's Syndrome Patients. Sruthi Srinivasan, Kara Menzies, Lyndon Jones. CCLR-School of Optometry, University of Waterloo, Waterloo, Ontario. Canada

37 Inter- and Intra-Observer Agreement and Repeatability of Imaging the Meibomian Glands with the Oculus Keratograph 4 and Keratograph 5M. William Ngo, Sruthi Srinivasan, Marc Schulze, Lyndon Jones. CCLR-School of Optometry, University of Waterloo, Waterloo, Ontario. Canada

38 Automated Measurement Of Meibomian Gland Dropout. Carolina M.E. Kunnen1,2,3, Percy Lazon de la Jara1,2, Brien A. Holden1,2,3, Eric B. Papas1,2,3. 1Brien Holden Vision Institute, Sydney, NSW, Australia; 2School of Optometry and Vision Science, University of New South Wales, Sydney, NSW, Australia; 3Vision CRC, Sydney, NSW, Australia

39 Meibomian Gland Function Cannot be Predicted by Meibography Unless There is Total Meibomian Gland Drop Out in Patients with MGD. Donald Korb1,2, Caroline Blackie1,2, Heiko Pult PhD3. Korb Associates, Boston, MA1, TearScience Inc, Morrisville, NC2, Cardiff University, UK3

40 Debridement-Scaling of the Line of Marx and Keratinized Lower Lid Margin is Effective in Increasing Meibomian Gland Function and Patient Comfort. Donald Korb 1,2, Caroline Blackie1,2. Korb Associates1, Boston, MA, TearScience Inc, Morrisville, NC2

41 Marx Line – Association with Parameters of the Ocular Surface System. Dieter Franz Rabensteiner1, Ingrid Boldin1, Gerold Schwantzer2, Manuela Fischl1, Christine Wachswender1, Haleh Aminfar1, Jutta Horwath-Winter1. Department of Ophthalmology1, Institute for Medical Informatics, Statistics and Documentation2, Medical University of Graz, Austria

42 A New Tearscope (Hosik's Tearscope) Made of Papter for Lipid Layer Evaluation in Tearfilm Related Disease. Man Soo Kim1, Kyoung Sook Cho2, Ho Sik Hwang3, Catholic University of Korea1, Kyoungwon University2, Hallym University3 Korea

43 Identification Of Lipid Biomarkers For Dry Eye Disease In Post-Menopausal Women Using Shot-Gun Electrospray Mass Spectrometry. Kelly K. Nichols1, Jianzhong Chen2, Kari B. Green2. College of Optometry, University of Houston, Houston, Texas, USA1, Mass Spectrometry and Proteomics Facility, The Ohio State University2, Columbus, OH, USA2

44 Protein Biomarkers In Post-Menopausal Dry Eye Disease. Kari B. Green1, Sasha Popova-Butler1, Liwen Zhang1, Kelly K. Nichols2. Mass Spectrometry and Proteomics Facility, The Ohio State University, Columbus, OH, USA1, College of Optometry, University of Houston, Houston TX, USA2

45 Is There a Relationship Between Serum Levels of 3α-diol-G and DHEA-S and Dry Eye Symptoms In Post-Menopausal Women? Hampel, Ulrike1, 2; Golebiowski, Blanka 1; Badarudin, Noor1; Stapleton, Fiona1. 1School of Optometry and Vision Science, University of New South Wales, Sydney, NSW, Australia. 2Department of Anatomy 2, Friedrich Alexander University Erlangen Nürnberg, Erlangen, Germany

46 Higher Plasma Levels of Oestradiol are Associated with Increased Ocular Discomfort in Women. Fiona Stapleton, Ulrike Hampel, Noor Badarudin, Moneisha Gokhale, Michele Madigan, Isabelle Jalbert, Blanka Golebiowski. School of Optometry and Vision Science, University of New South Wales, Sydney, Australia

47 Meibomian Gland Dysfunction: Endocrine Aspects. Ozlem G Sahin1, Elçin Kartal2, Nusret Taheri3. Dunya Goz Hospital, Department of Ophthalmology/Uveitis1, Middle East Technical University Department of Statistics2, Middle East Technical University Health Sciences Department of Biochemistry3, Turkey

48 Evaluation of Dry Eye Disease Among Office Workers Using New Instruments: The Osaka Study in Moriguchi. Motoko Kawashima1, Norihiko Yokoi2, Masaki Fukui1, Yoshiyuki Ichihashi1, Hiroaki Kato2, Motoko Yamatsuji3, Mitsuko Nishida3, Shigeru Kinoshita2, Kazuo Tsubota1. 1Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan, 2Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan, 3IT products business unit, AVC Networks Company, Panasonic Corporation, Osaka, Japan

49 Work Productivity Loss Among Visual Display Workers with Dry Eye Disease: Osaka Study. Miki Uchino1, Yuichi Uchino1, Murat Dogru1, Motoko Kawashima1, Norihiko Yokoi2, Aoi Komuro2, Yukiko Sonomura2, Hiroaki Kato2, Shigeru Kinoshita2, Debra A. Schaumberg3,4, Kazuo Tsubota1. Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan1; Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan2, Department of Ophthalmology & Visual Sciences, University of Utah, Salt Lake City, UT, USA3, Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA4

50 Why Psychological Complaints Complicate The Symptoms of Dry Eyes. Johannes Nepp. MD Department of Ophthalmology, Medical University Vienna, Austria; ÖGPPM Austrian Society for Psychosomatic and Psychotherapeutic Medicine

51 **Discussion**: Comparison Of Reading And Blink Rates In Dry Eye And Normal Subjects During Visual Function And Reading Tasks. George Ousler1, Keith Lane1, Endri Angjeli1, Colleen Heckley1, John Rodriguez1. Ora, Inc., Andover, MA, USA

52 A Series Of Pilot Studies To Assess Diurnal Variation In A Dry Eye And Normals Utlizing Continuous Blink Capture. George Ousler1, Keith Lane1, Endri Angjeli1, Colleen Heckley1, John Rodriguez1. Ora, Inc., Andover, MA, USA

53 Central Lid Margin Position And Tear Film Spreading During 'LID CLOSURE' in Spontaneous Blinking. Heiko Pult1,2, Donald Korb3, Paul Murphy4, Caroline Blackie3, Britta H. Riede-Pult1,2. 1Dr. Heiko Pult – Optometry & Vision Research, DE; 2Cardiff University, School of Optometry & Vision Sciences, UK; 3Korb Associates and TearScience Inc., USA; 4University of Waterloo, School of Optometry & Vision Sciences, CA

54 The Korb-Blackie Lid Light Test. Victor Finnemore1, Caroline Blackie1,2, Donald Korb1,2. Korb Associates, Boston, MA,1 TearScience Inc, Morrisville, NC.2

55 Eyelid Disorders Evaluation In The Ophthalmic Current Practice In Germany And Poland. The Meibum Study. Jose Manuel Benitez del Castillo1, Thomas Kaercher2, Zbigniew Zagórski3. 1Hospital Clinico San Carlos, Madrid, Spain; 2Dossenheimer Landstraße 48, 69121 Heidelberg, Germany; 3Pr. Zagorski Eye Surgery Centre, Al. Malachowskiego 5, 24-140 Naleczow, Poland

56 Experience From Running The First Dry Eye Clinic In Poland. Zbigniew Zagórski, Katarzyna Molenda, Agnieszka Kudasiewicz-Kardaszewska, Marta Piecyk-Sidor. Zagórski Eye Surgery Centers in Cracow and Lublin

57 Performance of Dry Eye Tests in a Tertiary Eye Unit in The UK. Francisco C Figueiredo, Philipp Baenninger Dept of Ophthalmology, Royal Victoria Infirmary, Newcastle University, Newcastle upon Tyne, UK

58 Correlations Between Severity of Dry Eye Symptoms and the Use of Systemic Prescription Drugs in a Cohort of Dry Eye Patients. Jon Roger Eidet1,2, Xiangjun Chen1,3, Tor Paaske Utheim1,2,3,4, Øygunn Aass Utheim1,2,4, Aleks Stojanovic1,3, Filip Stojanovic1,3, Sten Raeder1,3,5, Tørreøyneklinikken1. (Dry Eye Clinic, Oslo, Norway) Department of Medical Biochemistry, 2Oslo University Hospital, Oslo, Norway; SynsLaser Kirurgi, 3Oslo and Tromsø, Norway; Schepens Eye Research Institute, 4Massachusetts Eye and Ear, Harvard Medical School, Boston, MA, USA; Department of Clinical Medicine, 5Section of Ophthalmology, University of Bergen, Bergen, Norway

59 Predictors Of Tear Film-Associated Optical Degradation. Holly Hindman, Michael Chen, Christine Callan, Ranjini Kottaiyan, Gheorghe Salahura, Geunyoung Yoon, James Zavislan, & James Aquavella. University of Rochester, Rochester, NY

60 Optical Aberrations of the Corneal Surface: 3 Months Follow Up Investigation. Balamurali Vasudevan. College of Optometry, Midwestern University, Glendale, AZ, USA

61 Non-invasive objective metrics of bulbar hyperemia for clinical trial endpoints. Neha Gadaria-Rathod, Kyu-In Lee, Benyamin Y Ebrahim, Penny A Asbell. Dept. Ophthalmology, The Icahn School of Medicine at Mount Sinai. New York, NY, USA

62 Investigation of Screening Standards of Dry-Eye Patients Using Functional Visual Acuity Measurement System. Minako Kaido1, Miki Uchino1, Yu-ichi Uchino1, Norihiko Yokoi2, Dogru Murato1, Motoko Kawashima1, Masaki Fukui1, Yoshiyuki Ichihashi1, Aoi Komuro2, Yukiko Sonomura2, Hiroaki Kato2, Motoko Yamatsuji3, Mitsuko Nishida3, Shigeru Kinoshita2, Kazuo Tsubota1. 1Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan, 2Department of Ophthalmology, Kyoto Prefectural University, of Medicine, Kyoto, Japan, 3Health care section, IT Products Business Division, AVC Networks Company, Panasonic Corporation, Osaka, Japan

63 **Discussion**: Long-Term Results Of The Treatment By 3% Diquafosol Ophthalmic Solution In Dry-Eye Patients With Sjögren's Syndrome. Yukiko Sonomura1,2, Norihiko Yokoi2, Aoi Komuro2,3, Hiroaki Kato2, and Shigeru Kinoshita2. 1Department of Ophthalmology, Kyoto Yamashiro General Medical Center, Kyoto, Japan; 2Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan; 3Komuro Eye Clinic, Kyoto, Japan

64 Quantifying Tear Film Inflammatory Markers Using A Novel, Multiplex Electrochemiluminescent Technique. Lakshman Subbaraman, Mirunalni Thangavelu, David McCanna, Lyndon Jones Centre for Contact Lens Research, School of Optometry and Vision Science, Waterloo, ON, Canada

65 Glucose Metabolites Changes and Their Impact on the Changes in Dry Eye Induced Mouse Lacrimal Gland. Hyung Keun Lee1,2, Yong Woo Ji1, Hye Mi Noh1, Eun Ae Jung1. 1Institute of Vision Research, Department of Ophthalmology, Yonsei University College of Medicine, Seoul, South Korea, 2Severance Institute for Vascular and Metabolic Research, Yonsei University College of Medicine, Seoul, Korea

66 **Discussion**: RAB3D And RAB27 Play Distinct Roles In Regulating Tear Protein Secretion From Lacrimal Gland Acinar Cells. Zhen Meng1, Maria Edman-Woolcott1, Yu Zhou2, Ebrahim Zandi2, Sarah Hamm-Alvarez1. School of Pharmacy1, Keck School of Medicine2, University of Southern California, Los Angeles, CA, USA

67 Compositional Monosaccharide Analysis Of Marine Mammal Tears. Robin Kelleher Davis and Pablo Argüeso Schepens Eye Research Institute and Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, Massachusetts, USA

68 Correlations Of Clinical Signs In Dry Eye Disease With Corneal Immune Cell Influx: An In Vivo Confocal Microscopy Study. Shruti Aggarwal, Ahmad Kheirkhah, Bernardo Cavalcanti, Pedram Hamrah. Massachusetts Eye and Ear Infirmary, Department of Ophthalmology, Harvard Medical School, Boston, MA, USA

69 Autologous serum tear use results in early nerve regeneration by In Vivo Confocal Microscopy and significant improvement in symptoms of corneal neuropathy-related light sensitivity; Shruti Aggarwal, Ahmad Kheirkhah, Bernardo Cavalcanti, Pedram Hamrah. Massachusetts Eye and Ear Infirmary, Department of Ophthalmology, Harvard Medical School, Boston, MA, USA

70 Automatic Quantification of Optical Coherene Tomography Images for Evaluation of the Ocular Surface Shape Beyond the Limbus. Tina Hakimi1,2, Arthur Ho1,2,3, Fabian Conrad1,2, Brien

Holden1,2. 1Brien Holden Vision Institute; 2School of Optometry and Vision Science University of New South Wales, Sydney, NSW, Australia; 3University of Miami, Millers School of Medicine, Miami, Florida, USA

71 Laser in vivo Confocal Microscopy Detects Bilateral Changes In Corneal Immune Cell And Subbasal Nerve Plexus In Unilateral Herpes Zoster Ophthalmicus. Bernardo M. Cavalcanti, Andrea Cruzat, Deborah Pavan-Langston, Eric Samayoa, Pedram Hamrah. 1Ocular Surface Imaging Center and Cornea & Refractive Surgery Service, Massachusetts Eye & Ear Infirmary, Department of Ophthalmology, Harvard Medical School, 243 Charles Street, Boston, MA 02114, USA

72 Assessment of Langerhans Cell Density in the Central Cornea in Patients with Severe Dry Eye under Anti-Inflammatory Therapy with Cyclosporine A 0.05% Eye Drops. Christina Jacobi, Eva Schneider, Friedrich E. Kruse. Department of Ophthalmology, University of Erlangen-Nuremberg, Germany

Friday, September 20, 2013

Session II

Meibomian Gland Dysfunction: Update

Chairpersons - Juan Ding (USA), Erich Knop (Germany), Donald R. Korb (USA)

8:00 **Keynote Address:** Epidemiology and Risk Factors for Meibomian Gland Dysfunction (MGD). Debra A. Schaumberg. Moran Eye Center, University of Utah; Clinical & Epidemiologic Research at Moran Center for Translational Medicine, Salt Lake City, UT, USA; Harvard Medical School, Division of Preventive Medicine, Brigham & Women's Hospital, Boston MA, USA

8:25 **Keynote Address:** Pathophysiology, Diagnosis, And Treatment Of Meibomian Gland Dysfunction. Kelly K. Nichols. College of Optometry, University of Houston, Texas, USA

8:50 **Keynote Address:** Regulation of the human meibomian gland in health and disease. David A. Sullivan, Juan Ding, Wendy R Kam, Yang Liu, Raheleh Rahimi Darabad, Afsun Sahin, Shaohui Liu. Schepens Eye Research Institute, Massachusetts Eye and Ear and Harvard Medical School, Boston, MA, USA

9:15 A Molecular-Level View on the Organization of Lipids in the Tear Film. Alicja Wizert1, D. Robert Iskander1, Lukasz Cwiklik2. Wroclaw University of Technology, Wroclaw, Poland1, Academy of Sciences of the Czech Republic, Prague, Czech Republic2

9:30 **Poster Session II (with Coffee & Tea)**

Ocular Surface Senescence: Programmed or Preventable?

Chairpersons - Motoko Kawashima (Japan), Geraint Parfitt (USA), Eduardo M. Rocha (Brazil)

10:20 **Keynote Address:** The Role Of Nutrition And Exercise In Decelerating The Degenerative Diseases Of Aging. Christiaan Leeuwenburgh. University of Florida, Institute on Aging, Department of Aging and Geriatric Research, Gainesville, FL, USA

10:45 **Keynote Address:** The Effects of Age and Evaporative Stress on Meibomian Gland Function. James V. Jester, Geraint Parfitt, Mikhail Geyfman, Yilu Xie, Donald Brown. Gavin Herbert Eye Institute, University of California, Irvine, Irvine, CA

11:10 **TFOS Business Meeting**

12:10 **Poster Viewing & Lunch**

Poster Discussion II

Chairpersons - Pasquale Aragona (Italy), Wendy R. Kam (USA), Mark Rosenblatt (USA)

13:40 The Anti-Evaporative Effect of the Tear Film Wax Esters. Antti H. Rantamäki1, Susanne K. Wiedmer2, and Juha M. Holopainen1. Helsinki Eye Lab, Department of Ophthalmology1, Department of Chemistry2 University of Helsinki, Helsinki, Finland

13:45 Diurnal Changes of Lipid Inflammatory Mediators in Human Tears with and Without Contact Lenses. Simin Masoudi1, 2, Fiona Stapleton1, 2, Mark Willcox2. 1Brien Holden Vision Institute, Sydney, Australia; 2University of New South Wales, Sydney, Australia

13:50 Use Of Novel IL-1 Receptor Inhibitor (EBI-005) In The Treatment Of Patients With Moderate To Severe Dry Eye Disease. Michael Goldstein1,2, Jennifer Agahigian1, Gregory Zarbis-Papastoitsis1, Kathryn Golden1, Joseph Kovalchin1, Cameron Wheeler1, Siddhartha Chowdury1, Abbie Celniker1, Eric Furfine1. 1Eleven Biotherapeutics, Cambridge, MA, United States, 2Ophthalmology, New England Eye Center/Tufts Medical Center, Boston, MA, United States

13:55 ESC(1-21) A Novel Antimicrobial for Microbial Keratitis? AM McDermott1, SS Kolar1, V. Luca2, G. Mannino2, SM Recupero2, ML Mangoni2. 1The Ocular Surface Institute, University of Houston, College of Optometry, Houston TX, USA; 2Sapienza Università di Roma, Italy

Innate & Adaptive Immunity: For Whom the Bell Tolls

Chairpersons - Stefano Bonini (Italy), Terry Coursey (USA), Alison M. McDermott (USA)

14:00 **Keynote Address:** Role of epithelial cells in mucosal immunity. Richard S. Blumberg. Gastroenterology Division, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts

14:25 **Keynote Address:** Intrinsic Lipid Circuits: Key Regulators Of Ocular Surface Innate Immune And Reparative Responses. Karsten Gronert. Vision Science Program, School of Optometry, University of California Berkeley, CA

14:50 **Keynote Address:** Development And Regulation Of T Cell-Mediated Immunity In Dry Eye Disease. Reza Dana. Massachusetts Eye and Ear Infirmary, Boston MA USA

15:15 Untangling The Mysteries Of The Tear Film Neutrophil. Cameron Postnikoff1, Elena Kreinin1, Robert Pintwala1, Maud Gorbet1,2. 1Systems Design Engineering, 2School of Optometry, University of Waterloo, Waterloo, ON, Canada

15:30 **Poster Session II (with Coffee & Tea)**

Microbiome: Role in Inflammation & Infectious Disease

Chairpersons - James Chodosh (USA), Mihaela Gadjeva (USA), Rose Reins (USA)

16:20 **Keynote Address:** The Human Microbiome - A New Frontier in Human Health. Susan V. Lynch, Division of Gastroenterology, Department of Medicine, University of California, San Francisco, 513 Parnassus Ave., San Francisco, CA

16:45 **Keynote Address:** Ocular Surface Commensal Bacteria In Health And Disease And Innate Immunity On The Ocular Surface. Shigeru Kinoshita. Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan

17:10 **Keynote Address:** Relevance of the microbiome to the ocular surface. Suzanne M. J. Fleiszig. University of California Berkeley, School of Optometry, Berkeley CA USA

17:35 Adenovirus Evolution And The Emergence Of Corneal Pathogens. James Chodosh1, Christopher M. Robinson1, Gurdeep Singh1, Jeong Yoon Lee1, Shoaleh Dehghan2, Jaya Rajaiya1 Elizabeth B. Liu2, Morris S. Jones2, David W. Dyer3, Donald Seto2. Mass. Eye & Ear – Harvard Medical School1, School of Systems Biology, George Mason University2, Dept. Microbiology & Immunology, University of Oklahoma Health Sciences Center3

17:50 Getting To NET. Mihaela Gadjeva. Division of Infectious Disease, Brigham and Women's Hospital, Harvard Medical School

Poster Session II

Chairpersons - Pasquale Aragona (Italy), Wendy R. Kam (USA), Mark Rosenblatt (USA)

1 Observation Of Meibomian Gland Morfology In Acute-Phase Of Stevens Johnson Syndrome And Chemical Burn. Kozue Kasai1, Seika Shimazaki-Den1, Murat Dogru1,2, Jun Shimazaki1,2. Tokyo Dental College Ichikawa General Hospital1, Chiba, Japan, Keio University School of Medicine2, Tokyo, Japan

2 Comparison of Meibomian Gland Loss and Meibum Grade in Patients with Obstructive Meibomian Gland Dysfunction. Youngsub Eom, Kwang-Eon Choi, Su-Yeon Kang, Hyo Myung Kim, Jong-Suk Song Department of Ophthalmology, Korea University College of Medicine, Seoul,South Korea

3 Distribution of Meibomian Gland Loss along the Nasal, Central and Temporal Segments of the Lower Eye Lid. Heiko Pult1,2, Donald Korb3, Caroline Blackie3, Britta H. Riede-Pult1,2. 1Dr Heiko Pult – Optometry & Vision Research, Weinheim, DE; 2Cardiff University, School of Optometry & Vision Sciences, Cardiff, UK; 2Korb Associates and TearScience Inc., USA

4 Relation Between Meibography Of The Two Eyes, NIBUT And OSDI In Young Subjects. Alessandro Fossetti1,2, Simone Aru1, Carlo Falleni1,4, Alessandro Farini1,3 , Alessandro Landi1, Andrea Ramacciotti1. Istituto Regionale Studi Ottici e Optometrici (IRSOO)1, Vinci, Italy, Università degli Studi di Firenze2, Italy, INO - CNR, Firenze3, Italy, Università degli Studi di Torino4, Italy

5 Comparison Between Distorsion Of The Meibomian Glands And Inflammatory Reaction On The Ocular Surface In Patients With Ocular Discomfort. Ingrid Boldin1, Haleh Aminfa1, Dieter Franz Rabensteiner1, Gerold Schwantzer2, Manuela Fischl1, Christa Wachswender1, Jutta Horwath-Winter1. 1Department of Ophthalmology, 1Institute for Medical Informatics, Statistics and Documentation, 2Medical University Graz, Austria

6 A Preliminary Population-Based Association Study: Does EDAR Gene affect Meibomian Gland Development? Jianjiang Xu, Jingyu Yang, Jiaxu Hong. Department of Ophthalmology and Visual Science, Eye and ENT Hospital, Fudan University, Shanghai, China

7 Influence Of Azithromycin On Human Meibomian Gland Epithelial Cells. Yang Liu, Wendy R. Kam, Juan Ding and David A. Sullivan Schepens Eye Research Institute, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA, USA1

8 The Effects Of Insulin-Like Growth Factor-1 And Growth Hormone On Human Meibomian Gland Epithelial Cells. Juan Ding, David A. Sullivan. Schepens Eye Research Institute, Massachusetts Eye and Ear, and Department of Ophthalmology, Harvard Medical School, Boston, MA, USA

9 Are Mice Relevant Models For Human Meibomian Gland Research? Wendy R. Kam, Raheleh Rahimi Darabad, Shaohui Liu, David A. Sullivan Schepens Eye Research Institute, Massachusetts Eye and Ear, Harvard Medical School, Boston, MA, USA

10 First Results for a Three-Dimensional Culture System Using Immortalized Human Meibomian Gland Epithelial Cells. Nagayoshi Asano1,2, Daniel Abrar1, Ulrike Hampel1, Friedrich Paulsen1. Department of Anatomy II, Friedrich Alexander University Erlangen/Nuremberg, Erlangen, Germany1, Santen Pharmaceuticals. Co., Ltd, Nara, Japan2

11 Systemic Isotretinoin Side Effects On Humen Meibomian Gland. Suarez,M N Cátedra de Oftalmología Facultad de Medicina de Montevideo, Uruguay

12 The Relationship Between Contact Lens Use and Subtle Meibomian Gland Dysfunction. Costas Katsoulos1, Anastasios Charonis, MD1, George Charonis MD1. 1Athens Vision eye clinic, Athens, Greece

13 Efficacy Evaluation of a Novel Emulsion Based, Anionic Phospholipid Containing Artificial Tear in Meibomian Gland Dysfunction (MGD) Subjects. Gary Foulks1, Chris Sindt2, Joe Griffin3. 1Kentucky Lions's Eye Center, Lexington KY, 2U of Iowa, Iowa City IA, 3Alcon Research Ltd, Ft Worth, TX

14 Diquafosol Tetrasodium Stimulated Lipid Secretion in Rabbit Meibomian Glands. Masatsugu Nakamura, Asuka Sakamoto, Hidetoshi Mano, Takashi Nagano, Naoto Mori. Santen Pharmaceutical Co., Ltd., Research & Developement Center, Japan

15 Comparison Of Tear Film Lipid Profile Among Basal, Reflex And Flush Tears. Athira Rohit1, 2, Simon HJ Brown3, Fiona Stapleton1, 2, Mark Willcox2. Brien Holden Vision Institute, Sydney, Australia1, The School of Optometry & Vision Science, the University of New South Wales, Sydney2, The School of Health Sciences, University of Wollongong, Sydney3

16 **Discussion:** The Anti-Evaporative Effect of the Tear Film Wax Esters. Antti H. Rantamäki1, Susanne K. Wiedmer2, and Juha M. Holopainen1. Helsinki Eye Lab, Department of Ophthalmology1, Department of Chemistry2 University of Helsinki, Helsinki, Finland

17 Analysis of the Fatty Acid Composition of Human Meibum. Tomo Suzuki1,2, Sayaka Kamada1,2, Satoshi Fujiwara3, Tetsuya Tajika4, and Shigeru Kinoshita1. Department of Ophthalmology, Kyoto Prefectural University of Medicine1, Kyoto, Japan; Kyoto City Hospital, Kyoto, Japan2; Shimadzu Techno-Research, Inc., Kyoto, Japan3; Senju Pharmaceutical Co., Ltd., Kobe, Japan4

18 Correlation Between Quantitative Measurements of Tear Film Lipid Layer Thickness and Meibomian Gland Loss in Patients with Obstructive Meibomian Gland Dysfunction and Normal Controls. Jong Suk Song, Youngsub Eom, Jong Suk Lee, Su Yeon Kang, Hyo Myung Kim Department of Ophthalmology, Korea University College of Medicine, Seoul, South Korea

19 Analysis Of Spread Meibomian Films Alone And Seeded With Deuterated Wax Esters Or (Omega-Acyl)-Hydroxy-Fatty Acids Using Neutron Reflectivity. Thomas J Millar, Shiwani R Raju, Burkhardt Schuett. School of Science and Health, University of Western Sydney, Sydney, NSW Australia

20 Interactions of Meibomian and Polar Lipid Films with Hyaluronic Acid. Georgi As. Georgiev1, Norihiko Yokoi2, Slavyana Ivanova1, Rumen Krastev3, Zdravko Lalchev1. Department of Biochemistry, Sofia University, Bulgaria, 1Department of Ophthalmology, Kyoto Prefectural University of Medicine, Japan, 2Biomaterials Group, NMI Naturwissenschaftliches und Medizinisches Institut an der Universität Tubingen, Germany3

21 Localisation of Adult Stem Cells in the Mouse Meibomian Gland using State-of-the-Art Stem Cell Mouse Model. G.J. Parfitt, M. Geyfman, Yilu Xie, D.J. Brown, J.V. Jester. Gavin Herbert Eye Institute, University of California, Irvine, CA, USA

22 Predictivity of Tear Parameters in the Onset of Ocular Graft Versus Host Disease (GVHD) Dry Eye after Hematopoietic Stem Cell Transplantation (HSCT). Piera Versura1, Giuseppe Giannaccare1, Francesca Bonifazi2, Giuseppe Bandini2, Lorenza Ridolfi3. 1Ophthalmology Unit, 2Haematology section, DIMES, Alma Mater Studiorum University of Bologna, 3Emilia-Romagna Transplant Reference Center, S. Orsola-Malpighi Teaching Hospital, Bologna–Italy

23 Ocular Surface Disease Associated to Graft-Versus-Host-Disease after Allogeneic Stem Cell Transplantation. Monica Alves1, 2 MD PhD, Franscisco Penteado Aranha3 MD, PhD, Daniella Paiva2 MD, Afonso C. Vigoritto3 MD, PhD, Wilson Estevam Filho 3, Carmino A. Souza3 MD PhD, Eduardo Melani Rocha1 MD PhD Faculty of Medicine Ribeirão Preto, São Paulo University, 2Pontific Catholic University of Campinas, 3University of Campinas, Brazil

24 International Chronic Ocular Graft-Vs-Host-Disease (GVHD) Consensus Group: Proposal Of New Diagnostic Criteria For Chronic GVHD (Part I). I). Yoko Ogawa1, Stella K. Kim2, Reza Dana3, Janine Clayton4, Sandeep Jain5, Mark I. Rosenblatt6, Victor L. Perez7, Hasanain Shikari3, Anjo Riemens8, Kazuo Tsubota1. 1Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan; 2MD Anderson Cancer Center, Houston, TX, USA; 3Massachusetts Eye and Ear Infirmary, Department of Ophthalmology, Harvard Medical School, Boston, USA; 4National Institute of Health, USA;University of Illinois, Chicago, Illinois, USA; 5Weill Cornell

Medical College, New York, USA; 6Bascom Palmer Eye Institute, USA; 7University Medical Center Utrecht, the Netherlands

25 Dry Eye Related Quality of Life And Eye Manifestation in Ocular Chronic Graft Versus Host Disease Patients. Machiko Shimmura-Tomita, Hiroko Takano, Nozomi Kinoshita, Fumihiko Toyoda, Ayumi Ota, Katsuaki Tanaka, Akihiro Kakehashi. Department of Ophthalmology, Saitama Medical Center, Jichi Medical University, Saitama, Japan

26 Disrupted Induction Of Induced Regulatory T Cells In The Dry Eye Host Exacerbates Corneal Allograft Rejection. Jing Hua, William Stevenson, Yihe Chen, Thomas Dohlman, Takenori Inomata, Hyun Soo Lee, Sunil Chauhan, Reza Dana Immunology Laboratories, Schepens Eye Research Institute, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston MA USA

27 Upregulation of Th1-associated chemokine expression in experimental dry eye requires NK cell-produced IFN-γ. Terry G. Coursey, Stephen C. Pflugfelder, and Cintia S. de Paiva Ocular Surface Center, Cullen Eye Institute, Department of Ophthalmology, Baylor College of Medicine, Houston, Texas

28 Lifitegrast, an investigational ICAM-1 decoy, inhibits T-cell activation, adhesion and cytokine release. C.P. Semba1 1Shire Pharmaceuticals

29 **Discussion**: Use Of Novel IL-1 Receptor Inhibitor (EBI-005) In The Treatment Of Patients With Moderate To Severe Dry Eye Disease. Michael Goldstein1,2, Jennifer Agahigian1, Gregory Zarbis-Papastoitsis1, Kathryn Golden1, Joseph Kovalchin1, Cameron Wheeler1, Siddhartha Chowdury1, Abbie Celniker1, Eric Furfine1. 1Eleven Biotherapeutics, Cambridge, MA, United States, 2Ophthalmology, New England Eye Center/Tufts Medical Center, Boston, MA, United States

30 Association of Interleukin-17A and -17F Genes Single Nucleotide Polymorphisms with Dry Eye Diseases. Choun-Ki Joo1,2,3, Jee-Won Mok1, Je-Hyung Hwang1,2,3, Yong-Eun Lee1,2,3, Kyung-Sun Na1,2,3. 1Catholic Institutes of Visual Science, The Catholic University of Korea; 2Department of Ophthalmology & Visual Science, College of Medicine, The Catholic University of Korea; 3Seoul St. Mary's hospital Eye Institute (SSEI)

31 Genetic Variants in IL6 and IL6R Genes are Strong Associated with Korean Dry Eye Disease. Kyu Yeon Hwang, Kyung-Sun Na, Jee-Won Mok, Choun-Ki Joo Department of Ophthalmology and Visual Science, Catholic University of Korea, Catholic Institute of Visual Science, Seoul, Republic of Korea

32 Genetic Variants In The TNFα Are Associated With Korean Dry Eye Disease. Hyun Soo Lee1,2,3, Chang Rhe Rho1,2,4, Kyung-Sun Na1,2,3, Jee-Won Mok1, Choun-Ki Joo1,2,3. Catholic Institutes of Visual Science1, Department of Ophthalmology & Visual Science2, College of Medicine, The Catholic University of Korea, Seoul St. Mary's hospital Eye Institute (SSEI)3, Seoul, Korea, Daejeon St. Mary's Hospital4, Daejeon, Korea

33 Experimental Dry Eye-Associated Neuroinflammatory Changes in Trigeminal Ganglia. Hyun Soo Lee1,2, Kishore Reddy Katikireddy1, Thomas Dohlman1, William Stevenson1, Sunil K. Chauhan1. 1Schepens Eye Research Institute and Massachusetts Eye & Ear Infirmary, Harvard Medical School, Boston, Massachusetts, USA; 2Department of Ophthalmology&Visual Science, Seoul St. Mary's hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea

34 Cultivated Trigeminal Neurons from Dry Eye Mice Induce Higher Expression of Maturation Markers by Bone Marrow-Derived Dendritic Cells. Sang-Mok Lee1, William Stevenson1, Kishore Reddy Katikireddy1, Hyun Soo Lee1, Thomas Dohlman1, Sunil Chauhan1, Reza Dana1. Schepens Eye Research Institute, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA, USA1

35 Tear Cytokine Profiles And Their Clinical Correlations With Ocular Surface Parameters Of Different Ages. Hua Zhu1,2,3, Nisha Yeotikar1, Negar Babaei1, Amali Ariyavidana1, Maria Markoulli2, Daniel Tilia1, Brien Holden1,2,3, Eric Papas1,2,3. 1Brien Holden Vision Institute, 2School of Optometry and Vision Science University of New South Wales, 3Vision CRC, Sydney, Australia

36 Standardized Quantification Of Inflammatory Biomarkers For Multicenter Clinical Trials Of Ocular Surface Disease. Penny A. Asbell1, Seth P. Epstein1, Yi Wei1, Neha Gadaria-Rathod1, Maureen G. Maguire2. Department of Ophthalmology, Mount Sinai School of Medicine, New York, NY1 Department of Ophthalmology, University of Pennsylvania, Philadelphia, PA, USA2

37 Effect Of Inhibition Of SPLA2 On Ocular Surface Disease In Dry Eye Mice. Penny Asbell, Yi Wei, Pengcheng Li, Jun Zou, Seth Epstein, Neha Gadaria-Rathod. Department of Ophthalmology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

38 Co-Expression of COX-2 And MMP9 in Dry Eye Induced Mouse Lacrimal Glands. Yuri Seo, MD1, YongwooJi, MD1, Jongwoo Shim, MD1, Hyemi Noh, PhD1, HyungKeun Lee, MD1. Institute of Vision Research, Department of Ophthalmology, Yonsei University College of Medicine, Seoul, Korea1

39 Galectin-3 Contributes to the Ocular Surface Inflammatory Response. Yuichi Uchino1, Jerome Mauris1, Julia Dieckow1, Ashley M. Woodward1, Francisco Amparo1, Reza Dana1, Flavio Mantelli2, Pablo Argüeso1. Schepens Eye Research Institute, Harvard Med School, Boston, MA, USA1; Dept of Ophthalmology, University of Rome, Italy2

40 Toll-Like Receptor Agonists Increase In Vivo And In Vitro Expression Of Matrix Metalloproteinases. Carolina Lema, Rachel Redfern University of Houston, College of Optometry, Houston, TX, USA

41 Vitamin D Attenuates TLR3 Induced Inflammation in Human Corneal Epithelial Cells. Rose Reins, Alison McDermott. University of Houston College of Optometry, Houston, TX, USA

42 **Discussion**: Diurnal Changes of Lipid Inflammatory Mediators in Human Tears with and Without Contact Lenses. Simin Masoudi, PhD Candidate1, 2, Fiona Stapleton, PhD, Professor1, 2, Mark Willcox, PhD, Professor2. 1Brien Holden Vision Institute, Sydney, Australia; 2University of New South Wales, Sydney, Australia

43 Lipopolysaccharide Induction Of Pro-Inflammatory Gene Expression In Human Corneal, Conjunctival And Meibomian Gland Epithelial Cells. Afsun Sahin, Wendy R. Kam, Raheleh Rahimi Darabad, Yang Liu, David A. Sullivan. Schepens Eye Research Institute, Massachusetts Eye and Ear, Harvard Medical School, Boston, MA, USA

44 S100A8 and S100A9 Activate a Range of Disease Mediators Via Transcription in the Conjunctiva of Pterygium Patients. Louis Tong1,2,3,4, Wanwen Lan4, YoonPin Lim3, AiHua Hou4. Singapore National Eye Center,1 Duke-NUS Graduate Medical School, 2National University of Singapore,3 Singapore Eye Research Institute,4 Singapore

45 Significant Ocular Findings In Patients With Primary Sjögren's Syndrome. Priya M. Mathews, MPH1, Sarah Hahn, MD1, Alan N. Baer, MD2, Esen K. Akpek, MD1,2. 1Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland; 2Johns Hopkins Jerome L. Greene Sjögren's Syndrome Center, Baltimore, Maryland

46 Analysis Of Fibrosis in IGG4-Related Mikulicz's Disease. Masaki Fukui, Yoko Ogawa, Shigeto Shimmura, Shin Hatou, Yoshiyuki Ichihashi, Saori Yaguchi, Masatoshi Hirayama, Tetsuya Kawakita, Kazuo Tsubota. Department of Ophthalmology, Keio University School of Medicine

47 Ocular Surface Immunological Changes In Patients With Cicatricial Pemphigoid. Stefano Barabino1, Maurizio Rolando1, Elisa Montaldo2, Cristina Mingari2, Carlo Enrico Traverso1. 1Clinica Oculistica, DINOGMI, University of Genoa, 2DIMES, University of Genoa, Italy

48 Clinical and Pathological Characteristics of Nonpigmented Corneal Neoplasm. Yong Jae Cha, MD, Bo Hyuck Kim, MD, Soo Jeong Lyu, MD, Joon Young Hyon, MD, PhD, Won Ryang Wee, MD, PhD, Joo Youn Oh, MD, PhD Department of Ophthalmology, Seoul National University Hospital, Seoul, Korea

49 Vernal Keratoconjunctivitis Clinical and Therapeutic Aspects. Sihem Lazreg MD Cabinet d'ophtalmologie Blida Algeria

50 Anti-Inflammatory Effects of Rebamipide Eye Drops on Allergic Conjunctivitis . Mayumi Ueta1,2, Chie Sotozono1, Ayaka Koga1,2, Norihiko Yokoi1, and Shigeru Kinoshita1. 1Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan; 2Faculty of Life and Medical Sciences, Doshisha University, Kyoto, Japan

51 The Effect of 2% Rebamipide Eye Drops On The Ocular Surface of SOD-1 Knock Out Mice. Murat Dogru1,2, Takeshi Kojima1,2, Taeko Nagata2, Ayako Igarashi1, Kazunari Higa1, Seika Den Shimazaki1, Yoshiyuki Satake1, Kazuo Tsubota1, Jun Shimazaki1. 1Tokyo Dental College Ichikawa General Hospital, Department of Ophthalmology, Ichikawa, Chiba, Japan; 2Keio University School of Medicine, Department of Ophthalmology, Tokyo, Japan

52 **Discussion**:ESC(1-21) A Novel Antimicrobial for Microbial Keratitis? AM McDermott1, SS Kolar1, V. Luca2, G. Mannino2, SM Recupero2, ML Mangoni2. 1The Ocular Surface Institute, University of Houston, College of Optometry, Houston TX, USA; 2Sapienza Università di Roma, Italy

53 Production Of Contact Lens Induced Microbial Keratitis In An Animal Model Without Prior Damage To The Ocular Surface. Mark DP Willcox, Ajay K Vijay. School of Optometry and Vision Science, University of New South Wales, Sydney, NSW, Australia

54 Antimicrobial Activity of Multipurpose Disinfection Solution Soaked Contact Lenses. Debarun Dutta1,2, Hua Zhu1,2, Mark DP Willcox2. 1Brien Holden Vision Institute, Sydney, Australia; 2School of Optometry and Vision Science, University of New South Wales, Sydney, Australia

55 Membrane Permeability Of Staphylococcus Aureus Aggregates Exposed To Contact Lens Care Solutions. David McCanna, Lyndon Jones Centre for Contact Lens Research, School of Optometry & Vision Science, University of Waterloo

56 Efficacy Of Contact Lens Solutions Against Achromobacter Xylosoxidans Biofilms Using Confocal Microscopy. David J. McCanna, Jaclyn Chang, Lakshman Subbaraman, Lyndon Jones Centre for Contact Lens Research, School of Optometry & Vision Science, University of Waterloo

57 Effect of Pathogenic Compared to Commensal Bacteria on Conjunctival Goblet Cell NRLP3 Inflammasome Activation and Mucin Secretion. Darlene A. Dartt1, Victoria McGilligan1, Dayu Li1, Robin R. Hodges1, Michael Gilmore2, and Meredith S. Gregory-Ksander1. 1Schepens Eye Research Institute/Massachusetts Eye and Ear, Boston MA; 2Massachusetts Eye and Ear, Boston, MA

58 Demodex Infestation in Patients with Ocular Discomfort. Jutta Horwath-Winter1, Dieter Franz Rabensteiner1, Gerold Schwantzer2, Manuela Fischl1, Christa Wachswender1, Haleh Aminfar1, Ingrid Boldin1. Department of Ophthalmology, 1Institute for Medical Informatics, Statistics and Documentation,2 Medical University Graz, Austria

59 Tear Secretion Impairment as a Function of Severity of Herpetic Keratitis. M M'Garrech1, A Rousseau A1,2, G Kaswin1, A Sauer3, T Bourcier T3, E Barreau E1, M Labetoulle1,2. 1Ophthalmology Department, Hôpital de Bicêtre, Assistance Publique-Hôpitaux de Paris, 94275 Le Kremlin-Bicêtre Cedex, FRANCE; 2Laboratoire de Virologie Moléculaire et Structurale, CNRS, UPR 3296, Centre Nationale de la Recherche Scientifique, 91198 Gif-sur-Yvette; 3Ophthalmology Department, Nouvel hôpital Civil, hôpitaux universitaires de Strasbourg, BP 426, 67091 Strasbourg, France

60 Clinical, Confocal And Morphological Investigations On The Cornea In Human Mucopolysaccharidosis. Pasquale Aragona1, Edward Wylegala2, Ewa Wroblewska-Czajka2, Adrian Smedowski2, Anna Nowinska2, Anna M. Roszkowska1, Antonina Pisani3, Antonio Micali3, Domenico Puzzolo3. Department of Experimental Medical-Surgical Sciences1, Department of Ophthalmology, District Railway Hospital, Katowice, Poland2, Department of Biomedical Sciences and Morphofunctional Imaging, University of Messina, Messina, Italy3

61 Intermittent Fasting Prevents Lacrimal Hypofunction in Rat Visual Display Terminal Users Model : A Pivotal Role of Endogenous D-3-Hydroxybutyrate. Nakamura, Shigeru1, Hisamura, Ryuji1, Imada, Toshihiro1, Tsubota, Kazuo1. 1Keio university, Tokyo, Japan

62 Quercetin Intake Improves Lacrimal Function Remedial Effect of Quercetin Intake on Lacrimal Function. Yasuhisa Tanaka,1 Takaaki Inaba,1 Jun Shimazaki,2 Kazuo Tsubota,1 1Department of Ophthalmology, Keio Universiy School of Medicine, 2Department of Ophthalmology, Tokyo Dental College, Ichikawa General Hospital

63 The Effect Of Systane® Balance Artificial Tears On Corneal Sensitivity And Tear Film Stability. Illés Kovács1, Lóránt Dienes1, Huba Kiss1, János Németh1, Mari-Carmen Acosta2, Juana Gallar2. 1Department of Ophthalmology, Faculty of Medicine, Semmelweis University, Budapest, Hungary. 2Instituto de Neurociencias, Universidad Miguel Hernandez-CSIC, San Juan de Alicante, Spain

64 A New Examination for Assessing Ocular Surface Sensitivity: Maximum Opening Time of the Eye. Jun Shimazaki1, Murat Dogru1, Seika Shimazaki-Den1. Department of Ophthalmology, Tokyo Dental College Ichikawa General Hospital, Chiba, Japan

65 The Effect of Increased Periocular Humidity on Lipid Layer Thickness and Ocular Comfort of Symptomatic Contact Lens Wearers. Timothy Willis1, Caroline Blackie1,2, and Donald Korb1,2. TearScience Inc, Morrisville1, NC, Korb Associates, Boston, MA2

66 Responses Of The Ocular Surface Temperature And Lipid Layer Thickness To Stressed Environmental Conditions In Normal And Dry Eyes; Ranjini Kottaiyan1; Holly Hindman1, 2; Gheorghe Salahura1, 2; James M. Zavislan2, 1; Geunyoung Yoon1, 2, 3; James V. Aquavella 1Flaum Eye Institute, University of Rochester 2Center for Vision Science 3.The Institute of Optics, Rochester, NY, USA

67 Construct Validity Of The Current Symptoms Questionnaire. Ping Situ1, Carolyn Begley1, Trefford Simpson2, Robin Chalmers1, Ziwei Wu. 1School of Optometry, Indiana University, USA1, School of Optometry and Vision Science, University of Waterloo, CANADA2

68 Comparing Sensory Responses To Repeated Ocular Surface Stress With And Without Wearing A Contact Lens. Ping Situ, Jun Zhang, Carolyn Begley School of Optometry, Indiana University, USA

69 Is Lid-Wiper/ Lens Surface Friction The Mechanism Of Contact Lens Dryness And End-Of-Day Discomfort? Noel A. Brennan, Chantal M-L Coles. Johnson & Johnson Vision Care Inc, Jacksonville, FL, USA

70 Lens Care Influence On Ocular Comfort In Silicone Hydrogel Daily Wearers. Cécile Maissa1,2, Michel Guillon1,2, Renee Garofalo3, Jami Kern3. 1OTG Research & Consultancy, London, UK; 2School of Life and Health Science, Aston University, Birmingham, UK, 3Alcon Research. Ltd., Fort Worth, TX

71 Pre-Ocular Tear Film Dysfunction And Its Association With Peripheral Neuropathy In Diabetes Mellitus. Stuti Misra1, Dipika V Patel1, Charles NJ McGhee1, Monika Pradhan1, Dean Kilfoyle2, Geoffrey Braatvedt3, Jennifer P. Craig1. 1Department of Ophthalmology, New Zealand National Eye Centre. 2Department of Neurology; 3Department of Medicine, The University of Auckland, Auckland, New Zealand

Saturday, September 21, 2013

Session III

New Paradigms in Understanding Corneal Sensation & Pain

Chairpersons - Giulio Ferrari (Italy), Juana Gallar (Spain), Todd P. Margolis (USA)

8:00 **Keynote Address:** Neural Protective Mechanisms Of The Ocular Surface In Health And Disease. Carlos Belmonte. Instituto de Neurociencias UMH-CSIC, San Juan de Alicante and Fundación de Investigación Oftalmológica, Instituto Fernandez-Vega, Oviedo, Spain

8:25 **Keynote Address:** The Corneal Pain System And Dry Eye Disease. Perry Rosenthal. Boston Eye Pain Foundation, Boston, MA, USA

8:50 A Neural Basis of Cold-Induced Dry Eye Pain: Sensitizations of Dry-Sensitive Corneal Afferents Produced by Hyperosmolar Tears vs. Menthol. Harumitsu Hirata. Department of Neurology, Thomas Jefferson University, Philadelphia, PA, USA

9:05 The Effect Of Increasing Ocular Surface Stimulation On Blinking. Ziwei Wu1, Carolyn Begley1, Ping Situ1, Trefford Simpson2. Indiana University School of Optometry1, Bloomington, IN, USA. University of Waterloo School of Optometry and Vision Science2, Waterloo, Canada

9:20 **Poster Session III (with Coffee & Tea)**

Corneal Homestasis, Repair & Regeneration

Chairpersons - Julie T. Daniels (UK), Sarah F. Hamm-Alvarez (USA), Xio Wei Tan (Singapore))

10:10 **Keynote Address:** Novel Mechanisms for Ocular Surface Homeostasis and Protection. M. Elizabeth Fini and Shinwu Jeong. Institute for Genetic Medicine, University of Southern California, Los Angeles, California, USA

10:35 **Keynote Address:** Biological Parameters Determining The Clinical Outcome Of Autologous Cultures Of Limbal Stem Cells. Graziella Pellegrini1, M. De Luca1. 1Centre for Regenerative Medicine S. Ferrari, University of Modena and Reggio Emilia

11:00 **Keynote Address:** Insight into How to Reduce the Formation of Recurrent Erosions Gained from Corneal Debridement Studies in the Mouse. Sonali Pal-Ghosh1, Ahdeah Pajoohesh-Ganji1, Christophe Cataisson2, Daniel Saban3, and Mary Ann Stepp1. GWUMC, Department of Anatomy and Regenerative Biology and Department of Ophthalmology Washington DC, NCI/NIH, Bethesda, MD, Duke University Eye Center, Durham, NC

11:25 Biomimetic And Composite Corneas For High Risk Transplantation. May Griffith1, Chyan-Jang Lee1, Ranjithkumar Ravichandran1, Vijayalakshmi Rajendran2, John Forrester2, Lucia Kuffova2. 1Integrative Regenerative Medicine Centre, Linköping University, Linköping, Sweden; 2Section of Immunology and Infection (Ocular Immunology), Division of Applied Medicine, School of Medicine and Dentistry, University of Aberdeen, Aberdeen, Scotland

11:40 **Poster Viewing & Lunch**

Poster Discussion III

Chairpersons - Joseph B. Ciolino (USA), May Griffith (Sweden), Zhen Meng (USA)

13:10 Changes In Neural Activity Of Ocular Surface Sensory Nerves During Allergic Keratoconjunctivitis. MC. Acosta, C. Luna, S. Quirce, C. Belmonte, Juana Gallar. Instituto de Neurociencias, Universidad Miguel Hernandez-CSIC, San Juan de Alicante, Spain

13:15 Functional Mouse Lacrimal Gland Regeneration In Vivo. Masatoshi Hirayama1, Miho Ogawa2, Masamitsu Oshima3, Shigeto Shimmura1, Tetsuya Kawakita1, Takashi Tsuji2,3 & Kazuo Tsubota1

13:20 Lacritin Survival Signaling Restores Corneal Epithelial Health By Several Mechanisms And Is Reduceable To A Synthetic Mimetic. Ningning Wang1, Jeffrey Romano1, Robert L. McKown2, Gordon W. Laurie1. University of Virginia1, Charlottesville, VA; James Madison University2, Harrisonburg, VA, USA

13:25 The Role Of Secreted Frizzled-Related Protein 1 (SFRP1) in Corneal Epithelium. Jingjing You1, Li Wen1, Michele C Madigan2, Gerard Sutton1,3. 1Save Sight Institute, University of Sydney, Australia, 2School of Optometry and Vision science, University of New South Wales, Australia3. Vision Group, Australia

Prime Time: TFOS Debates

Chairpersons - Jing Hua (USA), Thomas J. Millar (Australia), Paolo Rama (Italy)

Debate: Is corneal staining an irrelevant complication of using preserved care systems?

13:30 **Keynote Address:** Agree. Frank V. Bright. Department of Chemistry, University at Buffalo, The State University of New York, Buffalo, New York

13:40 **Keynote Address:** Disagree, Maria Markoulli. School of Optometry and Vision Science, The University of New South Wales, Sydney, Australia

Debate: Is short BUT a different type of dry eye disease, or merely an early stage of aqueous deficiency?

14:00 **Keynote Address:** Different type. Norihiko Yokoi. Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan

14:10 **Keynote Address:** Early stage. Michael A. Lemp. Georgetown and George Washington Universities, Washington DC, USA.

Debate: Is inflammation the core mechanism in dry eye disease?

14:30 **Keynote Address:** Agree. Penny A. Asbell, Seth P. Epstein, Yi Wei, Neha Gadaria-Rathod Department of Ophthalmology, Mount Sinai School of Medicine, New York, NY, USA

Keynote Address: Disagree. Kazuo Tsubota. Keio University School of Medicine, Tokyo, Japan

15:00 **Poster Session III (with Coffee & Tea)**

TFOS Workshop on Contact Lens Discomfort: Reports

Chairpersons - Jason J. Nichols (USA), Kokoro Sano (Japan), Mark D. P. Willcox (Australia)

15:50 Introduction. Jason J. Nichols. College of Optometry, University of Houston, Texas, USA

15:55 **Keynote Address:** Definition & classification. Desmond Fonn. CCLR School of Optometry, University of Waterloo, Waterloo, ON, Canada

16:10 **Keynote Address:** Epidemiology. Kathy Dumbleton. CCLR School of Optometry, University of Waterloo, Waterloo, ON, Canada

16:25 **Keynote Address:** Neurobiology of discomfort & pain. Fiona Stapleton. School of Optometry and Vision Science, University of New South Wales, Sydney, NSW, Australia

16:40 **Keynote Address:** Contact lens interactions with the ocular surface & adnexa. Nathan Efron. Institute of Health & Biomedical Innovation, Queensland University of Technology, Queensland, Australia

16:55 **Keynote Address:** Contact lens interactions with the tear film. Jennifer P. Craig. Department of Ophthalmology, University of Auckland, Auckland, NZ

17:10 **Keynote Address:** Contact lens materials, design & care. Lyndon W. Jones. CCLR School of Optometry, University of Waterloo, Waterloo, ON, Canada

17:25 **Keynote Address:** Management & therapy. Eric B. Papas. Brien Holden Vision Institute, Sydney, Australia

17:40 **Keynote Address:** Trial design & outcomes. Gary N. Foulks. Dept of Ophthalmology & Visual Science, University of Louisville, Kentucky Lions Eye Ctr, Louisville, KY, USA

Closing Session

Chairperson: Florence Malet (France)

17:55 Academic perspective: Pablo Argüeso (USA)
18:00 Clinical perspective: Esen K. Akpek (USA)
18:05 Industry perspective: Richard E. Weisbarth (USA)

Closing Remarks

18:10 *Kazuo Tsubota (Japan)*

Poster Session III

Chairpersons - Joseph B. Ciolino (USA), May Griffith (Sweden), Zhen Meng (USA)

1 **Discussion:** Changes In Neural Activity Of Ocular Surface Sensory Nerves During Allergic Keratoconjunctivitis. MC. Acosta, C. Luna, Discussion: S. Quirce, C. Belmonte, J. Gallar. Instituto de Neurociencias, Universidad Miguel Hernandez-CSIC, San Juan de Alicante, Spain

2 Substance P and its Inhibition in Corneal Neovascularization. Giulio Ferrari1,2,3, Fabio Bignami1,3 Chiara Giacomini1,3, Paolo Rama1,2,3. San Raffaele Scientific Institute1, Cornea and Ocular Surface Unit2, Eye Repair Lab3

3 *In vitro* expansion of Activated Keratocytes from Human Corneal Stroma for Tissue Engineering Applications. Gary Hin-Fai Yam, Nur Zahirah Binte M Yusoff, Jodhbir S Mehta. Tissue Engineering and Stem Cell Group, Singapore Eye Research Institute, Singapore

4 Human Oral Mucosal Fibroblasts Support The Culture Of Epithelial Cells For Clinical Use In 2D Culture And On Plastic Compressed Collagen (RAFT). AR O'Callaghan1, MP Lewis2, M Dziasko1 and JT Daniels1. 1Department of Ocular Biology and Therapeutics, UCL Institute of Ophthalmology, London, UK. 2Musculoskeletal Biology Research Group, School of Sport, Exercise and Health Sciences, Loughborough University, UK

5 Re-Assembly Of The Stem Cell Niche In Long-Term Culture Of Limbal Epithelial Cell Sheets. Shigeto Shimmura, Hideyuki Miyashita, Satoru Yoshida, Tetsuya Kawakita, Kazuo Tsubota. Department of Ophthalmology, Keio University School of Medicine

6 Reprogramming Of Adipose Derived Stem Cells Into Epithelial Cells By Defined Chemicals For Ocular Surface Reconstruction. Xiao Wei Tan1, Melina Sentiwan1, Gwendoline Goh1, Gary Hin Fai Yam1, Donald Tan2,3,4, Jodhbir S. Mehta1,2,3,4. 1Tissue engineering and stem cell research group, Singapore Eye Research Institute 2Yong Loo Lin School of Medicine, National University of Singapore 3Singapore National Eye Centre 4Department of Clinical Sciences, Duke-NUS Graduate Medical School, Singapore

7 Extracellular Vesicles and The Stress Response in the Corneal Epithelium. Alexandra Robciuc1,2, Antti H. Rantamäki1, Matti Jauhiainen2 and Juha M. Holopainen1. 1Helsinki Eye Lab, Department of Ophthalmology, University of Helsinki, Helsinki, Finland; 2Public Health, Genomics Research Unit - National Institute for Health and Welfare, Helsinki, Finland

8 **Discussion:** The Role Of Secreted Frizzled-Related Protein 1 (SFRP1) in Corneal Epithelium. Jingjing You1, Li Wen1, Michele C Madigan2, Gerard Sutton1,3. 1Save Sight Institute, University of Sydney, Australia, 2School of Optometry and Vision science, University of New South Wales, Australia3. Vision Group, Australia

9 Modulation of Glycocalyx Barrier Function in Human Corneal Epithelial Cells. Pablo Argüeso1, Jerome Mauris1, Flavio Mantelli1, Ashley M. Woodward1, Ziyhi Cao2, Bertozzi CR3,4, Noorjahan Panjwani2, Kamil Godula4, Schepens Eye Res Inst/Mass Eye and Ear, Harvard University, Boston, MA1, Dept of Ophthalmology, Tufts University, Boston, MA2, Dept of Chemistry, University of California, Berkeley, CA3, and Lawrence Berkeley National Laboratory, Berkeley, CA4

10 Ocular Surface in Sjogren's Syndrome. Sihem Lazreg. Cabinet d'ophtalmologie, Blida, Algeria

11 Autologous Serum Eye Drops for Treatment Ocular Surface Disorder and Post-Operative Delayed Corneal Epithelial Healing. Kaevalin Lekhanont, Passara Jongkhajornpong, Rossanun Sikaringul Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

12 Effects of Prolonged Use of Oxybuprocaine 0,4% Eye Drops on Normal Murine Cornea. Chiara Giacomini1,2, Giulio Ferrari1,2 , Fabio Bignami1,2, Paolo Rama1,2. Cornea and Ocular Surface Unit - Eye Repair Lab1 San Raffaele Institute, 2Milan, Italy

13 Osteopontin And Associated Integrin And CD44 Receptor Expression In Human Cornea Epithel Cells Under Normal And Wound Healing In Vitro Conditions. Julia Lehmann1, Maximilian Schmalfuss1, Kathrin Hoffmann1, Fabian Garreis1, Friedrich Paulsen1, Michael Scholz1. 1Department of Anatomy II, Friedrich Alexander University Erlangen Nuremberg, Erlangen, Germany

14 Impact of environmental changes on in vitro corneal epithelial wound healing. Emma V. Dare, Lakshman Subbaraman, Lyndon Jones Centre for Contact Lens Research, School of Optometry & Vision Science, University of Waterloo

15 Quantification Of Lipocalin-1 In Tears And Contact Lens Deposits Using A Sandwich Elisa Technique. Lakshman Subbaraman, Rajan Mistry, Mirunalni Thangavelu, Lyndon Jones Centre for Contact Lens Research, School of Optometry & Vision Science, University of Waterloo

16 The Evaluation of Lid Wiper Epitheliopathy in Contact Lens Wearers in a Controlled Low Humidity Enivironmental Exposure Chamber. Lyndon W. Jones1, Jalaiah P. Varikooty1, Nancy J. Keir1, Fiona Soong2, Piyush Patel2. CCLR-School of Optometry, University of Waterloo, Waterloo1, Inflamax Research, Mississauga, 2 ON, Canada

17 Ocular Signs And Symptoms In Contact Lens Wearers In A Controlled Low Humidity Environmental Exposure Chamber (LH-EEC) A Natural Provocation Research Model. Piyush Patel1, Fiona Soong1, Anne Marie Salapatek1, Jalaiah P. Varikooty2 , Nancy J. Kier2 , Lyndon Jones2. 1Inflamax Research, Mississauga, 2CCLR, University of Waterloo, Waterloo, Canada

18 *In-Vivo* Wettability Of Contact Lenses Worn In A Low Humidity Environmental Exposure Chamber (LH-EEC) Show Comparable Changes To Traditional Field Trials. Anne Marie Salapatek1, Fiona Soong1, Jalaiah P. Varikooty2, Nancy J. Kier2, Lyndon Jones2, Piyush Patel1. 1Inflamax Research, Mississauga 2CCLR, University of Waterloo, Waterloo, Canada

19 A drug-eluting contact lens for the treatment of glaucoma, Joseph Ciolino Massachusetts Eye and Ear Infirmary, Department of Ophthalmology, Harvard Medical School, Boston, MA, USA

20 Efficacy of Two Different Artificial Tears for the Treatment of Dry Eye in Frequent Computer and Contact Lens Users. Caterina Gagliano1,2, Vincenzo Papa3, Giulia Malaguarnera4, Roberta Amato1,2, Maria Grazia Mazzone3, Teresio Avitabile1. 1University of Catania, Eye Clinic, Catania, Italy, 2Neurovisual Science Technology (NEST), Catania, Italy, 3SIFI S.p.A., Aci S.Antonio, Catania, Italy, 4Department of Molecular and Clinical Biomedicine (Pharmacology and Biochemistry), University of Catania, Catania, Italy

21 Tear Supplement Effect on Tear Physiology and Wettability in Contact Lens Wear. A. Tomlinson, R. Fagehi, K. Oliver Vision Sciences, Glasgow Caledonian University

22 Management Of Intractable Ocular Surface Disease With Newly Developed Scleral Contact Lens. Ryu Soo Jeong1, Hyuk Jin Choi2,3, Joon Young Hyon1,2, Sang Mok Lee4, Joo Youn Oh2, Won Ryang Wee2. Department of Ophthalmology, Seoul National University Bundang Hospital, Gyeonggi, Korea1,Department of Ophthalmology, Seoul National University College of Medicine, Seoul, Korea2, Seoul National University Hospital Healthcare System Gangnam Center, Seoul, Korea3, Schepens Eye Research Institute, Massachusetts Eye & Ear Infirmary, Harvard Medical School, Boston, MA, U.S.A.4

23 Prominent Decrease Of Tear Meniscus Height By SCL Wearing And Efficacy Of Eye Drops For Dry Eye To Prevent The Change. Yukiko Nagahara1,2 , Shizuka Koh2, Hitoshi Watanabe1,2. 1Kansai Rosai Hospital 2Osaka University Graduate School of Medicine

24 Comparison Of The Ocular Tolerance Of Hyabak® New Formula Compared To Standard Hyabak® In Contact Lens Wearers – A Pilot Study. Professor Christine Purslow1,2, Mr Philip Gibson1, Dr Katharine Evans1. Cardiff University1, Plymouth University2, United Kingdom

25 Migration Around The Eyelid Margin. Christine Purslow1,2, Clare Conaty2, Alison Ng2. 1Plymouth University, 2Cardiff University, United Kingdom

26 Evaluation Of P2Y2 Receptor Expression In The Sod1 Knock Out Mice. Takashi Kojima1,2, Taeko Nagata2,3, Ayako Igarashi3, Murat Dogru2,3, Kazuo Tsubota2, Jun Shimazaki3. 1Gifu Red Cross Hospital, Gifu, Japan 2Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan 3Department of Ophthalmology, Tokyo Dental College, Chiba, Japan

27 Damage in Cornea and Lacrimal Glands of Smoking Rat. Akihiro Higuchi1, Murat Dogru1, Masanori Kitamura2, Tetsuya Kawakita1, Yoko Ogawa1, Kazuo Tsubota1. Department of Ophthalmology, Keio University School of Medicine, Tokyo1 Department of Molecular Signaling, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi2, Japan

28 **Discussion:** Functional Mouse Lacrimal Gland Regeneration In Vivo. Masatoshi Hirayama1, Miho Ogawa2, Masamitsu Oshima3, Shigeto Shimmura1, Tetsuya Kawakita1, Takashi Tsuji2,3 & Kazuo Tsubota1

29 Decellularization of Porcine Lacrimal Gland Tissue for Development of A Lacrimal Gland Scaffold. K. Spaniol1, A. Kunze1, M. Metzger2, G. Geerling1, S. Schrader1. 1Department of Ophthalmology, University Hospital Dusseldorf, 2Institute for Tissue Engineering and Regenerative Medicine, University Hospital Wurzburg

30 **Discussion:** Lacritin Survival Signaling Restores Corneal Epithelial Health By Several Mechanisms And Is Reduceable To A Synthetic Mimetic. Ningning Wang1, Jeffrey Romano1, Robert L. McKown2, Gordon W. Laurie1. University of Virginia1, Charlottesville, VA; James Madison University2, Harrisonburg, VA, USA

31 Protective Effects Of Trehalose On Corneal Epithelium In Course Of Laser Subepithelial Keratomileusis. Pasquale Aragona1, Pietro Colosi2, Laura Rania2, Francesca Colosi2, Antonina Pisani3, Domenico Puzzolo3, Antonio Micali3. Department of Social and Environmental Health1, Department of Experimental Medical-Surgical Sciences2, Department of Biomedical Sciences and Morphofunctional Imaging, University of Messina3, Messina, Italy

32 Comparision Of Prednisolone Acetate And Loteprednol Etabonate For The Treatment Of Benzalkonium Chloride Induced Dry Eye Syndrome In Rats. Emrullah Beyazyıldız1, U ur Acar2, Özlem Beyazyıldız3, Emine R. Hekimo lu2, Ferda A. Pınarlı4, Nagihan U urlu5, Aynur Albayrak4, Figen Kaymaz6, Meral Tiryaki4, Tuncay Deliba ı4. Gazi Mustafa Kemal State Hospital1, Hacettepe University Kastamonu Faculty of Medicine2, Ulucanlar Eye Research Hospital3, Dıskapı Yıldırım Beyazıt Research and Training Hospital4, Ataturk Research and Training Hospital5 Hacettepe University Faculty of Medicine5 Ankara, Turkey 33 The Effect Of Topical Cyclosporine 0.05% On Dry Eye After Cataract Surgery. Sung Kun Chung, MD, Ye Jin Ahn, MD The Catholic University of Korea, St. Mary's Hospital, Seoul, Korea

34 Ocular Safety of Cationic Emulsion of Cyclosporine in in vitro and Animal Models. Christophe Baudouin1,2, Philippe Daull3, Jean-Sébastien Garrigue3, Françoise Brignole-Baudouin2,4. 1Quinze-Vingts National OphthalmologyHospital, Paris, France; 2Vision Institute, Paris, France; 3Santen France, Novagali Innovation Center, Evry, France; 4Paris Descartes University, Faculty of Pharmaceutical and Biological Sciences, ToxicologyDept., Paris, France

35 Safety and Efficacy of Topical Ophthalmic MIM-D3, a Novel TRKA Receptor Agonist, in a Phase 2 Clinical Trial for the Treatment of Dry Eye. Garth Cumberlidge1, Karen Meerovitch1, Reza Haque2, and George Ousler3 1. 1Mimetogen Pharmaceuticals 2Bausch+Lomb, 3Ora, Inc

36 Nerve Growth Fractor In Human Cultured Conjunctival Cells Under Dry Eye Mimiced Hyperosmoar Stress. Hungwon Tchah1, Soon-Suk Kang2, Eun-Soon Kim2, Jae Yong Kim1, Jin-A Sin2, In-Suk Song1. 1Department of Ophthalmology, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea. 2Asan Institute for Life Sciences, Asan Medical Center, Seoul, Korea

37 Effect of 0.05% Cyanocobalamine, 0.5% Taurine and 0.5% Long-Chained Hyaluronic Acid on the Ocular Surface in Dysmetabolic Patients Affected by Dry Eye. Caterina Gagliano1,2, Giuseppina Marrazzo3, Giulia Malaguarnera4, Roberta Amato1,2, Mario Toro1, Santo Stella2, Teresio Avitabile1. 1University of Catania, Eye Clinic, Catania, Italy, 2Neurovisual Science Technology (NEST), Catania, Italy, 3ALFA INTES, 1University Casoria (NA), Italy, 4Department of Clinical and Molecular Biomedicine (Pharmacology and Biochemistry), University of Catania, Italy

38 Long-Term Effect of 3% Diquafosol Sodium Ophthalmic Solution in Patients with Short Breakup Time Type Dry Eye with "Spot Break". Hiroaki Kato1, Norihiko Yokoi1, Aoi Komuro1, Yukiko Sonomura1, Rieko Sakai1, Minoru Shimoyama1, Akane Yamaguchi1, Masaki Baba1, and Shigeru Kinoshita1. Department of Ophthalmology, Kyoto Prefectural University of Medicine1, Kyoto, Japan

39 Diquafosol Sodium Ophthalmic Solution Increases Tear-Fluid Secretion In Dry-Eye Patients With Sjögren's Syndrome. Aoi Komuro, Norihiko Yokoi, Hiroaki Kato, Yukiko Sonomura, Shigeru Kinoshita. Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan

40 The 2% Rebamipide Eye Drop Treatment for Dry Eye. Hiroko Takano, Nozomi Kinoshita, Machiko Shimmura-Tomita, Fumihiko Toyoda, Ayumi Ota, Katsuaki Tanaka, Akihiro Kakehashi. Department of Ophthalmology, Saitama Medical Center, Jichi Medical University, Saitama, Japan

41 Therapeutic Effects Of Rebamipide Eye Drops For Short-But Type Dry Eye. Hisashi Hosotani, Hiroyuki Shirasaki. Department of Ophthalmology, Shakaihoken Kobe Central Hospital, Kobe, Japan

42 Study Of The Safety And Efficacy Of D-3-Hydroxybutyrate Eye Drop For The Treatment Of Dry Eye. Kawakita Tetsuya1, Uchino Miki2, Fukagawa Kazumi3, Yoshino Kenichi4, Shimazaki Seika5, Toda Ikuko6, Tanaka Mari7, Arai Hiroyuki8, Sakatani Keiko8, Hata Seiichiro9, Okano Takashi10, Tsubota Kazuo1. Ophthalmology, Keio Univ School of Medicine1, Ryogoku Eye Clinic2, Iidabashi Eye Clinic3, Yoshino Eye Clinic4, Shimazaki Eye Clinic5, Minamiaoyama Eye Clinic6, Tokyo, Japan. Yatsuekimaeazisai Eye Clinic7, Chiba, Japan. Minatomirai Eye Clinic8, Sky building Eye Clinic9, Smile Eye Clinic10, Kanagawa, Japan

43 Effect of Systane® Balance on Non-Invasive Tear Film Break Up Time (NITFBUT) in Dry Eye Subjects with Lipid Deficiency; Aguilar1 AJ, Márquez1 MI, Albera1 P, Tredicce1 J, Ogundele2 A, Berra1 A. 1Universidad de Buenos Aires, Buenos Aires, Argentina, 2Alcon Research, Ltd., Fort Worth, TX

44 The Ocular Surface Healing Action of Systane® Ultra Vs. Maxidex® or Saline. Asbell P1, Gadaria N1, & Ogundele A.2 1Department of Ophthalmology Mount Sinai Hospital, New York, NY, 2Alcon Research, Ltd., Fort Worth, TX

45 Quality Of Vision After The Instilation Of A Liposomal Formulation. Jose M. Benitez-del-Castillo1, Ana M. Muñoz1, Marta Vicario2, Maria Caballo2, Irene Molina2, Rocio Herrero2. Hospital Clinico San Carlos1, Facultad de Farmacia, UCM2, Madrid, Spain

46 Ophthalmic Formulation of Liposomes and an Omega-3 Fatty Acid for the Treatment of Dry Eye Disease. Marta Vicario-de-la-Torre1, Omar Avelino-Cruz1, Maria Caballo-Gonzalez1, Beatriz de-las-Heras2, Jose M. Benitez-del-Castillo3, Rocío Herrero-Vanrell1, Irene T. Molina-Martínez1. Department of Pharmaceutical Technology, 1Department of Pharmacology, 2Faculty of Pharmacy, Complutense University, 3Department of Ophthalmology, Hospital Clínico San Carlos, Madrid, Spain

47 Lipid Release From Lipid-Based Eye Drops Can Be Assisted By Tear Film Sodium Chloride. Joseph G. Vehige, Peter A. Simmons, Bereth J. Beard, Prem Mohant. Allergan Inc., Irvine, California, USA

48 Complex Lipid Mixtures that Resemble Tear Fluid Lipid Layer Do Not Decrease Evaporation. Pipsa Kulovesi, Antti H. Rantamäki, Juha M. Holopainen. Helsinki Eye Lab, Department of Ophthalmology, University of Helsinki, Helsinki, Finland

49 Comparison of Two Lacrimal Substitutes in Patients afer LASIK: Hylabak (Preservative Free) vs. Systane (Preserved). Yuri S. ASTAKHOV, Sergei Y. Astakhov, Alla B. Lisochkina Department of Ophthalmology, Saint-Petersburg I.P. Pavlov State Medical University, 6-8 Lev Tolstoy str., Saint-Petersburg, 197022, Russia

50 The Effect of Three Different Phospholipid-Containing Eye Sprays on Tear Film and Symptoms. Dr Heiko Pult1. 1Optometry & Vision Research, Weinheim, DE; 2Clinical practice in JosefCarrée, Bochum, DE; 3Cardiff University, School of Optometry & Vision Sciences, Cardiff, UK

51 Lipomimetic Prophylaxis Against Dry Eye In Adverse Environments. Akilesh Gokul, Stuti Misra, Jennifer P. Craig Department of Ophthalmology, New Zealand National Eye Centre, The University of Auckland, New Zealand

52 Toxicity And Irritation Potential Of A Novel Manuka Honey-Based Formulation For Managing Blepharitis. Jaeun Kim1, Ilva Rupenthal1, Simon Swift2, Jennifer P. Craig1. 1Department of Ophthalmology, NZ-NEC; 2Department of Molecular Medicine, The University of Auckland, New Zealand

53 Efficacy and Safety Assessment of Azithromycin 1.5% Eye Drops (Azyter®) in Chronic Blepharitis Patients. Serge Doan1, Christophe Baudouin2, Tristan Bourcier3, Marc Labetoulle4, Louis Hoffart5, Pierre-Jean Pisella6 for the AZI Blepharitis Study Group 1Bichat Hospital, 2Quinze-Vingts National Ophthalmology Hospital, 3University Hospital of Strasbourg, 4Bicêtre University Hospital, 5Timone University Hospital, 6University Hospital of Tours, France

54 Use of Tetracyclines and Macrolides in Dry Eyes and Blepharitis – A Systematic Review. Dr. Hiok Hong Chan1, Samantha Sze Lee2, Dr. Louis Tong1,2,3,4. 1Yong Loo Lin School of Medicine, National University of Singapore; 2Singapore Eye Research Institute, Singapore; 3Singapore National Eye Centre, Singapore; 4Duke-NUS Graduate Medical School, Singapore

55 Effect Of HPMC/Dextran 0.3% And Sodium Hyaluronate 0.18% In The Treatment Of Ocular Surface Disease In Glaucoma Patient. Pinnita Prabhasawat, Ngamkae Ruangvaravate, Maneerat Thewthong Department of Ophthalmology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

56 The effect of topical anti-glaucoma eye drops on human Tenon's capsule fibroblasts survival. Nilgun Yildirim1, Gokhan Kus2, Selda Kabadere2, Ruhi Uyar2, Afsun Sahin1. Eskisehir Osmangazi University Medical School, 1Department of Ophthalmology and 2Physiology

57 Recovery of Conjuctival Goblet Cell Density in Mild-Moderate Dry Eye Patients after Treatment with Hyaluronic Acid - Vitamin B12 Based Eyedrops. Piera Versura, Vincenzo Profazio, Giuseppe Giannaccare, Emilio C. Campos. Ophthalmology Unit, DIMES, Alma Mater Studiorum, University of Bologna, S. Orsola-Malpighi Teaching Hospital, Bologna, Italy

58 Optimizing Viscosity Of Ophthalmic Solutions With The Combination Of Two Polymers. Peter A. Simmons1, Bereth J. Beard1, Joseph G. Vehige1. 1Allergan Inc., Irvine, California USA

59 Altered Calcium Regulation by Temperature-Sensitive Transient Receptor Potential Channels (Thermo-TRPs) as a Pathophysiologic Effect in Pterygium. Fabian Garreis1, Stefan Mergler2, Antje Gaebel1, Stefanie Zoll2, Alexander Lucius2, Martin Straßenburg2, Anja Lude2 and Friedrich Paulsen1. 1Department of

Anatomy II, Friedrich Alexander University Erlangen-Nuremberg, Erlangen, Germany; 2Department of Ophthalmology, University Medicine Berlin, Campus Virchow Hospital, Berlin, Germany

60 Treatment of ocular rosacea with low dose doxycycline. Manfred Zierhut1, Deshka Doycheva1, Christoph Deuter1, Ines Pfeffer2, Martin Schaller2, Bianca Sobolewska1. 1Center for Ophthalmology, 2Department of Dermatology, University Tuebingen, Germany

61 Tear Secretion Change By A Daily Intraperitoneal Injection With AICAR For 5 Days In C57BL/6J AND DB/DB Mice. Kokoro Sano, Motoko Kawashima, Takaaki Inaba, Akiko Ito, Tetsuya Kawakita, Shigeto Shimmura and Kazuo Tsubota Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan

62 Dry Eye: Two Experimental Rodent Models For Drug Development. Nicolas Cimbolini1, Sophie Antonelli1, Stefano Barabino2, Laurence Feraille1, Philippe Margaron1 and Pierre-Paul Elena1. 1Iris Pharma, La Gaude, France. 2 Clinica Oculistica, University of Genoa, Italy

63 Sleep Deprivation and Tear Film. Young Joo Shin Department of Ophthalmology, Hallym University College of Medicine, Seoul, South Korea

64 "PLUNC", A New Surfactant Protein Of The Tear Film. Martin Schicht, Fabian Garreis, Lars Bräuer and Friedrich Paulsen. Institute of Anatomy, Friedrich-Alexander-University Erlangen-Nuernberg, Germany

65 The Effect of Temperature on the Surfactant Properties of Human and Rabbit Tears. Xiaojia Eric Wei1,2, Zhenjun Zhao1,2, Thomas J. Millar3, Mark DP. Willcox2. Brien Holden Vision Institute1, School of Optometry and Vision Science, University of New South Wales2, School of Natural Science, University of Western Sydney3 Sydney, Australia

7th International Conference on the Tear Film and Ocular Surface: Basic Science and Clinical Relevance

Abstracts

Taormina, Sicily, Italy

September 18-21, 2013

Title Sponsor:

Alcon

AUTOLOGOUS SERUM TEAR USE RESULTS IN EARLY NERVE REGENERATION BY IN VIVO CONFOCAL MICROSCOPY AND SIGNIFICANT IMPROVEMENT IN SYMPTOMS OF CORNEAL NEUROPATHY-RELATED LIGHT SENSITIVITY

Shruti Aggarwal, Ahmad Kheirkhah, Bernardo Cavalcanti, Pedram Hamrah.
Massachusetts Eye and Ear Infirmary, Department of Ophthalmology, Harvard Medical School, Boston, MA, USA.

Purpose: Patients suffering from corneal neuropathy present with debilitating light sensitivity, but normal examination. The purpose of this study is to evaluate the use of autologous serum tears (ATS) in these patients and to study changes in corneal sub basal nerve plexus using laser *in vivo* confocal microscopy (IVCM).

Methods: This retrospective study included 16 eyes of 16 patients suffering from extreme light sensitivity and no active clinical ocular surface disease. All patients received 20% ATS 6-8x/day and compared to 16 eyes of age-matched controls. Symptom severity (0 to 10) and treatment were recorded. Corneal images by IVCM (HRT3/RCM) were analyzed for total nerve length and number and morphological nerve changes by 2 masked observers before and after ATS.

Results: All patients had extreme light sensitivity of 8.8 ± 1.1 (range7-10). Baseline IVCM showed significantly decreased density and altered morphology of subbasal plexus as compared to controls, with total nerve length (9208 ±1264 µm/mm^2 vs. 24714 ±1056 µm/ mm^2) ($p<0.0001$), total nerve number (9.6±1.4 vs. 28.6±2.0) ($p<0.0001$), reflectivity (2.9±0.2 vs. 1.8±0.1) ($p<0.0001$) respectively. Tortuosity was increased (2.21±0.1 vs. 1.8±0.2) ($p=0.061$). After a 3.6 ± 2.1 months of ATS, all patients reported decrease in symptoms to 1.6± 1.7. Nine patients showed > 90 %, with remaining patients showing 40-60% improvement. IVCM showed a significant increase ($p<0.005$) in total nerve length (15451±1595 µm/mm^2) and number (13.9±2.1) and a significant decrease ($p = 0.001$) in reflectivity (1.9±0.1) and tortuosity (1.7±0.2

Conclusion: IVCM demonstrates profoundly altered and decreased corneal nerves in corneal neuropathy patients and shows significant improvement following ATS treatment, which paralleled symptomatic improvement.

Funding: NIH K08-EY020575, Research to Prevent Blindness Career Development Award, Falk Medical Research Trust, New England Corneal Transplant Research Fund

CORRELATIONS OF CLINICAL SIGNS IN DRY EYE DISEASE WITH CORNEAL IMMUNE CELL INFLUX: AN IN VIVO CONFOCAL MICROSCOPY STUDY

Shruti Aggarwal, Ahmad Kheirkhah, Bernardo Cavalcanti, Pedram Hamrah. Massachusetts Eye and Ear Infirmary, Department of Ophthalmology, Harvard Medical School, Boston, MA, USA.

Purpose: Immune changes play a role in pathogenesis of dry eye disease (DED), and clinical examination does not allow precise detection. In vivo confocal microscopy (IVCM) has recently been used to detect corneal immune response in several diseases. Therefore, this study was designed to evaluate corneal dendritic immune cell (DC) alterations in DED and to correlate IVCM parameters with clinical signs and severity.

Methods: We retrospectively studied 300 eyes of 150 patients with DED and 52 age-matched control eyes. The DED severity was graded based on Dry Eye Workshop (DEWS) classification. Corneal IVCM (HRT3/RCM) images were analyzed for DC density and morphology (cell size and the field covered) by two masked observers independently. The clinical signs and disease severity were correlated to IVCM.

Results: In DED, DC density (93±6 cells/mm^2) was significantly increased compared to controls (26±4) ($P<0.001$). Morphologically, DC size and DC field were significantly higher in DED (107±5µm/mm^2 and 404±20, respectively) than controls (64±5 and 248±23, respectively, $P<0.005$). Corneal and conjunctival staining correlated to increase of DC density, while DC size and field correlated with corneal staining (all $P<0.05$). Interestingly, significant increase of DC density was observed as early as DEWS severity grade 1 ($P<0.001$), with mild additional increase in more severe grades 2-4 ($P=0.24$). Significant morphological changes in DC were detected only in grades 2 to 4 ($P<0.05$).

Conclusion: IVCM parameters show correlation to clinical signs and severity of DED. Although, DC density changes are detected in mild DED patients, morphological changes are seen in more severe cases. Therefore, IVCM is a powerful tool to detect early ocular surface changes and may be used to complement clinical examination, stratifying patients for clinical trials and guide treatment.

Funding: NIH K08-EY020575 (PH), Research to Prevent Blindness Career Development Award (PH), Falk Medical Research Foundation (PH) and Allergan, Inc, Irvine, CA, USA.

EFFECT OF SYSTANE® BALANCE ON NON-INVASIVE TEAR FILM BREAK UP TIME (NITFBUT) IN DRY EYE SUBJECTS WITH LIPID DEFICIENCY

Aguilar[1] AJ, Márquez[1] MI, Paula[1] A, Jorge[1] T, Ogundele[2] A, Berra[1] A. [1]Universidad de Buenos Aires, Buenos Aires, Argentina, [2]Alcon Research, Ltd., Fort Worth, TX.

Purpose: Dry eye syndrome is defined by a disruption of the tear film due to defective aqueous, lipid, or mucin layers, or a combination of the three with or without other factors such as epitheliopathy, lid abnormalities or autoimmune disease. This study evaluated the ability of SYSTANE® BALANCE (SYSB) dosed 4 times a day for 30 days to increase NITFBUT over baseline compared to a saline (SAL) control.

Methods: This was a single site, parallel two-arm, controlled, single masked (investigator only), randomized treatment comparison of SYSB lubricant eye drops and SAL. Eligible patients (≥18 years) had NITFBUT ≤ 7 seconds in at least one eye, Meibomian Gland Expression (MGE) ≥ Grade 1 in both eyes, and evidence of missing meibomian glands in both eyes. The primary outcome measure assessed after 30 days was NITFBUT (seconds) in both eyes 60-120 min post-dose. Secondary assessments included corneal (NaFl) and conjunctival (Lissamine Green) ocular surface staining, and Impression Cytology (Goblet Cell Density).

Results: The mean NITFBUT at baseline (±SD) was 4.60 ±0.69 and 4.93 ±0.80 in the SYSB (n=25) and SAL (n=24) groups respectively. After 30 days of treatment, patients in the SYSB group had a significantly ($p<0.001$) greater increase in the mean NITFBUT (7.43±0.51) compared to the SAL group (5.59±0.66), with the mean treatment difference of 2.16 in favor of SYSB ($p<0.001$). The change in the total score from baseline for both conjunctival and corneal staining after 30 days was reduced in the SYSB (-7.52 & -1.16, respectively) vs. SAL (-1.83 & -0.13, respectively) groups, with the mean treatment difference being statistically significant ($p\leq0.001$). After 30 days, 84% of SYSB and 33% of SAL patients showed improved goblet cell density.

Conclusions: NITFBUT assessments show that SYSTANE® BALANCE provides long-term tear film stabilization and reduced ocular surface staining in dry eye subjects with lipid deficiency.

(This research was supported by Alcon Research, Ltd., Fort Worth, TX)

THE REPEATABILITY OF CLINICAL MEASUREMENTS OF DRY EYE AND MEIBOMIAN GLAND DYSFUNCTION.

Waleed Alghamdi[1,2,3] Maria Markoulli[2] Brien Holden[1,2,3] Eric Papas[1,2,3], [1]Brien Holden Vision Institute, Sydney, Australia, [2]School of Optometry & Vision Science, University of New South Wales, Sydney, Australia, [3]Vision Cooperative Research Centre, Sydney, Australia

Aim: To assess the repeatability of several procedures for determining Meibomian Gland Dysfunction (MGD) and dry eye in a sample of contact lens (CL) wearers and non-wearers.

Methods: The following procedures were conducted on two separate occasions by one examiner on 5 CL wearers, and 5 non-wearers (mean

age ±SD: 27±3.6): ocular surface disease index (OSDI), non-invasive tear break-up time (NITBUT) and lipid layer assessment using the Tearscope, the tear evaporation rate using the Vapometer, tear production rate using phenol-red thread (PRT), MG "drop-out" using infra-red meibography, MG expressibility of 15 MGs using the Korb MG evaluator (0 to 45 scale) and lissamine-green staining for lid-wiper epitheliopathy (LWE). Coefficients of Repeatability (CoR) were calculated for continuous measurements and Cohen's kappa coefficient (k) for categorical data.
Results: Coefficients of repeatability are as shown in Table1. Relating these values to the group mean scores suggest that the measurements for OSDI, MG expressibility, PRT, LWE, NIBUT, and lipid assessment were fairly repeatable for all subjects, while evaporation rate showed poor repeatability. Measuring gland drop-out was the measurement with the highest repeatability. For subgroups, CL wearers had better repeatability with the OSDI compared to non-wearers. Conversely, CL wearers were more variable for lipid layer assessment, MG expressibility and LWE than non-wearers. Other measurements were similar between CL wearers and non-wearers.
Conclusion: The repeatability of MGD/dry eye procedures varies and in some cases can be regarded as poor. CL wearers may display different degrees of variability than non wearers for these tests.

Table1: Repeatability of assessed parameters

Variable	Measure	Overall	CL Wearers	Non wearers
OSDI	CoR	15.6	8.4	19.5
NIBUT		6.9sec	9.7sec	7.2sec
Tear evaporation		89g/m²/h	82g/m²/h	73g/m²/h
PRT		11.5mm	8.4mm	9.5mm
MG expressibility		14.3	17	7.5
MG drop-out		4.5%	3.4%	5.8%
Lipid assessment	Cohen's K	0.63	0.23	0.64
LWE		0.33	0.29	0.32

OCULAR SURFACE DISEASE ASSOCIATED TO GRAF-VERSUS-HOST-DISEASE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

Monica Alves [1, 2] MD PhD, Franscisco Penteado Aranha[3] MD, PhD, Daniella Paiva[2] MD, Afonso C. Vigoritto[3] MD PhD, Wilson Estevam Filho [3], Carmino A. Souza[3] MD PhD, Eduardo Melani Rocha[1] MD PhD [1] Faculty of Medicine Ribeirão Preto, São Paulo University, [2] Pontific Catholic University of Campinas, [3] University of Campinas
Purpose: Allogeneic hematopoietic stem cell transplantation (AHSCT) can be a curative treatment for hematologic malignancies and nonmalignant conditions. Graft-versus-host-disease (GVHD) represents the major cause of morbidity and mortality affecting AHSCT long-term survivors. The aim of this study was to describe and compare ocular surface parameters in a cohort of chronic GHVD patients, as well as clinical aspects of the disease and treatment.
Methods: Ocular evaluation encompassed: (1) structured questionnaire (OSDI); (2) tear osmolarity; (3) tear film break-up time (TBUT); (4) fluorescein and lissamine staining; (5) Schirmer test (ST); (6) meibomian gland evaluation; (7) severity grading. To better understand the associations of ocular surface disease to AHSCT, we also analyzed treatment regimens, the occurrence of acute and chronic GHVD and baseline diagnosis.
Results: Twenty-seven patients were enrolled in the study, along with twenty-four age and gender matched controls. The majority of patients underwent myeloablative regimen (70%) to treat different diseases, such as chronic myeloid leukemia and other conditions. Acute GVHD occurred in 33.3% of cases, after 62.1±44.2 days and chronic GVHD was diagnosed after 214.3±92.7 days. Besides the eyes, it mainly involved the oral mucosa (85.2%), skin (70.5%) and liver (59.2%). Significantly scores were obtained in all performed diagnostic tests: OSDI was 49.3±20.9, fluorescein 4.4±3.8, lissamine was 2.5±2.5, ST 8.8±8.8 mm, TBUT 3.8±2.8 seconds, tear osmolarity 312±17.2mOsmol, 85% presented cicatricial MGD. Correlations were significant in fluorescein/lissamine (r^2 =0.89) and TBUT/ST (r^2=0.49). Severity grades vary from moderate to severe.
Conclusions: Ocular surface disease is part of chronic range of GVHD after AHSCT. Patients present symptoms and ocular surface damage related to DED and MGD. However, diagnostic tests parameters vary significantly and most of times do not strongly correlate among one other.
Support: Fapesp, FAEPA, CNPq.

COMPARISON OF OCULAR SURFACE DISEASE INDEX AND TEAR OSMOLARITY AS A MARKER OF OCULAR SURFACE DYSFUNCTION IN VDT WORKERS.

Pasquale Aragona MD, PhD[1], Rosaria Spinella MD, PhD[1], Laura Rania MD, PhD[1], Elisa Postorino MD, PhD[1] and Concettina Fenga MD[2]. [1]Department of Experimental Medical-Surgical Sciences, Section of Ophthalmology, University of Messina, Messina, Italy. [2] Department of Social and Environmental Health, Section of Occupational Health, University of Messina, Messina, Italy.
Purpose: Video display terminal (VDT) work is recognized as a high risk factor for ocular dyscomfort. This study compares the ability of two ocular surface tests, the ocular surface disease index (OSDI) questionnaire and tear osmolarity, to evidentiate subjects with ocular surface dysfunction in a population of VDT users.
Methods: 64 subjects undergoing a routine health surveillance programme, underwent OSDI and the following tests: TearLab for tear osmolarity (OSM), tear break-up time (BUT), fluorescein corneal stain and the assessment for meibomian glands. The results of the worst eye were considered for the statistical analysis. Main outcome measures were the results of OSDI and tear osmolarity. The ROC curves for OSDI and tear osmolarity were evaluated using BUT and corneal score as classification variables.
Results: Considering the BUT as classification variable, the ROC curve analysis demonstrated a statistically significant difference between the AUC of OSDI (0.518; 95% CI 0.385-0.645) and OSM (0.864; 95% CI 0.755-0.937) (p<0.0001). The OSDI, at the previously determined cut-off of 12 points, showed a sensitivity of 42.8%, a specificity of 50%, a positive predictive value (PPV) of 85.7% and a negative predictive value (NPV) of 12%. The OSM at the pre-determined cut-off of 308 mOsm/L showed a sensitivity of 68.4%, a specificity of 100%, a positive predictive value (PPV) of 100% and a negative predictive value (NPV) of 33%. With the corneal score as classification variable the AUC for OSDI was 0.579 (95% CI 0.449-0.702), for OSM was 0.748 (95% CI 0.624-0.848) (P<0.01). For the OSDI the sensitivity was of 71.4%, specificity 22.2%, the PPV was of 62.5% and the NPV of 75%. For the OSM the sensitivity was of 64.3%, the specificity of 47.2%, the PPV of 48.6% and the NPV of 63%.
Conclusions: The results showed that OSM can be considered a more reliable test than OSDI, when screening VDT workers for possible ocular surface alterations.
The authors declare no commercial relationships and no grant support for the present research.

PROTECTIVE EFFECTS OF TREHALOSE ON CORNEAL EPITHELIUM IN COURSE OF LASER SUBEPITHELIAL KERATOMILEUSIS (LASEK).

Pasquale Aragona,[1] Pietro Colosi,[2] Laura Rania,[2] Francesca Colosi,[2] Antonina Pisani,[3] Domenico Puzzolo,[3] Antonio Micali.[3] Department of Social and Environmental Health[1], Department of Experimental Medical-Surgical Sciences[2], Department of Biomedical Sciences and Morphofunctional Imaging, University of Messina[3], Messina, Italy
Purpose: Alcohol treatment is used to prepare corneal epithelium in laser subepithelial keratomileusis (LASEK). Aim of the present work was to evaluate the effects of the pretreatment with trehalose on the

corneal epithelium of LASEK-treated patients.
Methods: Twelve patients undergoing LASEK were consecutively included in the study. The right eyes were pretreated with 3% trehalose eye drops, whilst left eyes were used as control. Epithelial specimens were processed for light and transmission electron microscopy and from both groups a morphometric analysis was performed.
Results: In trehalose-untreated eyes (TUE), the corneal epithelium showed superficial cells with reduced microfolds, wing cells with intracellular vesicles and dilated intercellular spaces, and dark basal cells filled with vesicles, separated by wide clefts. In trehalose-treated eyes (TTE), superficial and wing cells showed a better preserved morphology, and basal cells were generally clear with intracytoplasmatic vesicles. Morphometry demonstrated that in TTE the epithelial height and the basal cells area were statistically significantly higher and the basal cells cytoplasm was significantly clearer. Furthermore, the distribution of desmosomes and hemidesmosomes were statistically significantly different between the groups.
Conclusions: Morphological and morphometric features of alcohol delaminated corneal epithelium were better maintained after trehalose administration, when compared to controls.
The authors declare no commercial relationships and no grant support for the present research.

CLINICAL, CONFOCAL AND MORPHOLOGICAL INVESTIGATIONS ON THE CORNEA IN HUMAN MUCOPOLYSACCHARIDOSIS IH-S.

Pasquale Aragona[1], Edward Wylegala[2], Ewa Wroblewska-Czajka[2], Adrian Smedowski[2], Anna Nowinska[2], Anna M. Roszkowska[1], Antonina Pisani[3], Antonio Micali[3], Domenico Puzzolo[3]. Department of Experimental Medical-Surgical Sciences,[1] Department of Ophthalmology, District Railway Hospital, Katowice, Poland[2], Department of Biomedical Sciences and Morphofunctional Imaging, University of Messina, Messina, Italy[3]
Purpose: To correlate the corneal confocal microscopy findings in type I Hurler Scheie mucopolysaccharidosis (MPS IH-S) with their structural and ultrastructural characteristics.
Methods: Both corneas from a MPS IH-S patient were examined in vivo with confocal microscopy and then removed and processed for light microscopy and transmission and scanning electron microscopy.
Results: Confocal microscopy showed basal epithelial cells with either diffuse or granular hyperreflectivity. Keratocytes were highly reflective determining a web-shaped stromal appearance. Endothelial cells were barely visible. The histopathological study demonstrated superficial cells with apical microfolds, small vesicles and evident intercellular junctions. The wing cells showed either well-evident tonofilaments and small peripheral vesicles, or large paranuclear vesicles. The basal cells showed polygonal shape, many small vesicles, and enlarged intercellular spaces. The Bowman layer was either normal or thinner and formed by variably electron dense material. In the stroma, irregularly oriented lamellae, many vesicles-filled keratocytes and intercellular granular material were present. The Descemet membrane was normal, while the corneal endothelium showed marked degenerative changes.
Conclusions: The confocal alterations appeared correlated to the anomalous accumulation of material. The histopathological images gave a clue to the better understanding of the corneal changes demonstrated by the confocal studies in MPS IH-S.
The authors declare no commercial relationships and no grant support for the present research.

MODULATION OF GLYCOCALYX BARRIER FUNCTION IN HUMAN CORNEAL EPITHELIAL CELLS.

Pablo Argüeso,[1] Jerome Mauris,[1] Flavio Mantelli,[1] Ashley M. Woodward,[1] Ziyhi Cao,[2] Bertozzi CR,[3,4] Noorjahan Panjwani,[2] Kamil Godula.[4] Schepens Eye Res Inst/Mass Eye and Ear, Harvard University, Boston, MA,[1] Dept of Ophthalmology, Tufts University, Boston, MA,[2] Dept of Chemistry, University of California, Berkeley, CA,[3] and Lawrence Berkeley National Laboratory, Berkeley, CA.[4]
Purpose: Transmembrane mucins interact with the multivalent carbohydrate binding protein galectin-3 to maintain the integrity of the ocular surface epithelial glycocalyx. Here, we aimed to determine whether disruption of galectin-3 multimerization and insertion of synthetic glycopolymers in the plasma membrane could be used to modulate barrier function in corneal epithelial cells.
Methods: Barrier function was assayed in human corneal epithelial cell cultures and galectin-3 null mice using the rose bengal penetration assay. Galectin-3 was abrogated *in vitro* using siRNA. Recombinant galectin-3 was produced by cloning the full-length cDNA followed by expression in *E. coli* Rosetta cells. Site-directed mutagenesis was used to obtain a dominant negative inhibitor of galectin-3 polymerization lacking the N-terminal domain. Galectin-3 was quantified by Western blot. Incorporation of Alexa Fluor 488-labeled glycopolymers into cellular membranes and galectin-3 affinity were determined by fluorescence microscopy and pull-down assays, respectively.
Results: Abrogation of galectin-3 biosynthesis resulted in significant loss of barrier function, as indicated by increased permeability to rose bengal, in cell culture and mouse corneas. Addition of -lactose, a competitive inhibitor of galectin-3 binding, to cell cultures transiently disrupted barrier function. In these experiments, addition of recombinant galectin-3 deletion mutant, but not full-length galectin-3, prevented the recovery of barrier function to basal levels. Both cellobiose- and lactose-containing glycopolymers incorporated into apical membranes of corneal epithelial cells independently of the chain length distribution of the densely glycosylated backbone. Membrane incorporation of cellobiose glycopolymers, which, contrary to their lactose-containing counterparts, failed to bind galectin-3, impaired barrier function.
Conclusions: Galectin-3 multimerization and surface recognition of lactosyl residues is required to maintain barrier function at the ocular surface. Transient modification of galectin-3 binding could be used to enhance the efficiency of topical drug delivery.

FIRST RESULTS FOR A THREE-DIMENSIONAL CULTURE SYSTEM USING IMMORTALIZED HUMAN MEIBOMIAN GLAND EPITHELIAL CELLS.

Nagayoshi Asano, [1,2] Daniel Abrar, [1] Ulrike Hampel,[1] Friedrich Paulsen.[1] Department of Anatomy II, Friedrich Alexander University Erlangen/Nuremberg, Erlangen, Germany[1], Santen Pharmaceuticals. Co., Ltd, Nara, Japan[2]
Purpose: We aimed to build up a simple 3D culture model using a meibomian gland cell line (Liu et al. 2010) and to compare the characteristics between classical monolayer (2D) and the 3D model.
Methods: To establish the 3D model, we decided to use a scaffold-based culture system. Our three-dimentional culture model was cultured initially submerged. To find an adaptable scaffold, we first tested several types of inserts consisting of different materials (Millicell-HA, Millicell-PCF, ThinCert and Alvetex). After that, we optimized the culture conditions in combination with an exposure to air (air-lift) and media for at longest 28 days. Finally, we performed Sudan III staining for lipid detection to characterize cell differentiation in our developed 3D model.
Results: 3D cultures using ThinCert yield the most multiple cell layers. In case of Millicell-HA and Millicell-PCF cells grew to just a few layers or they were not firmly adherent to insert. However, even in 3D culture using ThinCert, meibocytes grew in multilayers only when using air-lift. Otherwise, cells finally died during “long-term” culture. Alvetex is a kind of spongy membrane where cells grow inside the membrane. Although cells grew well in Alvetex, cell density was low. On the other hand, lipid labelling was increased depending on the culture period when using Alvetex.

Conclusions: From the present findings we favor ThinCert as scaffold to obtain a multi-layered meibocyte culture model that can be used for further investigations. Also Alvetex is useful but needs further improvement. The model can serve as a tool to better understand mechanisms involved in the development of meibomian gland dysfunction.

[The authors thank David Sullivan, Schepens Eye Research Institute, Harvard Medical School, USA for obtaining the human meibomian gland epithelial cell line. This research was supported in part by grants from the Johannes and Frieda Marohn foundation and Sicca research award of the DOG]

THE OCULAR SURFACE HEALING ACTION OF SYSTANE® ULTRA VS. MAXIDEX® OR SALINE

Asbell P,[1] Gadaria N,[1] & Ogundele A.[2] [1]Department of Ophthalmology Mount Sinai Hospital, New York, NY, [2]Alcon Research, Ltd., Fort Worth, TX.

Purpose: Dry eye disease (DED) is associated with tear deficiency or excessive evaporation causing damage to the exposed ocular surface and discomfort. Untreated, severe DED can lead to desiccation of the corneal epithelium, increased discomfort and possible loss of vision. This study assessed the corneal surface healing action of artificial tears Systane® ULTRA (SYS) vs. (Maxidex® [MAX] Dexamethasone ophthalmic) and (saline solution [SAL]) controls.

Methods: Study was a randomized, active- and placebo-controlled, single center trial. Eligible DED patients (≥18 years of age) had a score of at least 15 on the Ocular Surface Discomfort Index (OSDI) symptom questionnaire and moderate vital staining. Patients were randomized to SYS, MAX or SAL, 1 drop to each eye 4 times a day for 4 weeks. Clinical outcomes included the change from baseline at 2 and 4 weeks in the OSDI, Tear Film Break-up Time (TBUT), conjunctival (lissamine) and corneal (fluorescein) staining according to the National Eye Institute grading scale.

Results: Study participants (N=36) were mostly female (92%) with a mean age of 57.6 years. The OSDI at baseline was 52.0 (n=12), 56.2 (n=12), and 48.3 (n=12) in the SYS, SAL, and MAX groups respectively. The mean change from baseline in OSDI at 2 weeks was -13.6 for SYS compared to 2.0 for SAL patients (p=0.082) and -16.7 for MAX treated patients (p=0.726; SYS vs. MAX). Continued improvement was observed in the mean OSDI score at 4 weeks with SYS and MAX treated patients having a -17.7 and -16.6 change from baseline (p=0.911) compared to -11.5 for SAL patients (p=0.809; SYS vs. SAL). Pairwise comparison of TBUT, corneal and conjunctival staining scores did not show significant differences between the treatment groups.

Conclusions: Systane® ULTRA showed rapid improvement (at 2 weeks) in DED symptoms (OSDI) that was similar to MAX and numerically better than those treated with SAL with no statistical difference between treatments at either 2 or 4 weeks, possibly due to the small sample size.

(This research was supported by Alcon Research, Ltd., Fort Worth, TX)

STANDARDIZED QUANTIFICATION OF INFLAMMATORY BIOMARKERS FOR MULTICENTER CLINICAL TRIALS OF OCULAR SURFACE DISEASE.

Penny A. Asbell,[1] Seth P. Epstein,[1] Yi Wei,[1] Neha Gadaria-Rathod,[1] Maureen G. Maguire.[2] Department of Ophthalmology, Mount Sinai School of Medicine, New York, NY,[1] Department of Ophthalmology, University of Pennsylvania, Philadelphia, PA, USA.[2]

Purpose: To establish a standardized methodology for determining the amount of HLA-DR on ocular surface cells and for measuring tear inflammatory cytokine concentrations to validate the resulting profile as minimally invasive objective metrics for large scale multicenter clinical trials and translational research studies of ocular surface disease.

Methods: Standard Operating Procedures (SOPs) were established for the quantification of the expression of HLA-DR antigen by flow cytometry after immunofluorescent staining of human conjunctival (CNJ) impression cytology (IC) samples. Inflammatory cytokines (IL-1 , IL-6, INF- and TNF-) were quantified in human tears using a high sensitivity human cytokine multiplex kit.

Results: Validity of the SOPs was established: 1) collected sufficient numbers of cells; 2) relative biomarker expression quantified was repeatable; 3) results with pooled tear samples were reproducible; 4) cytokine standards statistically satisfied the manufacturer's quality control criteria; 5) personnel at distant sites were successfully taught to collect, store and ship samples; 6) samples were stored until processing was performed without affecting results; 7) the SOPs were successfully incorporated into clinical trials and validated.

Conclusions: We demonstrated the repeatability/effects of storage, ability to train/gather samples from distant sites and the feasibility of use in a randomized clinical trial. Both HLA-DR expression as determined from IC samples and tear cytokine profiling using these SOPs can serve as a minimally invasive objective metrics of inflammation for useful for diagnosing, classifying and analyzing treatment efficacy in inflammatory conditions of the ocular surface and may help further elucidate the underlying mechanisms important in the pathogenesis of ocular surface disease.

Supported in part by: The Martin and Toni Sosnoff Foundation, New York, New York; Research to Prevent Blindness, New York, New York; National Eye Institute.

ROLE OF INFLAMMATION IN DRY EYE DISEASE.

Penny A. Asbell, Seth P. Epstein, Yi Wei, Neha Gadaria-Rathod. Department of Ophthalmology, Mount Sinai School of Medicine, New York, NY, USA.

Dry eye disease (DED) is a multifactorial inflammatory disorder of the lacrimal functional unit that results in symptoms of discomfort, visual disturbance, and tear film instability and leads to chronic ocular surface disease, impaired quality of vision, and a wide range of complications, eventually causing a reduction in quality of life. It remains a frustrating disease due to the present scarcity of effective treatment modalities that can reverse, or at least stop its progression. The purpose of this presentation is to review the pivotal role of inflammation in dry eye disease and present recent findings of our Biomarker Laboratory substantiating this role of inflammation in DED.

A comprehensive literature survey of English-written scientific publications supporting the role of inflammation in DED in cell culture, animal models and humans will be presented. Our recent data demonstrating the presence of inflammatory biomarkers in DED and their change with treatment will also be provided.

Although multiple mechanisms may contribute to DED, inflammation of the ocular surface appears to be a pivotal part of its pathogenesis.

Supported in part by: The Martin and Toni Sosnoff Foundation, New York, New York; Research to Prevent Blindness, New York, New York; National Eye Institute.

EFFECT OF INHIBITION OF SPLA2 ON OCULAR SURFACE DISEASE IN DRY EYE MICE.

Penny Asbell, Yi Wei, Pengcheng Li, Jun Zou, Seth Epstein, Neha Gadaria-Rathod. Department of Ophthalmology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA.

Purpose: sPLA2-IIa is found in high concentrations in healthy tears. They are increased further in dry eye disease (DED) patients and is associated with ocular surface (OS) inflammation. sPLA2-IIa and its isoforms were shown to play an important role in the pathogenesis of experimentally induced DE mice by amplification of OS inflammation. Further, we demonstrated *in vitro* that sPLA2-IIa-specific inhibitors significantly decrease PGE2 concentration upon sPLA2 stimulation. The purpose of this study was to determine the role of topical sPLA2

on the symptoms of DED and inflammation in BALB/c DE mice.
Methods: DED was induced by scopolamine-air ventilation. Female BALB/c mice were divided into 6 groups: 1) normal control, no treatment; 2) DE control, no eye drops; 3) DE + inhibitors; 4) DE + dimethylsulfoxide (DMSO; solvent of the inhibitors); 5) DE + sPLA2 antibodies (against sPLA2-IIa, V or X); 6) antibody control, PBS + 0.1% BSA buffer. Eye drops containing inhibitors, solvent or antibodies were applied, to both eyes of groups 3-6 2x/day, starting at Day (D) 1. Pheno-Red Thread for tear volume and biomicroscopic examination were performed for SPK by masked ophthalmologists on D0, 4 and 7. Biopsies of mouse corneal, conjunctival and lacrimal gland were analyzed by immunofluorescent staining (IFA) at the termination of the study (D8).
Results: As compared to controls, all tested inhibitors reduced SPK scores on D4 and 7. The sPLA2 inhibitor V displayed better reduction (dose-dependent). No effect on tear secretion. All antibodies reduced SPK scores. Antibodies against sPLA2-IIa and V showed a peak of SPK-reduction (IIa: D4; V: D7), while that against sPLA2-X reduced SPK on D4 and 7. The reduction was accompanied by a concomitant decrease in sPLA2-IIa IFA in the conjunctiva.
Conclusions: These studies suggest that tear sPLA2 has a significant role in pathogenesis of DED. Inhibition of sPLA2 may provide new strategies for treatment of OS inflammation associated with DED and/or other inflammatory conditions.

THE IMPORTANCE OF TRANSLATIONAL RESEARCH IN MEDICINE AND OPHTHALMOLOGY

Dimitri Azar

The goal of translational research is to accelerate the translation of scientific discoveries into innovative diagnostics and therapies, disease prevention, improved health care delivery, and to provide cost-effectiveness prevention and treatment policies that are adopted in the community. This lecture will cover current and emerging challenges in the developing field of translational science as well as the importance of applying translational research in academic institutions through discussing our experience with the Center for Clinical and Translational Science at the University of Illinois at Chicago. Strategies that can be adopted to encourage translational research include development of physician-scientists, interdisciplinary collaborations, and implementation of technology will also be discussed. Successful application of translational research in ophthalmology will additionally be highlighted. Translational science offers an opportunity to deliver an unparalleled promise to advance patient care.

CHANGES TO THE TEAR FILM PROTEOME IN KERATOCONUS.

Siva Balasubramanian,[1] David Pye,[2] Mark Willcox.[2] Deakin School of Medicine, [1] University of New South Wales, Australia. [2]

Purpose: Keratoconus (KC) is a poorly understood degenerative disease of the eye, which causes an irregularly shaped cornea leading to severe impairment of vision. This study investigates the levels of proteins, proteases and cytokines in the tears of people with KC. We also sought to determine the influence of eye rubbing on protease expression, protease activity and concentration of inflammatory molecules in tears.
Methods: Basal tears were collected from controls (C), KC and from people who had undergone corneal collagen cross-linking (CXL) for the treatment of KC. ELISA, mass spectrometry, antibody arrays and activity assays were used to examine the changes in tear proteins between the different subject groups.
Results: There was approximately 2-fold decrease in total protein levels, lactoferrin, secretory IgA between KC and C tears. Increased levels of cathepsin B (2.7-fold) and decreased levels of cystatin S (2.1-fold) and cystatin SN (2-fold), polymeric immunoglobulin receptor (9.4-fold), fibrinogen alpha chain (8.2-fold) were observed in KC compared to C. Tears of people with KC had 1.9 times higher levels of proteolytic activity and over-expression of several matrix metalloproteinases (MMP) -1,-3,-7,-13 and interleukins (IL) -4,-5,-6,-7,-8 and tumour necrosis factor (TNF) - , - compared to tears from C. No significant difference in MMPs were observed between C and CXL groups, although the expression levels of TNF- was 1.5 times increased in CXL compared to C. The activities of tear proteases in CXL were not significantly different compared to either KC or C. The concentrations of MMP-13, IL-6 and TNF- were significantly increased in normal subjects after eye rubbing.
Conclusions: The increase in protease, protease activity and inflammatory mediators in tears after eye rubbing may be exacerbated in people with KC and this in turn may contribute to the progression of the disease. These novel findings might lead the way to the development of new therapeutic targets for KC.

OCULAR SURFACE IMMUNOLOGICAL CHANGES IN PATIENTS WITH CICATRICIAL PEMPHIGOID

Stefano Barabino,[1] Maurizio Rolando,[1] Elisa Montaldo,[2] Cristina Mingari,[2] Carlo Enrico Traverso[1]. [1] Clinica Oculistica, DINOGMI, University of Genoa, [2]DIMES, University of Genoa, Italy

Purpose: To test the hypothesis that patients with ocular cicatricial pemphigoid (OCP) have a significant degree of conjunctival inflammation and anatomical and immunological changes of the cornea.
Methods: Early stages OCP patients were identified, and Schirmer test, fluorescein and lissamine green staining, tear break-up time, and impression cytology of the conjunctiva were performed in 25 patients and in 25 age-matched controls. HLA-DR and CD45 expression on conjunctival cells was measured by flow cytometry. The central cornea was examined by *in vivo* confocal microscopy using a 40x lens and an axial resolution of 5 µm in both groups. Tear cytokines were analysed.
Results: Statistically significant changes in corneal fluorescein staining, lissamine green conjunctival staining, HLA-DR expression, and CD45 occurred in the study group compared to controls, while tear secretion did not show any differences. In patients with OCP confocal microscopy images obtained showed a significant lower number of epithelial cells compared to controls, superficial epithelial cell area considerably higher than normal, reduced nucleus/cytoplasm ratio, halos around the nuclei, and sharp borders. Numerous highly reflective dendritic-like cells were present in the epithelial cell basal layer, and their density correlated with HLA-DR expression. The stroma showed loss of keratocytes, the presence of lacunae, and tortuous subbasal nerves. No differences were recorded in endothelial cells density. A significant increase of IL8 was observed in the study group tears.
Conclusions In OCP the ocular surface is characterized by inflammation of the conjunctiva and immunological and structural changes of the cornea which confirm the importance of local and systemic anti-inflammatory therapy in the early stages of the disease.

IMAGING THE OCULAR SURFACE IN VIVO IN HEALTH AND DISEASE

Christophe Baudouin

The recently developed in vivo confocal microscopy technologies may provide in a noninvasive way excellent histologic-like patterns of the ocular surface structures, showing high-resolution images of the corneal, limbal and conjunctival epithelia, dendritic cells, new blood vessels, and in specific indications some infectious agents. Many ocular surface diseases may find important applications, such as dry eye, allergy, refractive surgery, glaucoma, infections, corneal neovascularization or limbal pathologies. Anterior segment OCT has also been widely developed and now provides tissue level images that may be useful for assessing corneal diseases, although the resolution is still insufficient to achieve cellular details. These complementary technologies could thus become routine methods to explore ocular surface disorders.

TRACKING THE PROGRESSION OF TEAR BREAK-UP.

Carolyn Begley,[1] Adam Winkeler,[1] Richard Braun.[2] Indiana University[1] University of Delaware[2]

Purpose: Events within areas of tear break-up (TBU) remain poorly understood. The purpose of this study was to monitor the progression of TBU using two independent methods to determine relative changes in slope as TBU formed and differences in tear thinning rates (TFT) within and outside areas of TBU.

Methods: Mydriacyl, proparicaine and 2 l of 2% FL were instilled into the eyes of 6 subjects, who kept one eye open as long as possible while a modified slit-lamp biomicroscope simultaneously imaged the tear film by fluorescence (FL) and retroillumination (RI). Areas of TBU were systematically selected for analysis. TFT rates inside and outside of areas of TBU were calculated assuming a 3µm initial thickness (Nichols et al, 2012). Surface profiles obtained from differentiated FL intensity and RI intensity were used to measure relative changes in slope of edges of TBU (Himebaugh et al, 2003).

Results: FL intensity decreased in all subjects over time, reaching a minimum within areas of TBU. Slope changes at edges of TBU were increasingly correlated between FL and RI methods, with high correlations at the end of trials (AVG r=0.73, p<0.001). The TFT rates for 2 subjects with very stable tears were 0.0063±0.0018 units/sec (Mean ± SD), compared to 0.0775±0.0055 units/sec from 4 other subjects with extensive TBU (almost 10x faster). The average TFT inside areas of TBU was 0.1038±0.0444 units/sec compared to 0.0576±0.0565 units/sec just outside and adjacent to TBU. This corresponded to estimated thinning rates of 2.913±1.327 µm/min inside TBU areas and 1.150±1.481 µm/min in adjacent areas.

Conclusion. Our results using two independent methods show a steady deepening of areas of TBU in most trials, with slope changes at the edges of TBU consistent with the concept of spatially local differences in TFT in and outside areas of TBU. These results suggest evaporation as a main mechanism in TBU, and if so, support the idea that "hot spots" of increased tear film hyperosmolarity may form within areas of TBU. This research was supported by a grant from NEI R01EY021794 (Begley).

THE EFFECT OF INCREASING OCULAR SURFACE STIMULATION ON BLINKING

Carolyn Begley,[1], Ziwei Wu,[1], Ping Situ,[1] Trefford Simpson.[2] Indiana University School of Optometry,[1] Bloomington, IN, USA. University of Waterloo School of Optometry and Vision Science,[2] Waterloo, Canada

Purpose: Ocular surface controls over blinking remain controversial because both attention to a task and ocular surface input affect blinking. In this investigation, we controlled attention to isolate the effect of increasing ocular surface air stimulation on blinking.

Methods: Ten healthy subjects played a video game to control attention while seated behind a slit lamp biomicroscope. Air flow (AF) was directed toward the central cornea (15mm distance, temperature= 24 Celsius) through a 0.5mm diameter tube. Using the ascending method of limits, the AF producing an approximate high blink rate was estimated and used to determine the stimulus intensities for a method of constant stimuli. Six levels of stimuli (0, 0.25, 0.5, 0.75, 1 and 1.25 multiples of this estimated AF) were randomly presented and 3 times each. Blinking and the AF were recorded simultaneously and custom MATLAB programs determined the blink rate (BR), interblink interval (IBI) and the corresponding AF for each subject.

Results: When increasing the AF, both mean and standard deviation of IBI were decreased. Mean (±SD) IBI were 5.69±3.92, 3.67±1.56, 2.77±0.81, 2.17±0.67, 1.36±0.37 and 1.02±0.37sec from no air applied to the maximum air stimulus. Different subjects required different AF to trigger significant IBI change that the mean (±SD) AF was 97.7± 25.9 ml/min (Permutation test, p<0.001). After log transformation, there was a significant linear function between AF and IBI (Pearson r= -0.944, p<0.001). A significant linear function was also found within each subject (Pearson r ranged from -0.859 to -0.987, p<0.05) with similar slope: mean (±SD) slopes were -0.00233±0.000647.

Conclusions: These results support the hypothesis that ocular surface stimulation increases blinking, following a linear dose response relationship. Both the frequency and regularity of the blink were increased in response to ocular surface stimulation, presumably to protect the ocular surface.

NEURAL PROTECTIVE MECHANISMS OF THE OCULAR SURFACE IN HEALTH AND DISEASE

Carlos Belmonte, MD, PhD
Instituto de Neurociencias UMH-CSIC, San Juan de Alicante and Fundación de Investigación Oftalmológica, Instituto Fernandez-Vega, Oviedo, Spain.

The ocular surface is continuously exposed to damage caused by environmental changes, chemical irritants, mechanical injury, infections or iatrogenic interventions. The various functional classes of trigeminal ganglion sensory neurons innervating eye surface tissues are differentially activated by this large number of potentially injurious stimuli, giving rise to immediate and long-term protective responses. These include acute and sustained conscious sensations of discomfort and pain, reflex responses such as blinking and tearing, enhancement of local inflammatory processes and stimulation of wound healing mechanisms.

Sensory neurons innervating the ocular surface tissues are functionally heterogeneous. Each neuron type detects preferentially a particular form of stimulating energy, according to the nature of the transduction receptor and channel proteins present at their peripheral receptor terminals. Based on the relative stimulus specificity, ocular surface neurons have been classified as mechano-nociceptor, polymodal nociceptor and cold thermoreceptor neurons. The quality of the sensation evoked by the excitation of each of these distinct neuronal classes, and their contribution to reflex protective responses is different. Moreover, the magnitude and time course of their impulse firing varies markedly depending on the characteristics of the stimulus, the level of accompanying inflammation and the degree of injury of peripheral terminals. Finally, each neuronal type contributes differently to the altered reflex blinking and tearing rate and to corneal and conjunctival cell proliferation and migration changes accompanying a particular pathological process.

The sustained changes in functional properties exhibited by each neuronal type depend ultimately on the specific changes in gene expression induced by a given pathological condition. For instance, prolonged dryness of the ocular surface due to reduced tearing, increases markedly background activity and responsiveness of corneal cold receptor neurons while impulse firing enhancement of polymodal nociceptor neurons is comparatively less pronounced. In contrast, during allergic or actinic ocular kerato-conjunctivitis, activity of polymodal nociceptors is augmented while the spontaneous and stimulus evoked impulse firing in cold thermoreceptors is depressed. Finally, corneal surgery impairs the impulse activity of both polymodal and cold neuronal classes. These disturbed pattern of nerve impulse responses reflect the differentially altered expression of transducing and voltage-dependent ion channels underlying generation and coding of nerve impulses by sensory nerve terminals, experienced by each functional type of sensory neuron in response to abnormal conditions. The variable participation of functionally distinct neuronal types in the response to injurious forces, helps to explain the characteristically distinct, innervation-related signs and symptoms appearing during different pathological conditions of the ocular surface.

QUALITY OF VISION AFTER THE INSTILATION OF A LIPOSOMAL FORMULATION

Jose M. Benitez-del-Castillo[1], Ana M. Muñoz[1], Marta Vicario[2], Maria Caballo[2], Irene Molina[2], Rocio Herrero[2]. Hospital Clinico San Carlos[1],

Facultad de Farmacia, UCM[2], Madrid, Spain.
Purpose: Dry eye disease (DED) is a multifactorial disease involving the ocular surface. It is accompanied by visual problems related with the tear film instability. Thus, medications directed to DED treatment should not only decrease discomfort but improve vision. The aim of the study was to evaluate the efficacy of a formulation based on liposomes. This formulation, destined to replace tear film layer, is composed by lipid components (liposomes) dispersed in a hypotonic solution of borates, trehalose and hyaluronic acid (0.2%).
Methods: Twelve subjects with occasional DED were evaluated before and after the instilation of 50 microliters of the test formulation. The following examinations were performed: symptomatology after instillation, TearLab osmolarity, objective tear film breakup time (TFBUT), objective redness, lipid layer study (Keratograph 5, Oculus, Germany), fluorescein staining, and objective scatter index (OSI)(OQAS, Visiometrics, Spain). Test were performed before, 15, 60 and 120 minutes after the instilation.
Results: After the instilation patients reported no adverse efects and felt comfortable. No objective redness and corneal staining appeared after the instilation. Objective TFBUT and 20 seconds after blinking OSI significantly decreased for at least 60 minutes ($p<0.05$). Osmolarity decreased 7% for 120 minutes. No statistical significant differences were found in the lipid layer pattern.
Conclusion: The test formulation has been proven to be safe and to improve visual parameters after restoring the tear film layer at least for one hour.
The authors have patented the formulation.
Research Group UCM 920415 (GR 35/10-A), IdISSC Ocular Surface, FIS PI10/00645 and FIS PI10/00993, and RETICs RD12/0034,

EYELID DISORDERS EVALUATION IN THE OPHTHALMIC CURRENT PRACTICE IN GERMANY AND POLAND; THE MEIBUM STUDY.

Jose Manuel Benitez del Castillo[1], Thomas Kaercher[2],, Zbigniew Zagórski[3].
[1]Hospital Clinico San Carlos, Madrid, Spain; [2]Dossenheimer Landstraße 48, 69121 Heidelberg, Germany; [3]Pr. Zagorski Eye Surgery Centre, Al. Malachowskiego 5, 24-140 Naleczow, Poland.
Purpose: To determine the incidence and characteristics of eyelid inflammatory disorders during general ophthalmological consultations and to demonstrate the association between palpebral pathologies and ocular surface pathologies in 2 of the 9 European countries involved in the Meibum survey.
Methods: Multi-centre, international, transversal and epidemiological survey. The primary objective was to assess the percentage of eyelid disorders in patients attending a current ophthalmologic consultation. The secondary objectives were to assess the association between palpebral pathologies and ocular surface pathologies, the impact of eyelid disorders on patient's daily life (on vision, on daily life activities/work, on leisure, on contact lens wearing, on emotions and on sleep) .
Results: 376 patients in Germany (DE) and 2635 patients in Poland (PL) were included by ophthalmologists. The mean age was 60.6 (±17.4) in DE and 56.2 (±18.6) in PL. At least an ocular history was found in 84.4 % of the patients in DE and 77.3% in PL. The main antecedents were "Dry eye" (52.2%), "Glaucoma" (19.9%) and "Cataract" (19.0%) in DE, and "Cataract" (32.6%) and "Dry eye" (29.8%) in PL.
The percentage of eyelid disorders was 75.8% in DE, and 72.8% in PL. The diagnostic of Meibomian Gland Dysfunction (MGD) was established in 60.4% of patients in DE and 46.6% in PL.
In DE, the associated pathologies were "Dry eye" (83.4% of MGD+ patients), "Eyelids disorders" (78% of MGD+), "Abnormal eyelid margin" (82.5% of MGD+). For PL the percentage were respectively, 61.4%, 61.1% and 77.6% of MGD+ patients.
Conclusions: Overall, the number of patients having eyelids disorders in the daily practice is high in both countries. . Once the eyelid disorders have been detected diagnostic steps for MGD have been added. Differences in the two countries were apparent.
DISCLOSURE: the research was sponsored by Laboratoires Théa.

HISTOLOGICAL CHANGES IN EYE-BANKED HUMAN CORNEAS AFTER SINGLE AND REPEATED CROSS-LINKING TREATMENT WITH RIBOFLAVIN AND UV-A.

Ithar M. Beshtawi,[1] Chantal Hillarby,[1] Clare O'Donnell,[1,2],Arun Brahma,[3] Fiona Carley,[3] Hema Radhakrishnan[1].[1] The University of Manchester, [2] Optegra, [3] Manchester Royal Eye Hospital, Manchester, UK.
Purpose: To evaluate the effect of repeated collagen cross-linking (CXL) treatment on corneal structure, in-vitro.
Methods: Thirty human corneas were included in the study, divided into three groups (five pairs each). In each group, one cornea was cross-linked [epithelium debrided, 30 min 0.1% riboflavin soaking and 30 min of UV-A (365nm, 3mW/cm^2) exposure while riboflavin was instilled], and the contralateral control was treated with riboflavin only. In group (A) five corneas were treated once, while in group (B) and (C), the corneas were treated twice and three times, respectively, 24 hours apart as described above. The endothelial cell density (ECD) was evaluated by trypan blue staining and ultrathin histological frozen sections were evaluated by haematoxylin and eosin (H&E) staining and TUNEL assay to detect apoptosis.
Results: There was no change in the corneal ECD after single treatment. The percentage of decrease in ECD after two and three times repeated treatment was by 2.00% and 15.50% in the CXL corneas and 1.60% and 4.12% in the control corneas. H&E and TUNEL analysis showed a variation of keratocyte cell loss in the CXL corneas among the three groups. The cell loss was found to be down to 250, 380 and 450 µm in group A, B and C respectively. Additionally, apoptotic signs were noticed at the endothelium level in the CXL corneas in group C. A shallow keratocyte cell loss, anteriorly, was noticed in the control corneas due to the epithelial scraping.
Conclusion. The single treatment showed that cross-linking effect is concentrated in the anterior zone of the cornea, while the repeated treatment showed deeper signs. A decrease in ECD and an expression of cell apoptosis signs at the endothelium level were noticed after repeating the treatment for three times which defines it unsafe.
[Ithar Beshtawi's PhD is supported by An-Najah National University, Palestine.]

COMPARISION OF PREDNISOLONE ACETATE AND LOTEPREDNOL ETABONATE FOR THE TREATMENT OF BENZALKONIUM CHLORIDE INDUCED DRY EYE SYNDROME IN RATS.

Emrullah Beyazyıldız[1], U ur Acar[2], Özlem Beyazyıldız[3], Emine R. Hekimo lu[2], Ferda A. Pınarlı[4], Nagihan U urlu[5], Aynur Albayrak[4], Figen Kaymaz[6], Meral Tiryaki[4], Tuncay Deliba ı[4] Gazi Mustafa Kemal State Hospital[1], Hacettepe University Kastamonu Faculty of Medicine[2], Ulucanlar Eye Research Hospital[3], Dıskapı Yıldırım Beyazıt Research and Training Hospital[4], Ataturk Research and Training Hospital[5] Hacettepe University Faculty of Medicine[5] Ankara,TURKEY
Purpose: Aim of this study is to compare the effects of topical prednisolone acetate (PA) and loteprednol etabonate (LE) application for the treatment of dry eye syndrome (DES) in a rat model.
Methods: DES was induced by topical administration of 0.2% BAC twice daily for one week. After DES was established, PA (n=10), LE (n=7), or phospate buffer solution (PBS) (n=7) were topically applied to 24 rats twice daily for one week. Schirmerr test, break up time score (BUT), Fluorescein and Rose Bengal staining (RBS) and inflammatory index scoring test (IIS) were performed at baseline, first week and 2 weeks. All rats were enucleated after 2 weeks and then analysed with light and electron microscopy.

Results: The mean aqueous tear volume and stability were significantly increased in both PA- and LE-treated rats and decreased in PBS-treated rats at 2 weeks ($p< 0.05$). The mean RBS score was significantly decreased in both PA and LE-treated rats and decreased in PBS-treated rats at 2 weeks ($p< 0.05$).The mean IIS and fluorescein scores were decreased in both PA- and LE-treated groups ($p< 0.05$). Histologically, diffuse inflammatory cell infiltration were observed in the PBS-treated group, whilst inflammation was almost resolved in both PA and LE-treated groups. Increase in the number of goblet cells and increased secretory granules in goblet cells were observed in PA and LE-treated groups.
Conclusions: In the current study, we showed that both PA and LE are effective treatments in a rat model of DES. No significant differences were observed between the two corticosteroids in the efficacy of DES treatment.
[This research was funded by medical research center.]

IN VIVO HIGH RESOLUTION MRI STUDY IN A CORNEAL ALKALI BURN MODEL

Fabio Bignami,[1] Giulio Ferrari,[1] Chiara Giacomini,[1] Eleonora Capitolo,[2] Linda Chaabane,[2] Paolo Rama[1]
Cornea and Ocular Surface Unit - Eye Repair Lab,[1] Institute of Experimental Neurology (INSpe) - Division of Neuroscience,[2] San Raffaele Scientific Institute, Milan, Italy.
Purpose: This pilot study was designed to evaluate the kinetics of inflammatory cell infiltration *in vivo* in a mouse model of alkali burn, using high resolution Magnetic Resonance Imaging (MRI).
Methods: The right eye of three CD1 mice was causticated with NaOH 1N. After 4 and 8 days, *in vivo* high resolution MRI was performed on a 7T-MRI scanner; maps of the T2 relaxation time were acquired 24hrs after iv. administration of ultrasmall particles of iron oxides (uspio) in order to detect macrophage infiltrate. After imaging at day 8, animals were sacrificed, eyes removed and embedded in OCT. Cryosections (7 µm) were fixed and stained with Prussian Blue, anti-CD45, anti-F4/80 and anti-CD206.
Results: Following uspio administration, a clear uptake of contrast was observed with MRI in the cornea of the causticated eye compared to the contralateral eye, suggesting an infiltration of macrophages. MRI detected a great contrast agent uptake on the right eye on day 4 in comparison to day 8. Prussian Blue staining confirmed uspio uptake within the right cornea (the left one was negative). Staining for CD45, F4/80 and CD206 identified the Prussian Blue positive cells as M2-macrophages.
Conclusions: MRI is a powerful and non-invasive tool that could be used to study corneal inflammation, in particular for the kinetics of M2-macrophages infiltration. Further studies are underway to test higher resolution MRI and different inflammatory conditions.
[The authors have no financial disclosures].

ROLE OF EPITHELIAL CELLS IN MUCOSAL IMMUNITY

Richard S. Blumberg, Gastroenterology Division, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts
Epithelial cells (EC) that line the various mucosal surfaces and separate the environment from the host have recently been recognized as a key regulator of both normal physiology (such as the recruitment and instruction of other cell populations within the adjacent epithelium and lamina propria) and the orchestration of pathophysiological responses to commensal microbes and pathogens. Similarly, ECs depend upon signals from the commensal microbiota as well as adjacent mesenchymal and hematopoietic cells for appropriate development as well as repair after injury. Further, it has recently become established that ECs can regulate the structure and function of the microbial composition. Moreover, the host's genetically imposed propensity to respond to these environmental (e.g. microbial) factors are an important determinant of whether inflammation will develop in the first instance to otherwise non-phlogistic environmental signals or contribute to the perpetuation of inflammation once initiated. Finally, this tri-directional relationship between the microbiota, epithelium and immune system evolves over the life of the individual making it possible that many diseases may be set into motion at the earliest periods of human development including the neonatal periods of life. This presentation will draw upon lessons related to these topics from observations in other mucosal tissues which might shed light on important biologic pathways associated with the wet mucosal tissues that are associated with ocular structures.

COMPARISON BETWEEN DISTORTION OF THE MEIBOMIAN GLANDS AND INFLAMMATORY REACTION ON THE OCULAR SURFACE IN PATIENTS WITH OCULAR DISCOMFORT.

Ingrid Boldin,[1] Haleh Aminfa,[1] Dieter Franz Rabensteiner,[1] Gerold Schwantzer,[2] Manuela Fischl,[1] Christa Wachswender,[1] Jutta Horwath-Winter.[1] Department of Ophthalmology,[1]
Institute for Medical Informatics, Statistics and Documentation,[2] Medical University Graz, Austria
Purpose Meibomian glands (MG) are an important part of the tear film and ocular surface system. Morphologic changes of MG can be observed in different subgroups of ocular surface disease. The aim of this study was to assess the relation between MG distorsion and inflammatory reaction in patients suffering from dry eye symptoms.
Patients and Methods In 524 consecutive patients with ocular discomfort from the dry eye unit of the Ophthalmological Department, Medical University Graz, Austria, non contact IR meibography of the lids (Heidelberg Retina Angiograph I) and cytological examination of the conjunctiva with giemsa staining were performed. The presence of inflammatory cells and the grade of gland distorsion were recorded.
Results: Distorsion of the meibomian glands was not correlated to the degree of inflammatory cells or the presence of eosinophilic cells.
Conclusion In our study population no correlation between inflammatory reaction of the conjunctiva and MG distorsion was found. Therefore other reasons for MG distorsion have to be investigated.
No financial interest.

COMPUTED TEAR FILM AND OSMOLARITY DYNAMICS ON AN EYE-SHAPED DOMAIN.

R.J. Braun,[1] Longfei Li,[1] W. D. Henshaw,[2] J.T. Banks,[2] and P.E. King-Smith.[3] [1]Department of Mathematical Sciences, University of Delaware, Newark, DE 19716-2553 USA. [2]Lawrence Livermore National Laboratory, Box 808, L-550, Livermore, CA 94551-0808 USA. [3]College of Optometry, The Ohio State University, Columbus, OH 43210-1280 USA.
Purpose: To develop mathematical models for simultaneous predictions of osmolarity and tear flow for the exposed ocular surface.
Methods: A model is derived for the thin tear film in two spatial dimensions on an eye-shaped domain. The model includes osmolarity (solutes), osmosis, surface tension, evaporation, supply and drainage of tear fluid, and wetting of the ocular surface. The mathematical model is solved numerically using sophisticated finite difference methods. Experimentally-determined parameters, including permeabilities and evaporation, are used in the model.
Results: The flow and evaporation of water in tears affects the distribution of solutes over the ocular surface. Flow preferentially occurs in the menisci. Elevated osmolarity is seen in the black lines and in the vicinity of the canthi. Evaporation causes elevated osmolarity in the interior of the exposed surface and under normal conditions new tear fluid does not penetrate into the interior without blinking. With prolonged eye opening after a blink, osmotic flow out of the ocular surface may increase to reach a dynamic equilibrium with evaporation

(steady tear thickness) under some conditions.
Conclusions: The model captures aspects of tear film and osmolarity dynamics that are expected from theories of solute transport and dry eye, as well as experiment. [Support: NSF 1022706 (Braun); R01 EY017951 (King-Smith).]

MATHEMATICAL MODELING OF TEAR BREAK-UP AND FLUORESCENT INTENSITY

R.J. Braun,[1] N. Gewecke,[1] C.G. Begley,[2] P.E. King-Smith,[3] J.I. Siddique.[4] [1]Dept of Mathematical Sciences, University of Delaware, Newark, DE 19716; [2]School of Optometry, Indiana University, Bloomington, IN; [3]College of Optometry, The Ohio State University, Columbus, OH 43210; [4]Dept of Mathematics, Pennsylvania State University, York, PA 17403.
Purpose: We study the dynamics of an evaporating tear film with fluorescein solution instilled. We aim to clarify the dynamics of the fluorescent intensity for an evaporating tear film for concentrations from dilute to quenching regimes. We compute the osmolarity during thinning as well.
Methods. A math model was developed and solved for changes in the tear film thickness (h), osmolarity (c) and fluorescein (FL, with math variable f) concentrations inside the tear film. FL concentration was converted to fluorescent intensity I using the expression involving h and the full range of f as described by Nichols et al. (IOVS, 2012; 53:5426-5432). The film model is for a spatially uniform (flat) film.
Results: The tear film thins to a steady state value that depends on the relative importance of the rates of evaporation and osmotic supply, and the resulting increase of osmolarity and fluorescein concentrations are calculated. Depending on the initial thickness and rate of osmotic supply, the osmolarity increase may be modest to quite large. Regarding fluorescent intensity, the boundary between the quenching regime and other regimes is delineated, and the quenching regime occurs for initial concentrations at or above the critical fluorescein concentration.
Conclusions: The osmolarity rise may be quite high for very small permeability, certainly enough to be felt by the subject, and in some cases enough to cause epithelial damage. The fluorescent intensity remains constant for a film thinning by evaporation in the dilute regime. In the quenching regime, the intensity decrease is dominated by the efficiency, which is quadratic with increasing fluorescein concentration. [Support: NSF Grant 1022706 (RJB,JIS), NEI Grant 1R01EY021794-01 (Begley), NEI Grant RO1EY17951 (King-Smith).]

COMPUTATIONAL MODELS OF TEAR FILM BREAKUP

R.J. Braun[1] and P.E. King-Smith,[2] and J.J. Nichols.[3] [1]Department of Mathematical Sciences, University of Delaware, Newark, DE 19716-2553 USA. [2]College of Optometry, The Ohio State University, Columbus, OH 43210-1280 USA. [3]College of Optometry, University of Houston, Houston, TX 77204 USA.
Purpose: To develop models for simultaneous predictions of osmolarity, fluorescence and tear film dynamics for the ocular surface.
Methods. A model is derived for the thin tear film in one spatial dimension. The model is solved numerically using Matlab. We use the code with specified evaporation profiles to find in detail when breakup occurs as a function of the peak value and width of the evaporation distribution, as well as the permeability of the cornea to water for osmosis. We compare the computed results with simultaneous measurements of decay of fluorescence due to quenching and interferometric imaging of the lipid layer. The mathematical model includes the effects of viscosity, surface tension, evaporation, osmosis, osmolarity (solute) transport and quenching.
Results: The minimum thickness obtained for the tear film is a complicated function of the evaporation rate and the permeability of the cornea. Theoretically, when permeability is small enough, the peak rate of evaporation high enough, and the evaporation wide enough, breakup occurs. When the evaporation distribution is narrow enough, corresponding to a small enough hole in the lipid layer, break up does not appear to occur.
Conclusions: The model captures aspects of tear film and osmolarity dynamics that are expected from theories of breakup due to dry eye. [Support: NSF 1022706 (Braun); R01 EY017951 (King-Smith).]

STUDY OF MEIBOMIAN GLAND DISTRIBUTION OF THE LOWER LID IN CHILDREN

Dominique Bremond-Gignac[1,2], Heiko Pult[3,4], Frédéric Chiambaretta[5]
[1]Ophthalmology, St Victor Center, University Hospital of Amiens, UPJV, Amiens, France.
[2]CNRS IRIS UMR8194, Paris V University, France.
[3]Optometry 1 Vision research, Weinheim, Germany
[4]Cardiff University, School of Optometry & Vision Sciencs, Cardiff, UK
[5]Ophthalmology, University Hospital of Clermont-Ferrand, France
Purpose: To evaluate in a retrospective study, the lower lid in children presenting ongoing MGD with an repartition analysis of meibomian glands. Tear film quality is conditionned by meibomian gland production
Methods: Our retrospective study included 12 children (22 eyes evaluated) with a mean age 11yo, range 4 to 17yo, of two groups that presented at ocular consultation. Group a, 6 with ongoing MGD, group b 6 control children who had been tested with no ocular surface impairment. All children underwent a Meibomian Gland Analysis with meibography images with infrared illumination acquired with Cobra system. An evaluation with Phoenix software calculated area of loss of glands. The repartition size and density of inferior eyelids Meibomian glands had also been evaluated.
Results: In group a, all children presented clinical signs of MGD due to ocular blepharitis or dry eye. In group b no ocular signs of conjunctiva or ocular surface anomalies were found. In group a area loss of meibomian gland was evaluated from 41% to 59%. Density of glands was low and desorganised. In group b area loss of meibomian gland was evaluated from 10% to 42% with an equal repartition of glands in the lid except in the medial part showing less glands. In younger children (4 to 7 yo) density of glands was tight and lenght size shorter.
Conclusions: In children, few data are known about Meibomian Gland development and function. Our study is useful in children with dry eye for the evaluation of repartition of Meibomian Glands and can be an interesting tool. A larger study has to be performed for a better understanding of dry eye ocular surface impairment in children.

IS LID-WIPER/ LENS SURFACE FRICTION THE MECHANISM OF CONTACT LENS DRYNESS AND END-OF-DAY DISCOMFORT?

Noel A. Brennan, Chantal M-L Coles. Johnson & Johnson Vision Care Inc, Jacksonville, FL, USA
Purpose: The cause of the dryness sensation in contact lens discomfort, and indeed dry eye, has proven to be elusive. Identification of this mechanism would be a breakthrough for developing methods for alleviating ocular dryness symptoms. Here, we test the association between contact lens comfort data and coefficient of friction.
Method: Two large sets of comfort data (C1: 30 day end-of-day comfort data- Brennan et al, OVS e-abstracts 90957; 2009) and C2: 2 hour comfort data- Andrasko staining grid website) were tested for correlation with the coefficient of friction data from Roba et al (Tribol Lett, 2011, 100 cycle data). Water content, manufacturer stated oxygen transmissibility and modulus (French, Silicone-hydrogel website, 2007) were also entered into multiple regression analyses to predict comfort.
Results: Both sets of comfort data correlate strongly with the coefficient of friction data (C1: $r = 0.91$, $P < 0.002$, C2: $r = 0.96$, $p < 0.001$). Multiple regression analyses saw all of the other lens-related properties aside from coefficient of friction drop out for both C1 and C2.
Conclusion: The two independently-gathered sets of comfort data

produce remarkably similar outcomes with coefficient of friction showing extraordinarily high correlation coefficients and yield multiple regression analyses with coefficient of friction remaining the only variable in both cases. This information strongly suggests that friction between the lens surface and the lid-wiper region is the principal driver of contact lens comfort, and is consistent with the earlier work of Korb et al (CLAOJ, 2002). The result may also be instructive for studies of symptoms in dry eye. Further research is required to establish the role of other factors, such as lens edge design, reproducibility, replacement frequency, care and maintenance solution interactions or combination of properties on contact lens comfort.
[The authors are employees of Johnson & Johnson Vision Care, Inc]

IS CORNEAL STAINING AN IRRELEVANT COMPLICATION OF USING PRESERVED CARE SYSTEMS? AGREE

Frank V. Bright, Department of Chemistry, University at Buffalo, The State University of New York, Buffalo, New York, 14260-3000

Solution induced corneal staining (SICS) is a transient phenomenon related to the use of certain contact lens care solutions. Small dyes can help eye care professionals (ECPs) visualize SICS.
Several years ago Morgan and Maldonado-Codina (Cont. Lens Anterior Eye 2009, 32, 48) wrote, "…there is remarkably little evidence-based research underpinning the mechanisms involved in the interaction of the superficial ocular cells and the dye itself which lead to the various ocular surface fluorescence patterns that we routinely observe. We urge that more fundamental research is carried out…." That is, we do not understand SICS, study it further. Markoulli, my debate opposition, recently wrote (contactlensupdate.com/2012/11/05/2706/), "Although the significance of SICS is still being debated, clinicians should aim to monitor for the development of SICS at the appropriate time points and also aim to minimise its occurrence by selecting appropriate lens-solution combinations. Further research is meanwhile warranted to understand the underlying aetiology and significance of SICS." To paraphrase, SICS merits further study, but change the patient's lens-solution combination if SICS develops. Efron (Clin. Exp. Opt. 2012, 1) wrote, "Due to the limitations of vital stains for definitive diagnosis, concomitant signs and symptoms in addition to a complete patient history are required." In essence, SICS in the absence of additional information is inadequate for definitive diagnosis.
Dyes are important tools at an ECP's disposal. However, should ECPs make a diagnosis based exclusively on SICS? Are multi-faceted test batteries including dye staining tests preferable prior to decision making? Are there ever cases of multi-purpose solution (MPS) use where SICS is transient, the patient lacks symptoms, and treatment is unwarranted? Perhaps the appropriate answer to this debate's query is not "Agree" or "Disagree", but "Maybe".
Our research is sponsored by B+L.

THE TEAR FILM AS A FLUID SHELL

Anthony J. Bron[1], Norihiko Yokoi[2], Georgi A. Georgiev.[3] Nuffield Laboratory of Ophthalmology, University of Oxford, UK,[1] Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan[2], Department of Biochemistry, University of Sofia, Sofia, Bulgaria.[3]

Purpose: To review the character of the human tear film during blinking and eye movements.
Methods: The pre-corneal tear film lipid layer (TFLL) was studied by video-interferometry.
Results: i. Despite compression and expansion of the TFLL with each blink, a single interference pattern may closely resemble that of a preceding blink, before degrading in a stepwise fashion with successive blinks. Then, at some point, it changes abruptly and the process is repeated. ii. After a horizontal saccade to one side during blink suppression, followed by a return saccade, the pattern is almost unchanged, but then degrades steadily if saccades are repeated in the blink interval. iii. If a period of full downgaze is followed by a return saccade to the primary position, the TFLL pattern can be disrupted below the arcuate line representing the earlier position of the lower lid margin.
Conclusions: i. The downstroke of the blink strips the TFLL from the aqueous layer but intermolecular cohesion between the lipid molecules allows it to be compressed and expanded while retaining its gross structure. ii. Little force is applied to the TFLL during a horizontal saccade so that it remains attached to the aqueous layer during the movement and moves with it as a *fluid shell*. iii. When the eye is held in downgaze, the lines of meniscus-induced thinning at each lid margin, imprint the tear film and influence its performance on the return saccade. It seems that, as the eye is restored to the mid position and the surfaces of the cornea and bulbar conjunctiva sweep across the tarsal mucosa, the tear film below the line of meniscus-induced thinning is variable disturbed. These events are relevant to the stability of the tear film during reading and computer work.

THE BASIS OF STAINING OF THE OCULAR SURFACE BY TOPICAL DYES

Anthony J. Bron,[1] Pablo Arguëso,[2] Murat Irkec,[3] Frank V. Bright,[4] Nuffield Laboratory of Ophthalmology, Nuffield Department of Clinical Neurosciences, University of Oxford, UK,[1] Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, MA, USA,[2] Department of Ophthalmology, Hacettepe University School of Medicine, Ankara, Turkey,[3] Department of Chemistry, The State University of New York, Buffalo, New York, USA,[4]

Purpose: We review here the mechanism of ocular surface staining.
Methods: A consideration of the literature.
Results: Water-soluble dyes are excluded from the epithelium by tight junctions and the surface glycocalyx. Shed cells can take up dye. A proportion of normal corneas, show time-dependent, punctate fluorescein uptake which we hypothesise is due to a graded loss of the glycocalyx barrier, permitting transcellular entry into pre-shed cells. In pathological staining, there is little evidence of 'micropooling' at sites of shedding and the term 'punctate erosion' may be a misnomer. It is more likely that the initial event involves transcellular dye entry and, in addition, diffusion across defective tight junctions. Different dye staining characteristics probably reflect differences in molecular size and other physical properties of dyes, coupled with differences in visibility under the conditions of illumination. This is most relevant to the rapid epithelial spread of fluorescein from sites of punctate staining, compared to the apparent confinement of dyes such as lissamine green. We assume that fluorescein, with the lowest molecular weight, spreads initially by a paracellular route and then by transcellular diffusion. Solution-Induced Corneal Staining (SICS), related to the use of certain contact lens care solutions, may have a different basis, involving the non-pathological uptake of cationic preservatives, such as biguanides, into epithelial membranes and secondary binding of the fluorescein anion. It is transient and may not imply corneal toxicity.
Conclusions: Understanding the mechanism of staining is relevant to grading, standardisation monitorin disease and the conduct of clinical trials.

LASER IN VIVO CONFOCAL MICROSCOPY DETECTS BILATERAL CHANGES IN CORNEAL IMMUNE CELL AND SUBBASAL NERVE PLEXUS IN UNILATERAL HERPES ZOSTER OPHTHALMICUS

Bernardo M. Cavalcanti, Andrea Cruzat, Deborah Pavan-Langston, Eric Samayoa, Pedram Hamrah.
[1]Ocular Surface Imaging Center and Cornea & Refractive Surgery Service, Massachusetts Eye & Ear Infirmary, Department of Ophthalmology, Harvard Medical School, 243 Charles Street, Boston, MA 02114, USA.

Purpose: Herpes zoster ophthalmicus (HZO), thought to be a unilateral disease, results in loss of corneal sensation, leading to neurotrophic keratopathy. This study aimed to analyze bilateral corneal immune cell and nerve changes in patients with HZO.
Methods: Laser in vivo confocal microscopy (Heidelberg Retina Tomograph/Rostock Cornea Module) and corneal esthesiometry (Cochet-Bonnet) of the central cornea were performed bilaterally in all 24 HZ) patients and 24 controls in a prospective fashion. Confocal images were evaluated for the presence and morphological changes of dendritic-form immune cells (DC) and subbasal nerve plexus, and correlated to clinical paramters.
Results: HZO affected and contralateral clinically unaffected eyes had a significant increase in DC influx of the central cornea (147.4 ± 33.9, 120.1 ± 21.2, and 23.0 ± 3.6cells/mm^2; p<0.0001). Further, affected HZO DC were larger in area (232.4 ± 47.4µm;p<0.001) and number of dendrites (4.1 ± 0.8 n/cell; p=0.01) as compared to controls (52.2 ± 11.7, and 2.3 ± 0.5). A significant decrease of subbasal nerve parameters in both eyes was found for total nerve length (9,052.6 ± 1151.4, 14,959.8 ± 903.2, and 22,851.4 ± 661.4 µm/mm^2 respectively; p<0.0001), total number of nerves (5.8 ± 0.9, 11.9 ± 1.2, and 26.6 ± 1.2 n/frame; p<0.0001), number of main nerve trunks (2.4 ± 0.3, 3.8 ± 0.3, and 4.4 ± 0.2;p<0.0001) and the number of branches (3.4 ± 0.7, 8.2 ± 1.1, and 22.2 ± 1.2;p<0.0001) as compared to controls. DC density and DC area showed moderate negative correlation with total nerve length (R=-0.43 and R=-0.57, respectively). Reduced nerve length and number of nerves were strongly correlated with corneal sensation across all subgroups (p<0.05). Further, LCM revealed specific findings in patients with pain.
Conclusions: Patients with chronic unilateral HZO demonstrated profound and significant bilateral increase in corneal dendritic-form cell density and decrease of the corneal subbasal nerve plexus as compared to controls. The results may explain bilateral ocular surface disease observed in patients with HZO and suggest a direct interaction between the immune and nervous system in the cornea and a bilateral nerve and immune alteration in an apparently unilateral disease.
Financial Support: NIH K08-EY020575 (PH), New England Corneal Transplant Research Fund (PH), Falk Medical Research Foundation (PH), Johnstone Research Fund (DP-L), Stevens Research Fund (DP-L)

CLINICAL AND PATHOLOGICAL CHARACTERISTICS OF NONPIGMENTED CORNEAL NEOPLASM

Yong Jae Cha, MD, Bo Hyuck Kim, MD, Soo Jeong Lyu, MD, Joon Young Hyon, MD, PhD, Won Ryang Wee, MD, PhD, Joo Youn Oh, MD, PhD Department of Ophthalmology, Seoul National University Hospital, Seoul, Korea
Purpose: In order to evaluate clinical and pathological characteristics of nonpigmented corneal neoplasm in Korean population
Methods: Medical records were reviewed for patients who underwent biopsy for nonpigmented corneal neoplasm.
Results: 25 eyes of 25 patients underwent en bloc excision for nonpigmented corneal tumors. Histologic examination revealed that 16 were dysplastic and 9 were nondysplastic. Tumors with dysplasia included corneal intraepithelial neoplasm (CIN, n=8), squamous cell carcinoma (SCC, n=7), and sebaceous cell carcinoma (n=1). Nondysplatic lesion included hyperplasia (n=6) and inflammation (n=3). Six out of 7 eyes with SCC were located at the nasal side, while 2 of 8 cases with CIN involved the nasal cornea. Vascularization was combined in all cases with SCC, 2 of 3 cases with inflammation, 2 of 8 with CIN, and in none with hyperplasia. The conjunctiva was involved in 6 of 8 cases with CIN, 7 of 8 with SCC, 2 of 3 with inflammation, and 2 of 6 with hyperplasia. When classified based on clinical appearance, 4 of 8 eyes with CIN were leukoplakic, and 4 of 7 cases with SCC were papilliform. Recurrence occurred in 6 of 16 dysplastic lesions: 3 of CIN and 3 of SCC. Nondysplastic lesions did not recur. The use of the postoperative chemotherapy including topical mitomycin C or interferon alpha 2b had a significant negative correlation with the recurrence.
Conclusions: Totally, 64% of nonpigmented corneal tumor had dysplasia with CIN being 32% and SCC being 28%. Among dysplastic lesions, 37.5% recurred after excision. The use of the postoperative chemotherapy was a significant factor negatively affecting the recurrence.

USE OF TETRACYCLINES AND MACROLIDES IN DRY EYES AND BLEPHARITIS – A SYSTEMATIC REVIEW

Dr. Hiok Hong Chan[1], Samantha Sze Lee[2], Dr. Louis Tong[1,2,3,4]
[1]Yong Loo Lin School of Medicine, National University of Singapore
[2]Singapore Eye Research Institute, Singapore
[3]Singapore National Eye Centre, Singapore
[4]Duke-NUS Graduate Medical School, Singapore
Purpose: To evaluate the current evidence on the effectiveness of macrolide and tetracycline in blepharitis and dry eye treatment available on PUBMED.
Methods: This study is a systematic review of interventional clinical trials on the effectiveness of macrolide and tetracycline in blepharitis and dry eye treatment. 2 independent reviewers analyzed the titles and abstracts of the 323
articles from the search. There were 6 interventional clinical trials with at least 1 comparison group, which met our inclusion criteria. They were analyzed and evaluated in greater detail.
Results: Despite differences in study design of various trials and various shortcomings, treatment with the oral tetracyclines (doxycycline, minocycline) and azithromycin results in improvement of the efficacy outcome. Depending on the trial, this may
be due to severity of subjective symptoms (such as irritation), objective signs such as tear function (tear break up time), ocular surface damage (dye staining), meibomian gland status (such as plugging), or a combination. Notably, low dose oral doxycycline is as effective as the high dose form and avoids severe gastrointestinal effects. With respect to contact lens-related dry eyes, topical azithromycin drops (Azasite®) is superior to contact lens re-wetting drops (Visine®). In moderate to severe blepharitis or blepharoconjunctivitis, topical tobramycin/dexamethasone combination drops (Tobradex®) is superior to topical azithromycin drops (Azasite®).
Conclusions: The macrolides and tetracycline have a major role in MGD and dry eye therapy, and should be part of the management plan in these patients. In severe blepharitis, clinicians should consider topical steroid or mixture of steroid
and antibiotics.
Conflict of interest: The authors have no proprietary interests in any of the products discussed in this manuscript.
Grant Support: NMRC/1206/2009, NMRC/CSA/013/2009, NMRC/CG/SERI/2010, BMRC/10/1/35/19/670

ADENOVIRUS EVOLUTION AND THE EMERGENCE OF CORNEAL PATHOGENS.

James Chodosh,[1] Christopher M. Robinson,[1] Gurdeep Singh,[1] Jeong Yoon Lee,[1] Shoaleh Dehghan,[2] Jaya Rajaiya,[1] Elizabeth B. Liu,[2] Morris S. Jones,[2] David W. Dyer,[3] Donald Seto.[2] Mass. Eye & Ear – Harvard Medical School,[1] School of Systems Biology, George Mason University,[2] Dept. Microbiology & Immunology, University of Oklahoma Health Sciences Center.[3]
Purpose: Human adenoviruses (HAdV) contain 7 species, designated A-G, and currently 69 types; two-thirds fall within species D (HAdV-D). Seventeen of the recognized types were described in the last decade, including 12 within HAdV-D, 3 of which were associated with epidemic keratoconjunctivitis (EKC), a highly contagious infection of the conjunctiva and cornea. The recent emergence of new and highly virulent HAdVs with corneal epithelial tropism underscores the need to determine their ontogeny.
Methods: We generated complete high quality genome sequences for all the previously unsequenced HAdV serotypes within HAdV-D (n=20), and analyzed them by bioinformatics in conjunction with another 40 HAdV previously sequenced prototypes, comprising all HAdV species.

Results: Bioinformatics sequence analysis identified stereotypical hypervariable regions within HAdV-Ds. The recombination/mutation ratio (rho/theta) among HAdV-Ds was higher than for other HAdV species examined. Proteotyping based on putative amino acid sequences for select proteins provided evidence for homologous recombination in every virus within HAdV-D. Patterns of alternating GC and AT rich motifs found in each viral genomic sequence correlated with hypervariable region recombination sites across all HAdV-Ds.
Conclusions: Homologous recombination among HAdV-Ds is their primary means of evolution. Foci of DNA instability may lead to formulaic patterns of homologous recombination and confer agility to adenovirus/host relationships.
Supported by NIH grants EY013124, EY021558, and EY014104, a Research to Prevent Blindness Senior Scientific Investigator Award (JC), the Massachusetts Lions Eye Research Fund, and Harvard Catalyst (UL1 RR 025758).

THE EFFECT OF TOPICAL CYCLOSPORINE 0.05% ON DRY EYE AFTER CATARACT SURGERY

Sung Kun Chung, MD, Ye Jin Ahn, MD
The Catholic University of Korea, St. Mary's Hospital, Seoul, Korea
Purpose: To evaluate the effectiveness of cyclosporine 0.05% for dry eye after cataract surgery.
Methods: Thirty-two newly diagnosed patients with dry eye syndrome, 1 week after cataract surgery, received a twice-daily treatment of cyclosporine 0.05% (Restasis®, Allergan, Inc., Irvine, CA, U.S.A.) for one eye and normal saline 0.9% for the other. Disease severity was measured at 2 weeks, 1 month, 2 months, and 3 months by Schirmer test I (ST-I), tear film break-up time (tBUT), corneal temperature, and a dry eye symptom questionnaire (OSDI).
Results: Both groups increased in ST-I and tBUT over time. ST-I in the cyclosporine 0.05% group showed a significant increase at 3 months, and tBUT in the cyclosporine 0.05% group showed an increase at 2 and 3 months. The dry eye symptom score was significantly reduced in the cyclosporine 0.05% group.
Conclusion: Cyclosporine 0.05% can be an effective treatment for dry eye after cataract surgery.

DRY EYE: TWO EXPERIMENTAL RODENT MODELS FOR DRUG DEVELOPMENT.

Nicolas Cimbolini[1], Sophie Antonelli[1], Stefano Barabino[2], Laurence Feraille[1], Philippe Margaron[1] and Pierre-Paul Elena[1]. [1]Iris Pharma, La Gaude, France. [2] Clinica Oculistica, University of Genoa, Italy.
Purpose: Dry eye syndrome is a relatively common disease with multifactorial causes and can afflict anyone of any age. In most cases, dry eye mostly results in mild discomfort but in more severe cases it can become painful and dryness and cause damage to ocular surface. It is thus is necessary to have an experimental model to test and select therapeutic candidates for this disease. Here we describe two experimental models of dry eye in which scopolamine, a tropane alkaloid drug with muscarinic antagonist effects, is employed to suppress lacrimation and induce dry eye symptoms.
Methods: The first model consisted in placing pigmented mice in a controlled environmental chamber (CEC, relative humidity <25%, air-flow 15l/min, temperature 20-22°C) with transdermal patches of scopolamine (0.5mg/72h). Animals were divided in three groups of five mice: the first group was placed in normal environmental condition (relative humidity >55%, temperature 20-22°C) without transdermal scopolamine administration. The other groups were placed in the CEC with transdermal scopolamine administration. In the second model albino rats receive scopolamine (20mg/day) via osmotic pumps implanted subcutaneously on day 1. Animals were divided in three groups of five rats: In the first group the pumps delivered saline solution. In the other groups the pumps delivered 20 mg/day of scopolamine over 21 days. In both models, the first two groups received saline instillation and the third group Restasis® eye drops (cyclosporine 0.05%), a drug approved in the USA for the treatment of dry eye syndrome.
Results: Symptoms of dry eye, including decrease in tear secretion, appearance of corneal defects and signs of inflammation, were observed in both models. Moreover Restasis® attenuated the severity of these symptoms.
Conclusions: The combination of scopolamine treatment and controlled environment in mice and scopolamine treated rats are therefore valuable models to mimic human, and test new therapeutic approaches for, dry eye syndrome.
[1,2] have commercial relationships.

A DRUG-ELUTING CONTACT LENS FOR THE TREATMENT OF GLAUCOMA

Joseph B. Ciolino, Cristina F. Stefanescu, Amy E. Ross, Borja Salvador, Fabiano Cade, Daniel S. Kohane
Purpose: To develop a drug-eluting contact lens designed for prolonged delivery of latanoprost for the treatment of glaucoma, the leading cause of irreversible blindness worldwide.
Methods: Latanoprost-eluting contact lenses were created by encapsulating latanoprost-poly(lactic-co-glycolic acid) films in methafilcon by ultraviolet light polymerization. *In vitro and in vivo* release kinetics were characterized and cytotoxicity studies were conducted.
Results: *In vitro and in vivo* studies demonstrated good correlation, and both showed an early burst of drug release followed by sustained release for one month. Contact lenses containing thicker drug-polymer films demonstrated more favorable controlled release properties with a greater amount of drug released after the initial burst. *In vivo*, contact lenses were able to achieve, for one month, latanoprost concentrations in the aqueous humor that were comparable to those achieved with topical latanoprost solution. The lenses appeared
safe in cell culture and animal studies.
Conclusion: This contact lens design can potentially be used as a treatment for glaucoma and as a platform for ocular drug delivery with widespread applications.

UPREGULATION OF TH1-ASSOCIATED CHEMOKINE EXPRESSION IN EXPERIMENTAL DRY EYE REQUIRES NK CELL-PRODUCED IFN-γ

Terry G. Coursey, Stephen C. Pflugfelder, and Cintia S. de Paiva
Ocular Surface Center, Cullen Eye Institute, Department of Ophthalmology, Baylor College of Medicine, Houston, Texas
Purpose: The chemokines CCL20 (binds CCR6), and CXCL-9, -10, -11 (binding CXCR3) coordinates migration of CCR6+Th17 cells and CXCR3+Th1 cells, respectively. Our previous studies have demonstrated the requirement of Th17 and Th1 cells in the pathogenesis of dry eye disease. The objective of this study was to evaluate the role of the innate immune system in the upregulation of chemokine expression in an experimental model of dry eye.
Methods: Desiccating stress (DS) was induced by subcutaneous injection of scopolamine and exposure to a drafty low humidity environment in RAG1KO, IL-17KO, IFN-γKO and wild-type (WT) mice, aged 6-8 weeks for 5 or 10 days (DS5, DS10) or were not treated. CCL20, CXCL-9, CXCL-10, and CXCL-11 expression in the cornea and conjunctiva were evaluated by real time quantitative PCR. RAG1KO mice were treated 100μg anti-NK1.1 (PK136) twice per week and compared with isotype (mouse IgG).
Results: Significant upregulation of CCL20 was observed in WT (> 30 fold) and RAG1KO (>7 fold) mice, however was not upregulated in IFN-γKO mice and significantly reduced in IL-17AKO mice (~2 fold). Similarly, CXCR3 ligands CXCL10 and -11 expression increased in WT (CXCL10- ~2 fold; CXCL11- ~2 fold) and RAG1KO (CXCL10- ~ 2 fold; CXCL11 ~ 2 fold) mice but not IFN-γKO or IL-17KO mice.

Both IFN-γ and IL-17 increased in RAG1KO mice. Expression of Th1-associated chemokines and IFN-γ was unchanged in NK cell-depleted RAG1KO mice compared to isotype controls.
Conclusions: Upregulation of CXCL9, CXCL10, and CXCL11 expression in experimental dry eye is independent of T cells and requires IFN-γ-producing NK cells.
Support: NIH Grant EY018888 (CSDP), EY11915 (SCP), Research to Prevent Blindness, the Oshman Foundation, William Stamps Farish Fund and the Hamill Foundation.

TOXICITY AND IRRITATION POTENTIAL OF A NOVEL MANUKA HONEY-BASED FORMULATION FOR MANAGING BLEPHARITIS

Jennifer P. Craig[1], Jaeun Kim[1], Ilva Rupenthal,[1] Simon Swift[2].
[1]Department of Ophthalmology, NZ-NEC; [2]Department of Molecular Medicine, The University of Auckland, New Zealand
Purpose: Methylglyoxyl (MGO) derived from New Zealand's native Manuka honey (MH) exhibits antimicrobial properties that may be useful in the treatment of blepharitis. Commercially, MH has been complexed with -cyclodextrin (CD) to increase bioavailability of the active ingredient (CycloPower™ (CP), Manuka Health, NZ). This project sought to undertake preliminary *in vitro* safety testing of novel formulations containing MH and CP in a microemulsion (ME) base.
Methods: MH, CP and CD, at concentrations between 1% and 30% w/v (honey solids) in aqueous solution and within the proposed ME-based formulation were tested, *in vitro*, on human corneal epithelial cells with a sulforhodamine B (SRB) assay, and with the Hen's Egg Test on Chorioallantoic Membrane (HET-CAM), to assess toxicity and potential for irritation, respectively. In the SRB assay, inhibition of cell growth after 15 minutes was evaluated relative to control (culture medium). In the HET-CAM, scores for solutions tested at 0.5, 2 and 5 min on 3 CAMs were averaged and each solution assigned an irritation potential (none, slight, moderate or strong), based on the score.
Results: Cell viability remained high following exposure to MH concentrations up to 30%, and CP and CD concentrations up to 3%. HET-CAM testing demonstrated that concentrations of ≥ 25% MH and ≥ 20% CP resulted in slight irritation, but no irritation was evident at lower concentrations.
Conclusions: HET-CAM testing, simulating *in vivo* conjunctival irritation, indicated that both MH and CP, at levels previously determined to be antimicrobial, can be considered non-irritant for ophthalmic application. Cytotoxicity testing, however, suggests that CP, due to the CD component, is better suited to eyelid application than application directly onto the ocular surface.
[Jaeun Kim was awarded a faculty summer student scholarship; research funding was received from Manuka Health Ltd, NZ]

PRE-OCULAR TEAR FILM DYSFUNCTION AND ITS ASSOCIATION WITH PERIPHERAL NEUROPATHY IN DIABETES MELLITUS

Jennifer P. Craig[1], Stuti Misra[1], Dipika V Patel[1], Charles NJ McGhee[1], Monika Pradhan[1], Dean Kilfoyle[2], Geoffrey Braatvedt[3].
[1]Department of Ophthalmology, New Zealand National Eye Centre; [2]Department of Neurology; [3]Department of Medicine, The University of Auckland, Auckland, New Zealand
Purpose: To compare tear film parameters in patients with type 1 diabetes mellitus (DM) and healthy controls and to investigate correlations between tear film characteristics and age, DM duration, and the presence of retinopathy and peripheral neuropathy.
Methods: Symptoms were evaluated for 53 patients with type 1 DM and 45 controls using McMonnies Dry Eye Questionnaire. All participants underwent HbA_{1c} testing to confirm the presence and/or status of DM. Ocular characteristics assessed included tear film lipid layer grade (LLG), non-invasive break up time (NIBUT), phenol red thread (PRT) test, laser scanning *in vivo* confocal microscopy (IVCM) and retinal photography. Individuals with DM also underwent a detailed neuropathy assessment, including a symptomatic neuropathy questionnaire, clinical neuropathy assessment, biothesiometry, and nerve conduction testing.
Results: Five control participants were excluded from the study due to high HbA_{1c}. There was no significant difference in age between the DM group (49 ± 12 years) and control group (44 ± 15 years, p=0.12), nor in dry eye symptom scores between the two groups (p=0.33). However, LLG (p=0.02), NIBUT (p<0.0001) and PRT (p=0.01) were significantly lower in the DM group compared to healthy controls. NIBUT correlated positively with corneal sub-basal nerve density (r=0.28, p=0.04) and inversely with total neuropathy score (r=-0.29, p=0.03). Decreased NIBUT was associated with increasing age (r=-0.28, p=0.05) and DM duration (r=-0.29, p=0.03) in the DM group.
Conclusions: The DM group exhibited significantly poorer tear film stability, secretion and lipid layer grades than did the control group. A correlation between tear film parameters and total neuropathy score suggests that ocular surface abnormalities may occur in parallel with diabetic peripheral neuropathy.

SAFETY AND EFFICACY OF TOPICAL OPHTHALMIC MIM-D3, A NOVEL TRKA RECEPTOR AGONIST, IN A PHASE 2 CLINICAL TRIAL FOR THE TREATMENT OF DRY EYE

Garth Cumberlidge[1], Karen Meerovitch[1], Reza Haque[2] and George Ousler[3]. 1. Mimetogen Pharmaceuticals 2.Bausch+Lomb 3.Ora, Inc.
Purpose: To assess the safety and efficacy of 1% and 5% MIM-D3 Ophthalmic Solutions compared to placebo for the treatment of the signs and symptoms of dry eye.
Methods: A multi-center, randomized, double-masked, placebo-controlled study included a 7-day run-in period and 28 days of BID dosing. For screening purposes, at Visits 1 and 2 subjects were exposed to the Controlled Adverse Environment (CAE) Model. Subjects were dosed BID with artificial tears between Visits 1 and 2. Eligible subjects had sufficient dry eye signs and symptoms pre-CAE at both visits as well as exacerbation in fluorescein corneal staining and ocular symptoms with CAE exposure. Patients were randomized (1:1:1): 1%, 5%, or placebo. Symptoms were recorded daily. CAE exposure was repeated at Days 14 and 28 to assess treatment efficacy. Results: In patients with moderate signs and symptoms of dry eye (n=150) both doses showed significantly less fluorescein staining than placebo after 28 days of treatment. Improvements in staining were observed in the post-CAE and changes from pre- to post-CAE analyses. Fluorescein staining in the 1% arm were significantly lower than in the placebo arm for the inferior, temporal, nasal, total cornea, conjunctiva and whole eye assessments (p=0.0039 to 0.0422). The mean dryness score in the symptom diaries over the 28-day treatment period demonstrated a treatment effect for both MIM-D3 groups with the 5% arm being significantly lower than the placebo arm (p=0.0342). In a subgroup defined by higher symptom scores during the run-in period, significant treatment effects for dryness (1% p=0.0150, 5% p=0.0637) and worst symptom (1% p=0.0368, 5% p=0.0533) were noted. Both doses of MIM-D3 were safe and well tolerated.
Conclusions: Topical ophthalmic MIM-D3 showed robust protection against the exacerbation of signs in the CAE model, and patient reported diary symptoms in the environment, with a favorable safety profile. MIM-D3 demonstrates promising results as a novel treatment for dry eye disease and will be further evaluated in a multi-center Phase 3 trial.

DEVELOPMENT AND REGULATION OF T CELL-MEDIATED IMMUNITY IN DRY EYE DISEASE

Reza Dana, MD, MPH, MSc., Massachusetts Eye and Ear Infirmary, Boston MA USA

Today, all but the most recalcitrant skeptics have acquiesced to the plethora of evidence linking immune mechanisms to dry eye disease (DED) pathogenesis and amplification.
Over the last several years data from several groups, including ours, has underscored the importance of CD4+, principally IL-17-secreting T helper-17 (Th17), T cells in mediating the immunopathogenic mechanisms that induce epithelial damage in DED. A lingering question, however, has been whether the immune response generated after desiccating stress is merely phenomenologic as a consequence of surface dryness, or whether it can indeed cause chronic autoimmune-based epitheliopathy even after termination of desiccation. Recently, we have shown in experimental DED that mice that are taken out of the dry environmental chamber to a normal-humidity environment retain long-term epithelial disease for months after termination of desiccating stress, and despite normal/supra-normal tear secretion (Chen Y et al, Mucosal Immunology 2013). We have also demonstrated that chronic DED is mediated primarily by effector memory Th17 (CD4+CD62L-CD44hiIL-17A+) cells, which can even be adoptively transferred to other (healthy) animals to induce autoimmune epithelial pathology.
Despite these discoveries, there are many critical questions that remain unanswered in this field, including how the local microenvironment leads to failure of immune homeostasis in DED—an observation supported by both laboratory and clinical evidence. This presentation will provide an overview of adaptive immune mechanisms in DED, including recent insights into immunological memory in chronic disease and the failure of T regulatory cells in controlling ocular surface inflammation.

EFFECT OF PATHOGENIC COMPARED TO COMMENSAL BACTERIA ON CONJUNCTIVAL GOBLET CELL NRLP3 INFLAMMASOME ACTIVATION AND MUCIN SECRETION

Darlene A. Dartt[1], Victoria McGilligan[1], Dayu Li[1], Robin R. Hodges[1], Michael Gilmore[2], and Meredith S. Gregory-Ksander[1]. [1]Schepens Eye Research Institute/Massachusetts Eye and Ear, Boston MA; [2]Massachusetts Eye and Ear, Boston, MA

Purpose: Conjunctival goblet cells have developed multiple mechanisms to protect the ocular surface from bacterial infection. One mechanism is the secretion of the gel-forming mucin MUC5AC. Another mechanism is activation of the NLRP3 inflammasome. NLRP3 is a member of the multi-protein complex termed the NLRP3 inflammasome that activates the caspase 1 pathway, inducing the secretion of biologically active IL-1β, a major initiator and promoter of inflammation. The purpose of the present study was to determine if pathogenic compared to commensal bacteria activate mucin secretion and the NRLP3 inflammasome as protective functions.
Methods: Cultured human goblet cells were incubated for 4 h with the toxigenic strain *Staphlococcus aureus* RN6390, the non-toxigenic strain, *S. aureus* ACL135, and the commensal bacterium, *S. epidermidis* all at an MOI of 20. High molecular weight glycoconjugate (includes MUC5AC) secretion was measured by enzyme-linked lectin assay. NRLP3 activation was determined by measuring caspase-1 activity by FLICA assay, pro-IL1β synthesis by western blotting analysis, and mature IL-1β secretion by ELISA.
Results: Toxigenic, but not non-toxigenic *S. aureus*, nor commensal *S. epidermidis*, stimulated MUC5AC secretion from cultured goblet cells. Similarly toxigenic, but not non-toxigenic *S. aureus* (nor commensal *S. epidermidis*) activated the NRLP3 inflammasome as measured by activation of caspase-1, increased synthesis of pro-IL-1β, and elevated secretion of mature Il-1β.
Conclusion: Conjunctival goblet cells are triggered to secrete mucin and activate the NLRP3 inflammsome by toxigenic, but not non-toxigenic *S. aureus*, nor commensal *S. epidermidis*, implicating the importance of these pathways in the prevention of infectious keratitis and conjunctivitis. Supported by NIH EY022415

COMPOSITIONAL MONOSACCHARIDE ANALYSIS OF MARINE MAMMAL TEARS

Robin Kelleher Davis and Pablo Argüeso. Schepens Eye Research Institute and Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, Massachusetts, USA

Purpose: It is well known that different species have different susceptibilities to ocular disease, and that the tear film is vital to protection of the ocular surface against pathogenic invasion and other challenges. The tear film of marine mammals lacks the lipid layer found in humans. We hypothesize that in lieu of lipids, mucin-type glycoproteins are an important protective component of the marine mammal tear film. In a previous study, we determined that high molecular weight O-glycans were present in the tears of marine mammals. In this study, we sought to identify the monosaccharides in tears of cetaceans and pinnipeds as a preliminary step to determining the specific types of glycoproteins that are present in the marine mammal tear film.
Methods: Tears were collected, using IRB and ACUC approved protocols, from dolphins, seals, sea lions, and humans. Protein and carbohydrate concentrations of tear samples were determined using standard bicinchoninic acid and sulfuric assays. Monosaccharides were cleaved and released from tear glycoproteins using acid hydrolysis. Samples were incubated at 100°C for 4.5 hours with a final concentration of 2N trifluoroacetic acid and then subjected to high performance anion exchange chromatography (HPAEC) on a Dionex CarboPac PA-20 column using isocratic gradient elution.
Results: Carbohydrate to protein ratios in marine mammal tears were 5-11 times greater than in humans. By HPAEC, marine mammal tears contained fucose (0.08-0.21 nmol/μg total protein), N-acetylgalactosamine (0.55-6.17 nmol/μg), N-acetylglucosamine (1.43-6.17 nmol/μg), galactose (0.52-2.78 nmol/μg), glucose (0.07-0.90 nmol/μg), and mannose (0.68-2.36 nmol/μg). N-acetylgalactosamine and N-acetylglucosamine were present in higher amounts in dolphin tears (6.57 and 6.17 nmol/μg respectively) than in any of the other species analyzed, including human (0.89 and 1.86 nmol/μg respectively).
Conclusions: Carbohydrate to protein ratios differed across species, and HPAEC profiles of hydrolyzed monosaccharides from tear glycoproteins revealed species-specific chromatographic patterns. We hypothesize that tear film glycan composition will correlate with species-specific differences in vulnerability to ocular surface disease. Support: We thank Dolphin Quest Oahu (Oahu, HI), Gulf World Marine Park, (Panama City Beach, FL), Aquarium of Niagara (Niagara, NY), and New England Aquarium (Boston, MA), for contributions of marine mammal tears; and David A. Sullivan, Schepens Eye Research Institute and Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA, for advice. Grant Support: NIH EY014847 (PA), R01EY05612, the Margaret S. Sinon Scholar in Ocular Surface Research Fund; Arey's Pond Boat Yard, S. Orleans, MA.

USE AND MISUSE OF AMNIOTIC MEMBRANE TRANSPLANTATION IN OPHTHALMOLOGY

Sophie X. Deng, M.D., Ph.D., Jules Stein Eye Institute, University of California, Los Angeles

The use of amniotic membrane transplantation in ophthalmology has increased dramatically over the last two decades. The rationale of using amniotic membrane is because of its proposed multiple unique properties, such as anti-inflammation, anti-bacterial effect, and surrogate extracellular matrix and basement membrane. Therefore, amniotic membrane transplantation has been widely used in ocular surface reconstruction ranging from forniceal reconstruction, persistent epithelial defect, corneal ulcer, and high risk keratoplasty. Amniotic membrane is also used in the ex vivo expansion of limbal stem cells and oral mucosal epithelium for transplantation. However, despites hundreds of publications on the use of amniotic membrane transplantation, the question of whether amniotic membrane transplantation is superior to the conventional therapies remains to be confirmed.

(SXD receives grant supports from California Institute for Regenerative Medicine and National Eye Institute)

DRY EYE DISEASE AND TEAR OSMOLARITY FOLLOWING BOTULINUM TOXIN INJECTION IN PATIENTS WITH BLEPHAROSPASM.

A Denoyer(1-3), C Virevialle(1,2), G Rabut(1,2), A Labbé (1-4), and C Baudouin(1-4).
Quinze-Vingts National Ophthalmology Hospital, Paris, France.
Clinical Center for Investigation, INSERM CIC 503, Paris, France.
Institut de la Vision, University Paris 6, INSERM UMRS968, CNRS U7210, Paris, France.
University of Versailles, Versailles, France.

Purpose: Prospective evaluation and follow-up of ocular dryness in patients undergoing botulinum toxin injections (BTIs) for blepharospasm.

Methods: Thirteen patients with blepharospasm have been assessed for dry eye through clinical evaluation, tear osmolarity measurement (TearLab, USA), corneal sensitivity measurement (Cochet-Bonnet aesthesiometer, Luneau, France), and the Ocular Surface Disease Index questionnaire. The evaluations were conducted before BTI, and 2 and 6 weeks after BTI. Thirteen patients with Sjögren Syndrome dry eye disease and 13 healthy subjects were also enrolled as controls.

Results: Mean age was 64.3 ± 9.4 years (mean ± SD), and the sex ratio Female/Male was 0.75. In patients with blepharospasm, tear film break-up time was 3 ± 0.75, 3.9 ± 1.2 and 3.8 ± 1.8 s, before, 2 weeks and 6 weeks after BTI, respectively, and Schirmer's Test I was 9.3 ± 6.4, 7.7 ± 5.9 and 5.8 ± 5.7 mm/5 min, before, 2 weeks and 6 weeks after BTI, respectively (P>.05 for each as compared to baseline). Corneal sensitivity was 3.6 ± 0.8, 3.8 ± 0.9 (P>.05 compared to baseline) and 4.8 ± 0.7 mm (P<.05 compared to baseline) at the same time points, respectively. Tear osmolarity was significantly reduced at 2 weeks and 6 weeks after BTI (307.4 ± 12.8 and 312.4 ±12.4 mOsm respectively, P<.01 for each) as compared to baseline value before BTI (324.4 ± 13.1mOsm).The OSDI overall score did not significantly change over the study period (22 ± 8, 23.6 ± 13 and 20.6 ± 12, P>.05 for each).

Conclusions: Patients with blepharospasm presented with mild- to moderate-severity dry eye disease as assessed by clinical evaluation as well as by the patient-reported quality of life questionnaire. BTI, which is commonly used to decrease blink rate and intensity in blepharospasm patients also caused a significant decrease in tear osmolarity, and hence may improve ocular dryness in these patients.

THE EFFECTS OF INSULIN-LIKE GROWTH FACTOR-1 AND GROWTH HORMONE ON HUMAN MEIBOMIAN GLAND EPITHELIAL CELLS.

Juan Ding, David A. Sullivan. Schepens Eye Research Institute, Massachusetts Eye and Ear, and Department of Ophthalmology, Harvard Medical School, Boston, MA, USA

Purpose: Recent research has shown that dry eye disease (DED) is the most common side effect of cancer treatment with figitumumab, an antibody that prevents insulin-like growth factor-1 (IGF-1) binding to its receptor. We hypothesize that the mechanism underlying this effect is the inhibition of IGF-1 action in meibomian gland epithelial cells. To begin to test this hypothesis, we examined whether IGF-1 acts on human meibomian gland epithelial cells. We also examined whether possible effects are analogous to those of growth hormone (GH), which is known to act in concert with IGF-1 to influence sebaceous gland function.

Methods: Immortalized human meibomian gland epithelial cells were cultured with and without serum and exposed to IGF-1, GH or vehicle for varying time intervals. Analytical procedures included proliferation assessments, extensive Western blotting, and LipidTox staining for neutral lipids.

Results: Our results demonstrate IGF-1 activates the phosphoinositol 3-kinase (PI3K)/Akt pathway and modulates the phosphorylation of forkhead box O1 (FoxO1) in meibomian gland epithelial cells. IGF-1 also stimulates cellular proliferation, increases sterol regulatory element binding protein-1 expression and promotes lipid accumulation, but has inconsistent effects on the mitogen-activated protein kinase (MAPK) pathway. In contrast, GH does not activate Akt, FoxO1, MAPK, Janus kinase (JAK) 2/ signal transducers and activators of transcription (STAT) 5, or induce cell proliferation, or enhance lipid generation.

Conclusions: Our results are consistent with our hypothesis that the mechanism by which figitumumab induces DED is by inhibiting IGF-1 action in human meibomian gland epithelial cells. Such an inhibition could reduce glandular lipid accumulation, lead to a lipid insufficiency on the ocular surface, and ultimately cause evaporative DED. [This research was supported by NIH grant R01EY05612, the Margaret S. Sinon Scholar in Ocular Surface and the AFER/Vistakon Dry Eye Fellowship]

EEFFICACY AND SAFETY ASSESSMENT OF AZITHROMYCIN 1.5% EYE DROPS (AZYTER®) IN CHRONIC BLEPHARITIS PATIENTS.

Serge Doan, [1] Christophe Baudouin, [2] Tristan Bourcier, [3] Marc Labetoulle, [4] Louis Hoffart, [5] Pierre-Jean Pisella, [6] for the AZI Blepharitis Study Group. [1] Bichat Hospital, [2] Quinze-Vingts National Ophthalmology Hospital, [3] University Hospital of Strasbourg, [4] Bicêtre University Hospital, [5] Timone University Hospital, [6] University Hospital of Tours, France

Purpose: A pilot phase II study was conducted to assess the efficacy and safety of azithromycin 1.5% (Azyter®) *versus* placebo (polyvidone) eye drops in patients suffering from moderate to severe anterior and/or posterior chronic blepharitis.

Methods: 93 blepharitis patients were included in this multicentre, double-masked, randomised, controlled study. After a 2-week wash-out period with eyelid care, patients with persisting blepharitis received either azithromycin (AZM) or placebo eye drops for 7 days (2 drops on D1, then one drop daily), followed by 2 weeks without treatment. This scheme was repeated twice. The primary endpoint was the change from D0 to D63 in the global ocular discomfort assessed on a 100-mm visual analogue scale (VAS). Secondary outcomes included the evaluation of ocular symptoms (irritation, itching, crusting, sticking, light sensitivity, blinking) and signs (redness margin, swelling, dysfunction and quality of meibomian secretion; semi-qualitative VAS).

Results: Mean ocular discomfort reduction (D0 to D63) was -33.9 ± 20.2 mm (-52.2%) on AZM and -26.1 ± 24.0 mm (-41.1%) on placebo in the Full Analysis Set (FAS). The estimated mean difference between treatments in discomfort score, using a mixed model for repeated measures (MMRM), was -8.01 mm in the FAS (p=0.072) and -10.37 mm in the Per Protocol Set (p=0.026). AZM-treated patients also had a lower total symptom score (*vs.* placebo-treated patients; p=0.005) and lower VAS score for blepharitis signs (p=0.092) on D63. Both treatments were well tolerated.

Conclusions: Azithromycin 1.5% eye drops were shown to be effective and safe for the management of moderate to severe blepharitis, and appear as a promising therapeutic option with notably its anti-inflammatory activity, over the standard eyelid hygiene care.
[This study was sponsored by Laboratoires Théa]

THE EFFECT OF 2% REBAMIPIDE EYE DROPS ON THE OCULAR SURFACE OF SOD-1 KNOCK OUT MICE

Murat Dogru,[1,2], Takeshi Kojima,[1,2], Taeko Nagata,[2], Ayako Igarashi,[1],Kazunari Higa,[1], Seika Den Shimazaki,[1], Yoshiyuki Satake,[1], Kazuo Tsubota,[1], Jun Shimazaki[1] [1]Tokyo Dental College Ichikawa General Hospital, Department of Ophthalmology, Ichikawa, Chiba, Japan [2] Keio University School of Medicine, Department of Ophthalmology, Tokyo, Japan

Purpose: We previously reported the SOD-1 knock out (KO) mice to be a model for age related dry eye disease with marked decrease in ocular surface muc5AC mRNA expression. Rebamipide is a quinolinone analogue which has been shown to have anti-inflammatory, anti-oxidative and mucin secretagogue properties. In this study, we investigated the effect of 2% rebamipide eye drops on the tear functions and ocular surface epithelial differentiation marker: SAM pointed domain-containing Ets transcription factor (SPDEF) of the SOD-1 KO mice.
Methods: Fourteen C57BL6 strain 50 week male wild type (WT) mice were divided as: Group I (7 mice): No rebamipide administration Group II (7 mice): 2 weeks of 2% rebamipide eye drop instillation q.i.d. Seven 50 week SOD-1 KO male mice also underwent 2% rebamipide eye drop instillation q.i.d. for 2 weeks. The mice underwent tear break up time (BUT), corneal sensitivity measurement (CS), corneal vital stainings and phenol red test measurement for tear quantity before and after 2 weeks eye drop instillations. The mice were then sacrified and conjunctival specimens underwent immunohistochemistry (IHC) stainings with SPDEF antibodies, RT-PCR for SPDEF mRNA expression and evaluation with electron microscopy.
Results: The mean BUT, CS, and corneal fluorescein vital staining scores showed a significant improvement in the SOD-1 KO mice with rebamipide administration. The phenol red test did not show significant changes in all mice groups. IHC SPDEF staining showed a significant decrease in staining intensity in the SOD-1 KO mice compared to the WT mice and significant increased staining intensity after 2 weeks of eye drop instillation. SPDEF mRNA expression showed a significant increase after 2 weeks' of 2% rebamipide application in the SOD-1 KO mice.
Conclusion: SOD-1 KO mice appeared to have conjunctival epithelial differentiation disturbances compared to the WT mice. Instillation of 2% rebamipide eye drops for 2 weeks appeared to improve the tear stability, corneal epithelial damage, corneal sensitivity and epithelial differentiation in this age related dry eye mouse model.

ANTIMICROBIAL ACTIVITY OF MULTIPURPOSE DISINFECTION SOLUTION SOAKED CONTACT LENSES

Debarun Dutta[1,2], Hua Zhu[1,2], Mark DP Willcox[2]
[1]Brien Holden Vision Institute, Sydney, Australia;
[2]School of Optometry and Vision Science, University of New South Wales, Sydney, Australia
Purpose: To evaluate antimicrobial activity of Etafilcon A or Senofilcon A contact lenses after soaking in OPTI-FREE® PureMoist® (containing the disinfectants polyquaternium-1 and ALDOX; the surfactants Tectronic 1304 and EOBO-41™), Biotrue™ (polyquat and PHMB; Poloxamine and sulfobetaine) or RevitaLens OcuTec™ (polyquat and alexidine; Tectronic 904) multipurpose disinfection solutions (MPDS).
Method: Contact lenses were soaked in 2 ml of MPDS or phosphate buffer solution (PBS), as negative control, and the MPDS or PBS was changed each day. After 1 or 10 days of soaking, the antimicrobial activity associated with the lenses was evaluated against *Pseudomonas aeruginosa* 6294 and *Staphylococcus aureus* 31 by viable plate count.
Results: No significant antimicrobial ($p>0.05$) activity was observed after 1 day soaking in any MPDS or PBS. After 10 days, a majority of contact lenses soaked in MPDS showed significantly ($p<0.001$) higher antimicrobial activity (range 1 log to 4 log reduction in bacterial numbers) compared to control PBS-soaked lenses. Soaked Etafilcon A showed higher antimicrobial effect than Senofilcon A ($p<0.001$) contact lenses.
Conclusion: Contact lenses can adsorb/absorb antimicrobial components from MPDS during a 10 days soak. Differences in lens material properties are essential with antimicrobial efficacy. Retention of MPDS derived antimicrobial activity is higher for hydrogel than silicone hydrogel contact lenses.
[There are no conflicts of interest for any of the authors that could have influenced the results of this work. The research is supported by grants from UIPA award, University of New South Wales and Brien Holden Vision Institute.]

CORRELATIONS BETWEEN SEVERITY OF DRY EYE SYMPTOMS AND THE USE OF SYSTEMIC PRESCRIPTION DRUGS IN A COHORT OF DRY EYE PATIENTS.

Jon Roger Eidet,[1,2] Xiangjun Chen,[1,3] Tor Paaske Utheim,[1,2,3,4] Øygunn Aass Utheim,[1,2,4] Aleks Stojanovic,[1,3] Filip Stojanovic,[1,3] Sten Raeder.[1,3,5]
Tørreøyneklinikken[1] (Dry Eye Clinic, Oslo, Norway); Department of Medical Biochemistry,[2] Oslo University Hospital, Oslo, Norway; SynsLaser Kirurgi,[3] Oslo and Tromsø, Norway; Schepens Eye Research Institute,[4] Massachusetts Eye and Ear, Harvard Medical School, Boston, MA, USA; Department of Clinical Medicine,[5] Section of Ophthalmology, University of Bergen, Bergen, Norway.
Purpose: The use of systemic prescription drugs is a significant risk factor for symptomatic dry eye disorder. The purpose of the study was to investigate which category of systemic drugs showed the strongest correlation with the severity of dry eye symptoms.
Methods: Two hundred and eleven consecutive patients diagnosed with dry eye disorder at the Norwegian Dry Eye Clinic were included. Symptomatic severity, assessed by the MDEIS and OSDI self-report questionnaires, was correlated with the use of twelve different categories of systemic drugs. Statistical analyses included calculation of Pearson's correlation coefficient (r). Bonferroni correction was performed to adjust for multiple comparisons. A P-value of <0.0021 (0.05/24) was considered significant.
Results: Seventy-one percent of the patients were female. Three percent were under 25 years of age, 32 percent were between 25 and 45, while 65 percent were above 45 years of age. Mean MDEIS score was 14.0±5.1 and mean OSDI score was 14.4±9.4. The MDEIS score correlated significantly with the use of anxiolytics (r=0.32; P<0.001), antipsychotics (r=0.28; P<0.001), benign prostatic hyperplasia drugs (r=0.22; P=0.001), antihistamines (r=0.22; P=0.002), urinary incontinence drugs (r=0.22; P=0.002), alpha/beta-blockers (r=0.21; P=0.002) and anti-Parkinson's drugs (r=0.21; P=0.002). The OSDI score correlated significantly with the use of anti-Parkinson's drugs (r=0.22; P=0.001).
Conclusions: Anxiolytics and antipsychotics showed the strongest correlation with severity of dry eye symptoms, assessed by MDEIS. The OSDI score was only correlated with the use of anti-Parkinson's drugs.
[The authors have no conflicts of interest.]

THE EFFECT OF SURGICAL TECHNIQUE ON THE PERFORMANCE OF CELL-FREE COLLAGEN TYPE III CORNEAL IMPLANTS.

Rodolfo A. Elizondo[1], Monika Kozak Ljunggren[1], David Olsen[2], Kimberley Merrett[1,3], Chyan-Jang Lee[1,3], Göran Salerud[4], James Polarek[2], Per Fagerholm[1] May Griffith[1,3] Integrative Regenerative Medicine Centre & Dept. of Clinical and Experimental Medicine, Linköping University, Sweden[1]; FibroGen Inc., San Francisco, CA, USA[2]; Ottawa Hospital Research Institute, Ottawa, Ontario, Canada[3]; Integrative Regenerative Medicine Centre & Dept. of Biomedical Engineering, Linköping University, Sweden[4]
Purpose: Our aim was to determine the effect of surgical technique, specifically graft retention, on biomaterial implant performance.
Methods: Twelve mini-pigs were implanted with 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC)/ N-hydroxysuccinimide (NHS) crosslinked, cell-free, recombinant human collagen type III (RHCIII) hydrogels as substitutes for donor corneal allografts using overlying sutures versus interrupted sutures, with or without human amniotic membrane. The effects of surgery were compared for implants with a 15% RHCIII content. A change in the composition of the implants, 13.7% versus 15%

RHCIII using overlying sutures only was also investigated.
Results: All implanted corneas showed initial haze that cleared with time, resulting in corneas with optical clarity matching those of untreated controls. Biochemical analysis showed that by 12 months post-operation, the initial RHC\z\III implants had been completely remodeled, as type I collagen, the most abundant collagen present in normal, healthy corneas, was the major collagenous protein detected. All implanted corneas showed regeneration of epithelial and stromal layers and nerves, along with touch sensitivity and tear production. Most neovascularization and macrophages were seen in corneas stabilized by interrupted sutures.
Conclusions: The study showed that the surgical technique used does have a small but noticeable effect on the overall performance of corneal implants.
[This research was funded by the Canadian Stem Cell Network and Swedish Research Council and the County of Östergötland.]

THE EVALUATION OF MAXIMAL OPENING TIME-BREAK UP TIME INDEX IN CONTACT LENS WEARERS EXPOSED TO ADVERSE ENVIRONMENT CONDITIONS

Akiko Endo,[1], Murat Dogru,[1] Naoki Toriyama,[1], Kozue Kasai,[1],Daisuke Tomida,[1],Takefumi Yamaguchi,[1],Kazunari Higa,[1], Seika Den Shimazaki,[1], Yoshiyuki Satake,[1],Jun Shimazaki[1] [1]Tokyo Dental College Ichikawa General Hospital, Department of Ophthalmology, Ichikawa, Chiba, Japan
Purpose: We previously evaluated the efficacy of Maximal opening time-break up time (MOT-BUT) index in diagnosing dry eye disease from normal healthy individuals. We aimed to study the changes of the same index in contact lens (CL) wearers exposed to adverse environment conditions and compared the results with subjects not wearing CLs.
Methods: Seven silicon hydrogel CL (Acuvue Moist, Johnson and Johnson, USA) wearers (4 males, 3 females; mean age: 34.6 years) and 7 age sex matched non CL wearers participated this study. Maximal opening time (MOT) was defined as the maximal eye opening time without blinking while viewing a 10 minute arc Landolt C optotype at 5 meters. MOT-BUT index was defined as BUT subtracted from MOT. All subjects underwent symptom assessment using visual analog scale (VAS) scores, blink counts, MOT, tear evaporation, strip meniscometry, BUT, corneal sensitivity (CS), vital staining measurements and Schirmer test-1. Subjects were exposed to 10 minutes of wind exposure (7m/sec) at 25 and a humidity of 40%. Changes in all test results before and after wind exposure were compared using Wilcoxon t-test. P value less than 5% was considered as statistically significant.
Results: The mean BUT, MOT, MOT-BUT values, foreign body sensation and tiredness VAS scores deteriorated significantly both in CL and non CL wearers ($p<0.05$).The mean number of blinks increased significantly both in CL and non CL groups ($p<0.05$). The mean tear evaporation rate showed a significant 5 fold increase ($p<0.05$) in the non CL wear group and a 2 fold non-significant increase in the CL wear group. CS did not reveal significant changes in both groups with wind exposure. The SM value showed a significant decrease in the non CL wearers and no significant change in CL wearers.
Conclusions: MOT and MOT-BUT are useful indices in the evaluation of the effect of adverse environment conditions on the tear functions of CL wearers.

COMPARISON OF MEIBOMIAN GLAND LOSS AND MEIBUM GRADE IN PATIENTS WITH OBSTRUCTIVE MEIBOMIAN GLAND DYSFUNCTION.

Youngsub Eom, Kwang-Eon Choi, Su-Yeon Kang, Hyo Myung Kim, Jong-Suk Song. Department of Ophthalmology, Korea University College of Medicine, Seoul, South Korea
Purpose: To evaluate the correlation between meibomian gland loss and other grades according to clinical feature such as meibum grade, tear break-up time (TBUT), and corneal staining score in patients with obstructive meibomian gland dysfunction (MGD).
Methods: Twenty-one eyes of 21 patients with obstructive MGD were enrolled. Upper and lower meibomian gland losses were evaluated using a non-contact meibography (Topcon, Tokyo, Japan). Quality of expressed meibum was assessed in eight glands of the central third of the both upper and lower eyelid on a 0-3 scale for each gland (total score range, 0–24). Tear film stability was evaluated by TBUT and corneal staining was graded according to the National Eye Institute (NEI) scale (range, 0-15). The correlations among variables were evaluated.
Results: Lower meibomian gland loss (21.9±7.6%) was significantly larger than upper meibomian loss, 12.7±5.4 ($P<0.001$). Meibum grade of lower eyelid (16.2±4.5) was significantly larger than that of upper eyelid, 10.8±4.6 ($P<0.001$). In the lower eyelid, meibomian gland loss was correlated with meibum grade ($r=0.484$, $P=0.026$). In the upper eyelid, however, there was no correlation between them. Meibum grades of both upper and lower eyelids were negatively correlated with TBUT ($rho=-0.475$, $P=0.030$ and $rho=-0.477$, $P=0.029$, respectively), and that of lower eyelid was correlated with corneal staining score ($rho=0.462$, $P=0.035$). However, upper and lower meibomian gland losses were not correlated with TBUT or corneal staining score.
Conclusions: A positive correlation between meibomian gland loss and meibum grade was observed only in the lower eyelid which has more prominent meibomian gland loss than upper eyelid. Quality of expressed meibum grades were more correlated with the tear film and ocular surface state than meibomian gland losses in patient with obstructive MGD.
[The authors have no proprietary or commercial interest in any materials discussed in this study.]

SUBSTANCE P AND ITS INHIBITION IN CORNEAL NEOVASCULARIZATION

Giulio Ferrari,[1,2,3] Fabio Bignami,[1,3] Chiara Giacomini,[1,3], Paolo Rama[1,2,3]. San Raffaele Scientific Institute,[1] Cornea and Ocular Surface Unit[2], Eye Repair Lab[3]
Purpose: To quantify expression of Substance P in corneal neovascularization (CNV) and to test whether topical administration of a Substance P receptor antagonist (NK1R-a) is toxic to the ocular surface and may reduce vessel growth in two different animal models of CNV.
Methods: Five-week old C57/BL6 male mice were used. To quantify substance P, eight mice received a corneal caustication with 1N NaOH in their right eye and the cornea of six mice was sutured intrastromally. Nine days later, the cornea was removed, homogenized and prepared for the Substance P EIA assay.
To test whether inhibition of Substance P may reduce CNV, twelve mice received corneal caustication and twelve mice received corneal sutures. Animals were divided into two groups: the first received normal saline; the second received NK1R-a dissolved in saline six times a day topically. Four days after treatment was started, animals were sacrificed, corneas removed and stained for CD31 and LYVE-1. Images of corneal whole-mounts were obtained with a confocal microscopy and mounted. Neovascular area was measured as a ratio between the area of the cornea covered by neovessels and the total area of the cornea using ImageJ software. Finally, four normal mice were used to test toxicity of topical NK1R-a.
Results: Substance P corneal concentration was 6,336 pg/ml in control corneas and increased to 8,702 pg/ml ($P<0.05$) in causticated corneas and 12,320 pg/ml in sutured corneas ($P<0.001$).
Following topical administration of an NK1-R antagonist in the caustication model, both lymphatic and hematic neovascular area in the cornea was significantly reduced ($P<0.01$). Corneal epithelial defect area significantly improved when compared with vehicle-treated eyes at 9 days ($P<0.05$). No significant difference was seen in the suture model of CNV. Topical administration of NK1R-a was not toxic when administered to normal eyes up to 9 days.

Conclusion: Corneal Substance P is increased in two different animal models of corneal neovascularization. Topical administration of an NK1R-a is not toxic to the ocular surface and reduces CNV and epithelial defects in an alkali burn model. No significant effect on corneal neovascularization was seen in the suture model. Studies are underway to test whether different concentration and/or administration routes may affect NK1R-a efficacy.
The authors have no financial disclosures.

PERFORMANCE OF DRY EYE TESTS IN A TERTIARY EYE UNIT IN THE UK.

Francisco C Figueiredo, Philipp Baenninger. Dept of Ophthalmology, Royal Victoria Infirmary, Newcastle University, Newcastle upon Tyne, UK
Purpose: To evaluate the performance of commonly used dry eye tests in a cohort of patients from a tertiary eye unit. **Methods:** 50 patients from a general National Health Service patient population were evaluated for dry eye. In addition to OSDI, bilateral osmolarity, Schirmer I, break up time (TBUT), corneal and conjunctival staining, meibomian dysfunction assessment, and visual acuity (VA) were performed.
Results: Data from 45 patients (43.3±12.0 yr, 12M/33F) were included in the study, as 5 patients had incomplete records. Using a composite scale, 25 patients were found to be normal, 14 were classified as moderate, 6 were severe, suggesting an overall prevalence of 44%, which is consistent with other studies. Snellen visual acuity demonstrated a worsening trend from normal: 20/19±4.3, moderate: 20/21.7±6.9 to severe patients: 20/25.0±8.4, p=0.024 vs. normal. VA was not part of the composite scale. Also confirmed was the lack of correlation between the commonly used tests and symptoms, with an average r^2 of 0.17 across the signs. Patients with Sjögren's/RA (343.2±19.1 mOsm/L) had the highest average osmolarity value as compared to patients with hypothyroidism (321.8±14.6), hypertension (321.5±50.2), diabetes mellitus (312.3±14.0), or those without an associated medical condition (311.1±23.6 mOsm/L). Of note, while signs such as osmolarity (301±14, 329±21, and 351±14 mOsm/L), TBUT (4.7±2.0, 2.2±1.3, 1.7±0.9 seconds) and corneal staining (1.0±1.4, 2.1±1.9, 6.5±2.8 grade) were shown to steadily worsen across the three levels of disease severity, OSDI symptoms were far lower than in other studies (2.6±3.3, 7.7±10.3, 10.8±9.7), potentially revealing a cultural component to symptoms.
Conclusion: The data suggest that the general patient population within the NHS exhibits a large prevalence of moderate to severe dry eye with a trend towards worsening visual acuity with worsening disease. This emphasizes the need for objective dry eye testing in the UK in order to help diagnose DED, stratify disease severity and possibly identify otherwise asymptomatic early stage dry eye before subjects progress.

NOVEL MECHANISMS FOR OCULAR SURFACE HOMEOSTASIS AND PROTECTION

M. Elizabeth Fini and Shinwu Jeong, Institute for Genetic Medicine, University of Southern California, Los Angeles, California, USA
The ocular surface epithelium is a wet tissue that is directly exposed to the environment, thus it is especially subject to desiccation and injury, and it is endangered by a large number of antigens and pathogenic microorganisms. As a consequence, numerous protective mechanisms are required.
Matrix metalloproteinases (MMPs) are a family of at least 20 (depending on species) zinc-dependent endoproteinases that serve as both regulators and effectors of a wide array of normal and pathological processes. MMPs are needed to maintain the ocular surface, but also serve as key mediators of ocular surface damage. In particular, accumulating evidence implicates Matrix Metalloproteinase-9 (MMP-9) in inflammatory damage due to dry eye disease of diverse etiologies. We recently identified a novel role for the multi-functional stress protein, clusterin (CLU) as an MMP inhibitor. Our studies suggest that CLU protects the ocular surface epithelium from the damaging effects of dry eye disease by this mechanism. In addition, new preliminary data indicates that CLU inhibits activity of TNF-alpha. These mechanisms will be discussed.
Mucins are also of central importance to ocular surface health, and changes in their expression are associated with dry eye disease. Two novel mucin genes, MUC22 (also called PBMUCL1) and HCG22 (also called PBMUCL2), were recently identified within the Major Histocompatibility (MHC) Class I locus on chromosome 6, expressed in the lung (Hu Genet 129:117, 2011). The inflammatory cytokine IL-1 stimulates expression of MUC22 and HCG22, while TGF-beta or glucocorticoids inhibits basal and IL-1-stimulated expression. Conceptual translation indicates that MUC22 mRNA encodes a large plasma membrane-associated protein of 174 kDa. Transient expression of a cloned variant of MUC22 cDNA with a truncated mucin repeat region (57 kDa) tagged with GFP (27 kDa) enriches in the plasma membrane, migrating at ~ 200 kDa on westerns. In contrast, conceptual translation indicates that HCG22 mRNA encodes a small, secreted protein of 26kDa. We show that a FLAG-tagged HCG22 cDNA is expressed as a ~40 kDa cytoplasmic form and a ~67 kDa secreted form. O-glycosylation predicted by sequence analysis explains the larger than expected sizes.
The new genes are part of an uncharacterized mucin gene cluster that includes SFT2 (a surfactant protein), DPCR1 (a zonadhesin domain-containing protein), MUC21, MUC22, and HCG22. We show by RT-PCR that corneal epithelial cells express the mRNAs for all of these genes. Interestingly, the mRNAs for DPCR1, MUC22 and HCG22 (but not SFT2 and MUC21) are also expressed in trabecular meshwork cells of the eye's aqueous outflow pathways. Of all the MUC-type mucins, MUC22 contains the largest number of mucin-type repeats, a domain that has been implicated in cell adhesion. Immunostaining demonstrates that MUC22 is expressed in the upper layers of the epithelium at the ocular surface. We will discuss possible roles for the novel mucins at the ocular surface and in the aqueous outflow pathways.
This work was supported by NIH grant EY09828 and a Shaffer Grant for Innovative Glaucoma Research from the Glaucoma Research Foundation.

THE KORB-BLACKIE LID LIGHT TEST.

Victor Finnemore,[1] Caroline Blackie,[1,2] Donald Korb,[1,2] Korb Associates, Boston, MA,[1] TearScience Inc, Morrisville, NC.[2]
Purpose: To investigate the possibility that apparently normal, closed, lids fail to create the necessary protective seal to prevent ocular surface desiccation during sleep.
Methods: Inclusion criteria: willingness to participate in the study, over the age of 18, no lid/closure (e.g. lagophthalmos) abnormalities, no current ocular inflammation/disease, no ocular surgery within the last 6 months, no history of lid surgery, no current use of eye ointment at night. 116 patients were enrolled. After completing a questionnaire regarding their eye comfort upon awakening, patients were asked to rest in a semi-reclined exam chair, to close their eyes and relax as if asleep. The transilluminator was lightly placed against the closed outer upper lid, centrally, at the juncture of the superior tarsal plate with the superior tarsal muscle. The closed lids were examined for any light emanating from the lid margins between the lashes. The lids were examined from an inferior position, looking up, to optimize viewing of the designated area. The lids were divided into three sections: temporal (T), central (C) and nasal (N). The amount of visible light in each section was quantified on a scale of 0 – 3 (0 = no light, 1 = minimal, 2 = moderate and 3 = severe). Eye discomfort upon awakening was quantified from 0 – 2 (0 = no discomfort, 1 = mild and 2 = significant discomfort).
Results: Right eyes only are presented. The mean age of the patients was 52.6±16.8 years (49 males; 67 females). The C section had a positive Lid-Light score (54.3%) significantly more frequently than the N (37.9%) or T (21.6%) lid sections ($p<0.0001$). The C section was also the most likely to have the most severe lid-light score compared to the N and T lids sections ($p<0.0001$). Patients with a positive Lid-Light test were significantly more likely to have symptoms upon awakening compared to those with a negative Lid-Light test ($p<0.0001$).

Conclusion: The KB Lid-Light test reveals that light emanating from between closed lids, which is correlated to symptoms of ocular discomfort upon awakening, may be linked to the inability of the lids to create the necessary protective seal to prevent ocular surface desiccation during sleeping.

RELEVANCE OF THE MICROBIOME TO THE OCULAR SURFACE

Suzanne M. J. Fleiszig (USA)

In recent years, the human microbiome has evolved into a hot topic as information about its many roles in maintaining health beyond protecting against infectious diseases has emerged. Certainly, our exposed body surfaces are exposed to a vast array of microbes, many of which are critical to normal function. Whether or not the healthy ocular surface harbors a significant microbiome, and if so whether it plays roles in protecting against ocular surface disease, is a subject of much debate. While culture studies have revealed too few bacteria and not enough consistency for the microflora of the ocular surface to perform critical functions, the possibility of a significant live non-culturable population has been proposed. Indeed, data generated using methods for identification of non-culturables that detect bacterial derived nucleic acids or other microbial derived debris have suggested a rich and diverse microflora at the ocular surface. Yet, it is of concern that some of the species identified are considered culturable, casting doubt on whether the presence of microbial nucleic acid/debris necessarily reflects the presence of live organisms. Ocular surface washing (using an irrigation chamber) or tear fluid collection and subsequent staining with acridine orange has enabled direct visualization of factors present at the ocular surface, including sloughing epithelial cells, leukocytes, mucin and cellular debris. Acridine orange labels both live and dead bacteria, culturable or not, yet bacterial forms are only observed in significant numbers when samples are deliberately inoculated with bacteria in vitro prior to visualization. Studies with cultured corneal epithelial cells and the ocular surface of mice provide insights into the lack of visible bacterial forms at the human ocular surface. Live imaging reveals that the apical surface of cultured but polarized corneal epithelial cells repels bacteria, as does the apical surface of other mucosal epithelia that have been studied. If the ocular surface (of rodents) is challenged in vivo with enormous numbers of highly resistant and highly adaptable bacteria (e.g. P. *aeruginosa* added at 1011 cfu/ml), all of the bacteria are cleared within a few hours. In the gut, which has a rich live microbiome, there is a 50-micron clear zone between the resident bacteria and the tissue epithelial surface (both intestine and colon), due to the highly antimicrobial nature of the apical cell surface. If corneal epithelial cell surfaces were only as antimicrobial as gut epithelial cells, we would expect the entire thickness of the 7-micron tear film to be clear of bacteria. While directly relevant data and other evidence suggests the lack of a stable live microbiome at the ocular surface, it remains important to define the microbial debris derived from bacteria killed prior to or after entry into the eye, since it could participate in shaping host immunity via mechanisms similar to a live microbiome.

THE EFFECT OF CONTACT LENS ON TEAR OSMOLARITY DEPENDS ON THE TEAR OSMOLARITY ITSELF

Alessandro Fossetti[1,2], Francesco Cozza[1], Carlo Falleni[1,4], Alessandro Farini[1,3], Jacopo Siroki[1]. Istituto Regionale Studi Ottici e Optometrici (IRSOO) [1], Vinci, Italy, Università degli Studi di Firenze[2], Italy, INO - CNR, Firenze[3], Italy, Università degli Studi di Torino[4], Italy

Purpose: The 2007 Report of the Dry Eye Workshop (DEWS) states that contact lens (CL) wear is a significant etiological cause of dry eye. The measurement of the tear osmolarity (TO) is the best single test for the diagnosis of dry eye (Khanal et al, 2008). Wetting agents are used to improve comfort during the wearing period. The aim of the study was to investigate the impact of CL wear on TO, and the effect of commercial wetting drops (WD).

Methods: 26 subjects, non CL wearers, were recruited from the students of the Course of Optometry at IRSOO. Measurement of the TO (Tear Lab), NIBUT (Sirius, CSO) and BUT were performed in the afternoon of the day 1. Two CL (Nelfilcon A) were fitted in the morning of the day 2, and the subjects were instructed to use WD only on one eye (randomly assigned) every 3 hours. NIBUT on contact lenses and TO were taken after 8 hours wear, then the CL were removed, and NIBUT and BUT were measured.

Results: Wearing of CL did not modify TO highly, neither for lens alone (r2=0,54, p=0,634) nor for lens plus WD (r2=0,70, p=0,179). The effect of WD on TO was not significant (p=0,4695). Grouping subjects by baseline TO (BTO): ≤316 and >316, limit suggested by Tomlinson et al (2006) to indicate dry eye, differences become significant. The mean difference in TO was +6,6 ± 20,2995 for the group with BTO ≤316 (n=35) and -30,588 ± 29,7007 for BTO >316 (N=17), with p=0000045.

Conclusions: In young subjects CL wear does not modify TO in case of BTO ≤316 and significantivelyreduce TO for BTO >316. The use of wetting drops does not lead to a major benefit in term of reduction of TO. The fitting of CL in patients who have dry eye with BTO >316 can increase TO and should not be preventively discouraged.

RELATION BETWEEN MEIBOGRAPHY OF THE TWO EYES, NIBUT AND OSDI IN YOUNG SUBJECTS

Alessandro Fossetti[1,2], Simone Aru[1], Carlo Falleni[1,4], Alessandro Farini[1,3], Alessandro Landi[1], Andrea Ramacciotti[1] . Istituto Regionale Studi Ottici e Optometrici (IRSOO) [1], Vinci, Italy, Università degli Studi di Firenze[2], Italy, INO - CNR, Firenze[3], Italy, Università degli Studi di Torino[4], Italy

Purpose: This is a common opinion that Meibomian Gland Dysfunction (MGD) is the leading cause of dry eye (DE). The assessment of the ocular surface and of the MG conditions is the basis for the identification of the dry eye (DE). Except for Tear Osmolarity, most of the existing assessment methods for DE have limitations as regards the lack of coincidence between the symptoms and the observed signs. The Meibography has been called to provide an in-vivo means to assess the structure of the MG (Pult H et al, 2011) and to indicate DE. The aim of this study was to investigate the correlations between Meibography, NIBUT and OSDI for the detection of DE.

Methods: 87 students were recruited at the Optics and Optometry School in Vinci, Florence. After the compilation of the OSDI questionnaire, evaluation of the MG loss in the upper lid (UL) and lower lid (LL) of both eyes was performed with Cobra (CSO). MG loss was measured by means of the Phoenix Meibography software (CSO). In a second day NIBUT was assessed by means of Sirius (Scheimpflug with a Placido disc, CSO)

Results: MG loss was significantly higher in the lower lid (LL) than in the upper lid UL) for both eyes (p<0,001). MG loss was not different between the UL of the two eyes (p=0,8839) and significantly different (p<00001) between the two LLs. The comparison of MG loss with NIBUT and OSDI does not lead to a significant correlation (OD r= -0,06948, OS r= -0,1335 for NIBUT; OD r=0,37022, OS r=0,22477 for OSDI)

Conclusions: The major MG loss for LL in comparison with UL as reported in other studies (Pult H et al, 2012) was confirmed. Significant difference in MG loss for LLs was found; no difference was found for ULs. Contrary to other studies, no correlation for MG loss with OSDI and with NIBUT was found. This dissimilar findings could be due to the young age of the subject observed (22,89 ± 3). For these subjects is questionable that MG loss can be taken as an indicator for DE.

EFFICACY EVALUATION OF A NOVEL EMULSION BASED, ANIONIC PHOSPHOLIPID CONTAINING ARTIFICIAL TEAR IN MEIBOMIAN GLAND DYSFUNCTION (MGD) SUBJECTS

Gary Foulks[1], Chris Sindt[2], Joe Griffin[3], [1]Kentucky Lions's Eye Center, Lexington KY, [2]U of Iowa, Iowa City IA, [3]Alcon Research Ltd, Ft Worth, TX.

Purpose: The objective of this study was to evaluate the efficacy of a novel emulsion based, anionic phospholipids containing artificial tear

product vs habitual therapy in diagnosed MGD subjects.
Methods: 49 subjects with MGD (defined by specific symptoms and evidence of gland dropout and aberrant meibum) were evaluated to determine the efficacy of a novel emulsion based artificial tear. Detailed habitual therapy was used as baseline comparison and collected by dose tracking with the Medication Event Monitoring System (MEMS) automated dose tracking device. Study endpoints including subject reported symptomatic relief, TFBUT, corneal staining, meibum expression quality and drop usage were collected at baseline and after 28 continuous days of treatment with the test drops. Each endpoint was compared to baseline (i.e. habitual use).
Results: From the patient reported symptomatic questionnaire, eighty six (86%) of subjects reported that the novel tear provided fast symptomatic relief, 79% reported satisfaction with drop comfort and 77% reported overall satisfaction. There was a statistically significant improvement in TFBUT (33%) and reduction in corneal staining (26%) in subjects treated with the test product ($p = 0.032$ and $p < 0.001$, respectively). There was a mild, but statistically significant improvement (17%) in meibomian gland expression ($p = 0.005$) and a moderate, but significant (24%) decrease in drop usage ($p = < 0.001$).
Conclusion: Taken collectively, the emulsion based, anionic phospholipid containing artificial tear was shown to be effective in treating the signs and symptoms in MGD subjects when compared to habitual use. The use of the MEMS dose tracking device for objective evaluation provided an improved system for measuring habitual drop usage.

ANALYSIS OF FIBROSIS IN IGG4-RELATED MIKULICZ'S DISEASE.

Masaki Fukui, Yoko Ogawa, Shigeto Shimmura, Shin Hatou, Yoshiyuki Ichihashi, Saori Yaguchi, Masatoshi Hirayama, Tetsuya Kawakita, Kazuo Tsubota. Department of Ophthalmology, Keio University School of Medicine
Purpose: IgG4-related disease (IgG4-RD) whose cause is unknown, is a fibro-inflammatory condition with a marked development of mass-forming lesions, which are characterized by a dense lymphoplasmacytic infiltrate, and IgG4+ plasma cells. The bilateral lacrimal glands and pancreas are frequently involved in IgG4-RD. However, the fibrotic mechanism of IgG4-RD is not unknown. Here, we investigated the pathogenesis of IgG4-RD by analyzing the fibrosis of the lacrimal gland tissues of three IgG4-RD patients.
Method: We compared the lacrimal gland tissues of four IgG4-RD cases with those of Sjögren's syndrome by histological, immunohistologival and transmission electron microscope (TEM) analyzes.
Results: In the IgG4-RD samples, they showed storiform fibrosis and abundant IgG4+ plasma cells. TEM observation revealed an unusual collagen fiber bundle pattern, collapse of the basement membrane of myoepithelial cells, myoepithelial cell invasion into the stroma, and the appearance of basement membrane and hemidesmozome on fibroblast like cells in the stroma. Immunohistology showed a reduction of E-cadherin on acinar epithelia, and an increased phalloidin staining and -SMA on myoepithelial cells around the acini of lacrimal gland, and Snail expression in the nucleus of acinar epithelial cells and HSP47 on the acinar and acinar ducts.
Conclusion: The results suggested that some of fibrosis in IgG4-RD occurs by epithelial-mesenchymal transition (EMT).
The authors have no commercial relationship. This research has no grant support.

TOWARD ATISSUE AND GENE THERAPY OF HERPES RELATED CORNEAL BLINDNESS

Eric E. Gabison,[1, 2]; Marc Labetoulle,[4]; Marine Gailledrat,[5]; Jose A. Sahel[3]; Benoit Chapellier [3]
INSTITUTIONS (ALL): 1. Cornea Department, Fondation A. de Rothschild, Paris, France.
2. Ophthalmology Department, Hôpital Bichat, Paris, France. 3. Institut de la vision, Paris, France.
4. Ophthalmology Department, Hôpital du Kremlin Bicêtre, Paris, France. 5. Cellectis, Paris, France.
Purpose: Herpetic Keratitis is a leading cause of decreased best corrected visual acuity in developed and developing countries. The aim of this study is to assess the antiviral property of a meganuclease targeting HSV in the prevention of HSV Keratitis
Methods: Rabbit models of corneal HSV stromal or endothelial keratitis was developed. Intracamerular or intrastromal injection of HSV F(1) were performed in rabbit eyes Corneal edema, keratic precipitates, ocular inflammation and infection rate for plaques or cells were analyzed in these model.
Results: In vivo experiments demonstrated a significant decreased in endothelial plaque formation and stromal infection. The rate of corneal edema and keratic precipitates was reduced in Megnuclease treated eyes as compared to controles.
Conclusions: Our in vivo model of herpetic corneal infection are reproducible and efficient to quantify viral proliferative capacity. Meganuclease transduction confers a significant inhibition of viral pathogenic effect. Meganuclease gene therapy targeting HSV-1 DNA may be an effective treatment to protect corneal transplant against HSV infection
This research was supported by OSEO ACTIVE GRANT FRANCE

GETTING TO NET

Mihaela Gadjeva
Division of Infectious Disease, Brigham and Women's Hospital, Harvard Medical School
Purpose: Recently-discovered complexes of DNA and proteins, termed Neutrophil Extracellular Traps (NETs) are novel, neutrophil-mediated, innate immune response to close-proximity extracellular pathogens. Here, we investigate the molecular mechanisms of neutrophil extracellular trap release in response to *P. aeruginosa* infection in their relevance to ocular keratitis.
Methods: Primary human neutrophils were exposed to different doses and strains of *P. aeruginosa* and the contribution of NET-mediated killing analyzed. The molecular composition of NET fragments released in response either invasive or cytotoxic strains was compared using LC/MS spectrometry. The presence of NETs during ocular keratitis was examined *in vivo* using mouse keratitis model.
Results: Both, invasive and cytotoxic *P. aeruginosa* strains induced NET release. As expected the cytotoxic strains were more efficient in triggering NETosis than the cytotoxic strains. NET-mediated killing ranged between 20 to 40 percent with the invasive strains being more sensitive to killing than the cytotoxic strains. Overall, NET killing appeared to be less efficient than the opsonophagocytic killing. NETs were decorated with the "classical" NET members (e.g., elastase, lysosyme, myeloperoxidase), novel proteins, and *P. aeruginosa*-derived proteins. The molecular make-up of NETs suggests that NET-induced killing is based on metabolic-control over *P. aeruginosa* growth.
Conclusions: NETosis is an innate, evolutionary conserved mechanism for protection which elicits metabolic control over bacterial proliferation. Gaining in-depth understanding of how NETs kill will provide the community with novel "innate" bactericidal molecules.

EFFICACY OF TWO DIFFERENT ARTIFICIAL TEARS FOR THE TREATMENT OF DRY EYE IN FREQUENT COMPUTER AND CONTACT LENS USERS.

Caterina Gagliano,[1,2] Vincenzo Papa,[3] Giulia Malaguarnera,[4] Roberta Amato,[1,2] Maria Grazia Mazzone,[3] Teresio Avitabile.[1] [1]University of Catania, Eye Clinic, Catania, Italy, [2]Neurovisual Science Technology

(NEST), Catania, Italy, [3]SIFI S.p.A., Aci S.Antonio, Catania, Italy, [4]Department of Molecular and Clinical Biomedicine (Pharmacology and Biochemistry), University of Catania, Catania, Italy.
Purpose: To compare the effect on tear functions of a lipid-based artificial tear acting on lipidic and aqueous layer (Emustil®, SIFI SpA, Italy) with that of a polymeric-based artificial tear acting on mucin and aqueous layer (Optive®, Allergan, USA) in women with dry eye (DE) symptoms associated to external causes.
Methods: Thirty women wearing contact lens (CL) and working on visual display terminal (VDT) were enrolled in this prospective randomized study. Participant were treated with Emustil® (group A, n=10) or Optive® (group B, n=10) *t.i.d.* for 30 days; a third untreated group was used as control (group C, n=10). Evaluation included ocular surface and tear function tests: vital staining (fluorescein and lisamine green), Schirmer test, tear meniscus height and osmolarity measurement, tear film break-up time. Ocular Surface Disease Index (OSDI) score was evaluated. All parameters were evaluated twice (at 8:00 am and at 8:00 pm-after VDT work) at baseline (Day 0) and at Day 7, Day 14, and Day 30. Data were analyzed by analysis of variance (ANOVA).
Results: At 30° day of treatment, only patients treated with Emustil® had a significant improvement of the tear meniscus height ($p<.001$) and a significant decrease of OSDI scores ($p<.001$). Osmolarity and corneal staining were significant decreased at 8:00 pm ($p<.001$) in both groups of treatment but not in the control group. Statistically significant ($p<0.05$) decrease in osmolarity at 8:00 am was observed only in the broup treated with Emustil®.
Conclusions: Use of a specific lipid composition as that present in Emustil® produces a significant reduction in evaporation and improvement in symptoms of DE.

EFFECT OF 0.05% CYANOCOBALAMINE, 0.5% TAURINE AND 0.5% LONG-CHAINED HYALURONIC ACID ON THE OCULAR SURFACE IN DYSMETABOLIC PATIENTS AFFECTED BY DRY EYE.
Caterina Gagliano,[1,2] Giuseppina Marrazzo,[3] Giulia Malaguarnera,[4] Roberta Amato,[1,2] Mario Toro,[1] Santo Stella,[2] Teresio Avitabile.[1]
[1]University of Catania, Eye Clinic, Catania, Italy, [2]Neurovisual Science Technology (NEST), Catania, Italy, [3]ALFA INTES, Casoria (NA), Italy, [4]Department of Clinical and Molecular Biomedicine (Pharmacology and Biochemistry), University of Catania, Italy.
Purpose: To study the effect of 0.05% cyanocobalamine, 0.5% taurine and 0.5% long-chained hyaluronic acid (IALUVIT® ophthalmic solution, Alfa Intes, Italy) on the functional recovery of the ocular surface and patient comfort in patients affected by metabolic syndrome and dry eye.
Methods: 40 patients affected by metabolic syndrome (according to the WHO definition) with moderate to severe dry eye, partecipate in a randomized, single center, single masked study. The patients were divided into 2 groups A and B and treated 4 times a day for all the monitoring time with IALUVIT® and saline solution (control) respectively. Symptoms of DE were evaluated using the Ocular Suface Disease Index (OSDI). Data from Schirmer test, BUT, fluorescein and lissamine green staining to highlight the epithelial suffering of cornea and conjunctiva, tear osmolarity were collected at day 0 and at 1,2 and 3 months after the treatment. Also, corneal sensitivity (Cochet-Bonnet) and confocal microscopy were performed.
Results: Schirmer test, BUT, fluorescein and lissamine green staining, tear osmolarity, showed a significant differences between each time point of Ialuvit treated patients vs control treated ($p<.001$). Significant decrease of OSDI scores ($p<.001$) was observed. Also, we found a persistent abnormal architecture of the nerve plexus of the ocular surface in the control group compared to the Ialuvit treated patinets.
Conclusion: Local treatment with the ophthalmic solution containing 0.05% vitamin B12 plus 0.5% taurine and 0.5% sodium hyaluronate could represent a powerful strategy to treat the ocular discomfort of dysmetabolic patients.
Commercial disclosure: Giuseppina Marrazzo is an employees of ALFA INTES (Casoria, NA, Italy); all other authors have no commercial interest.

CHANGES IN NEURAL ACTIVITY OF OCULAR SURFACE SENSORY NERVES DURING ALLERGIC KERATOCONJUNCTIVITIS.
J. Gallar., MC. Acosta, C. Luna, S. Quirce, C. Belmonte,
Instituto de Neurociencias, Universidad Miguel Hernandez-CSIC, San Juan de Alicante, Spain.
Purpose: The activity of polymodal, mechano-nociceptors, cold thermoreceptors and low-threshold mechanoreceptors innervating the ocular surface is in the basis of sensations arising at the eye surface. Allergic keratoconjunctivitis (AKC) is typically accompanied by edema, erythema and increased tearing, being irritation and itching sensation cardinal symptoms. The peripheral neural mechanisms underlying irritation, discomfort and itch sensations accompanying the eye allergic response have not been hitherto analyzed. To address this question, we recorded in vitro the changes in the electrical activity of corneo-conjunctival sensory nerve fibers in an experimental model of AKC in the guinea pig.
Methods: AKC was induced by i.p. injection of ovalbumin followed by topical application of ovalbumin into the eye 14-18 days afterwards. Edema, erythema, and increased blinking and tearing rate was observed after the allergen challenge. Spontaneous and stimulus-evoked (mechanical, thermal, chemical) sensory nerve activity from corneas of allergic and naïve animals were compared.
Results: Corneal mechano-nociceptors (reduction of mechanical threshold) and polymodal nociceptors (increased impulse response to acidic stimulation) were sensitized after exposure to the allergen. In contrast, a significantly reduced on-going activity and response to cooling was found in cold thermoreceptors. Pretreatment with the TRPV1 channel blocker capsazepine reversed the augmented blinking tearing rate produced by the allergen challenge, and prevented the sensitized response of polymodal nociceptors and the decreased activity of cold thermoreceptor observed after the allergic challenge.
Conclusions: The unpleasant sensations experienced during AKC are caused by the increased responsiveness (sensitization) of corneal polymodal nociceptors, likely attributable to the inflammatory mediators released by the allergic challenge and mediated by changes in TRPV1 channel activity. The reduced activity of cold thermoreceptors may enhance the discomfort sensations evoked by nociceptor sensitization, since the input of peripheral cold thermoreceptors inhibits in part the pain sensory pathways at higher levels of the CNS.
CR: None. Support: GV/2007/030, SAF2011-22500, BFU2008-04425, CSD2007-00023.

NON-INVASIVE OBJECTIVE METRICS OF BULBAR HYPEREMIA FOR CLINICAL TRIALS ENDPOINTS.
Neha Gadaria-Rathod, Kyu-In Lee, Benyamin Y Ebrahim, Penny A Asbell. Dept. Ophthalmology, The Icahn School of Medicine at Mount Sinai. New York,NY, USA
Purpose: Bulbar hyperemia is a prominent feature of ocular irritation associated with dry eye disease (DED), infection and allergy. The aims of this study were to evaluate the precision of a modified topographer (OCULUS Keratograph 5M) and its correlation with clinician and subject grading.
Methods: 39 eyes of 20 patients were evaluated for bulbar redness independently by 2 ophthalmologists, by patients' self-assessment and by the keratograph. CCLRU grading scales were used to score the bulbar redness on a scale of 0-4. To establish precision of the keratograph, 2 pictures of the same eye were taken by the same clinician, 2 minutes

apart. The repeatability of measurements was then tested with one-way analysis of variance and the intraclass correlation coefficient. Bivariate correlation analysis between keratograph scores, subject scores and clinician scores was done using the SPSS Statistical Software and the Spearman's correlation coefficient was calculated.
Results: The keratograph grading showed high repeatability; r=0.90, p<0.001.Both KR1 and KR2 showed high correlation with each other (r=0.93; p<0.01) and with subject scores (r1=0.63, r2=0.70, p<0.01), but variable correlation with clinical scores; clinician 1 scores (r1=0.59, r2=0.61, p<0.01); clinician 2 scores (r1=0.22, p1=0.1; r2=0.31, p=0.05). The clinician scores (mean=1.58+/-0.6) and subject scores (mean=2.3+/-0.7) were significantly higher as compared to the keratograph scores (mean=1.0+/-0.5) (p<0.01).
Conclusion: The keratograph showed high precision in measuring bulbar redness. Subjective scores were on an average higher than keratograph scores, in line with results of previous studies and may be due to the features of the grading scale used. The keratograph takes into account proportion of bulbar area occupied by vessels, number of vessels and the proportion of area occupied by thin vessels and thus reduces overestimation of hyperemia. This novel instrument may prove to be a non-invasive, objective biomarker of ocular surface inflammation in clinical trials of ocular surface disease.

ALTERED CALCIUM REGULATION BY TEMPERATURE-SENSITIVE TRANSIENT RECEPTOR POTENTIAL CHANNELS (THERMO-TRPS) AS A PATHOPHYSIOLOGIC EFFECT IN PTERYGIUM

Fabian Garreis[1], Stefan Mergler[2], Antje Gaebel[1] Stefanie Zoll[2], Alexander Lucius[2], Martin Straßenburg[2], Anja Lude[2] and Friedrich Paulsen[1]
[1]Department of Anatomy II, Friedrich Alexander University Erlangen-Nuremberg, Erlangen, Germany; [2]Department of Ophthalmology, University Medicine Berlin, Campus Virchow Hospital, Berlin, Germany
Purpose: Pterygium is a benign fibrovascular lesion with an appearance of triangular or wing-shaped over-growth on the corneal surface. It is associated with ocular surface inflammation, tearing, astigmatism and impair of vision. The exact mechanism of pathogenesis is still unknown. Ca^{2+} permeable temperature-sensitive transient receptor potential channels (thermo-TRPs) play an important role in regulation of calcium homoeostasis as well as various cell biological functions such as cell growth and cell migration. We hypothesize that thermo-TRPs might be involved in the pathophysiology of pterygium.
Methods: First, we characterized a spontaneously immortalized pterygium cell line by different bio markers for pterygium, limbal stem cells, corneal and conjunctival epithelial cells with RT-PCR, Western blot and immunofluorescence analysis. Cultivated human corneal (HCE) and conjunctival epithelial (HCjE) cell lines as well as primary pterygium cells from surgical removal were used as controls. Additionally, we analyzed expression and calcium regulation through thermo-TRPs by measurements of intracellular free Ca^{2+} with fura-2 and a planar patch-clamp system.
Results: RT-PCR and Western blot analysis revealed expression of pterygium markers vimentin, and matrix metalloproteinase 7 (MMP-7) as well as proliferation marker Ki-67 in spontaneously immortalized pterygium cell line compared to controls. In addition, this study demonstrates expression of thermo-TRPs in immortalized pterygium cell line and in cultivated primary pterygium cells from patients. Immunohistochemistry results verified thermo-TRPs expression in paraffin section from pterygia. Ca^{2+} analysis and analysis of whole-cell currents indicated an increased activity of the heat sensor TRPV1 (capsaicin receptor) and also of the cold sensor TRPM8 (menthol receptor) in pterygium cells compared to cultivated HCE and HCjE cells.
Conclusion: For the first time, we could demonstrate gene-, protein- and functional expression of TRPV1 and TRPM8 and an altered Ca^{2+} regulation by these thermo-TRPs in pterygium cells. This may provide a future target for pharmacological treatment of pterygium.

INTERACTIONS OF MEIBOMIAN AND POLAR LIPID FILMS WITH HYALURONIC ACID.

Georgi As. Georgiev,[1] Norihiko Yokoi,[2] Slavyana Ivanova,[1] Rumen Krastev,[3] Zdravko Lalchev.[1] Department of Biochemistry, Sofia University, Bulgaria,[1] Department of Ophthalmology, Kyoto Prefectural University of Medicine, Japan,[2] Biomaterials group, NMI Naturwissenschaftliches und Medizinisches Institut an der Universität Tübingen, Germany[3]
Purpose: It is widely considered that solutions of high molecular weight non-surface active hydrophilic polymers like hyaluronic acid, HA, are capable to solely replenish the aqueous layer of tears. However as such polymers are known to interact with the lipid headgroups via charge/dipole forces or hydrogen bonds it can be expected that they can also modify the lipid layer properties. Here we studied the interactions of HA (Mw $1x10^{+6}$) with films by human meibum (hMGS) or by polar lipids: Dipalmitoylphosphatidylcholine (DPPC), Sphingomyelin (SM), Dipalmitoylphosphatidylserine (DPPS). Experiments were also performed with the binary non-polar lipids mixture (nPL) ethyl oleate/cholesterol myristate (8/2).
Methods: The lipids were spread at the surface of Langmuir trough and HA (0.01, 0.1, 0.3 %) was applied in the phosphate buffered saline subphase. The surface pressure/area compression isotherms were analyzed with two dimensional virial equation of state. The dilatational rheology of the films was examined by the little deformations method. The morphology of the surface layers was monitored via Brewster angle microscopy.
Results: Segments of HA incorporated in the lipid films headgroup region. This resulted in increase of the apparent area per lipid molecule and increase in the lateral repulsion forces between the lipids. The spreading of the lipid layers at the air/water interface was improved and thicker and more homogeneous films were formed. HA also increased the elasticity of the surface layers. HA effects were particularly strong in the case of human meibum and DPPS films.
Conclusions: HA can modify the surface properties of films by meibomian and polar lipids. The results suggest that non-surface active hydrophilic polymers like HA can be used as an ingredient to lipid replenishment formulations.
(The research was supported with grant by Santen Pharmaceutical, Osaka, Japan.)

EFFECTS OF PROLONGED USE OF OXYBUPROCAINE 0,4% EYE DROPS ON NORMAL MURINE CORNEA.

Chiara Giacomini,[1,2] Giulio Ferrari,[1,2] Fabio Bignami,[1,2] Paolo Rama.[1,2] Cornea and Ocular Surface Unit - Eye Repair Lab,[1] San Raffaele Institute,[2] Milan, Italy
Purpose: The abuse of topical anaesthetics may result in corneal alterations such as corneal epithelial defects, stroma opacity and lead to neurotrophic keratopathy. This study was designed to investigate whether the prolonged use of oxybuprocaine 0,4% eye drops — the most widely used anaesthetic in Italy — may induce pathological changes suggestive of neurotrophic keratopathy in a murine cornea.
Methods. Six CD1 mice were used for this study. Oxybuprocaine 0,4% was instilled in the right eye and saline in the left eye 4 times a day for 11 days. Tear production and blink reflex were evaluated. Immunostaining for ß3-tubulin was performed to examine corneal innervation.
Results: No corneal alterations were detected in normal corneas treated with either oxybuprocaine 0,4% or saline. The anaesthetic had no influence on tear secretion quantified with phenol red thread test (1.8 +/- 0.40 for saline, 2.4 +/- 0.72 for oxybuprocaine, p=0.20) and on blink reflex. Corneal innervation was similar in both groups.
Conclusions: The use of oxybuprocaine 0,4% eye drops 4 times a day for 11 days do not appear to induce any toxic effect on the normal murine

cornea. We did not find any statistically significant difference in the tear production and reflex index in the anaesthetic-treated versus saline-treated group. There were no changes in corneal innervation. It is suggested that 11 days of treatment were not sufficient to induce neurotrophic changes in the cornea.
The authors have no financial disclosures.

OCULAR SURFACE TEMPERATURE CHANGES AFTER CATARACT SURGERY.

Giannaccare G, Versura P, Strobbe E, Cellini M, Bravetti GO, Campos EC. Ophthalmology Unit, DIMES, Alma Mater Studiorum University of Bologna, S. Orsola-Malpighi Teaching Hospital, Bologna – Italy
giuseppe.giannaccare@gmail.com

Purpose: To measure ocular surface temperature changes after cataract surgery and to correlate values with ocular inflammation and surgical parameters.

Methods: This prospective study included 26 patients affected by senile cataract who underwent standard phacoemulsification in one eye. Both eyes were evaluated before (V0) and 7 days after surgery (V1) with respect to subjective symptoms of discomfort (OSDI questionnaire), ocular surface inflammation (graded by conjunctival cytology score and dosage of exudated serum albumin in tears), tear stability (Break Up Time, BUT), epithelial damage (Oxford grading), intraocular anterior chamber inflammation (Kowa 500F laser cell flare meter). Ocular surface temperature was measured with dynamic infrared non-contact thermal imaging (Tomey TG-1000, Nagoya, Japan) in central cornea (CC), nasal conjunctiva (NC) and temporal conjunctiva (TC, in the incision area). Temperature was measured immediately after blinking at eye opening (T0) and after ten second of sustained eye opening (T1), difference between the two values (T) was calculated. T0, T1 and T values recorded in all regions were correlated (Pearson's r) with all the above and intraoperative surgical parameters. Postoperative treatment was equivalent in all patients.

Results: OSDI score, BUT values, epithelial damage, tear exudated albumin were shown in the pathological ranges already at V0, and significantly worsened only in the operated eye. Only in the operated eye a significant ocular surface cooling was found in CC and NC while a temperature raise was observed in TC; T in CC was related to BUT and OSDI, T in TC was related to intraocular flare and tear exudated albumin. No difference was found in flare values at V0 between eyes, while a significant increase was shown in the operated eyes still at day 7 postoperatively. Surgical parameters did not apparently affect neither temperature shifts nor surface parameters.

Conclusions: Data suggest that temperature shifts after cataract surgery may be considered hallmarks for either surface and intraocular inflammation.

LIPOMIMETIC PROPHYLAXIS AGAINST DRY EYE IN ADVERSE ENVIRONMENTS

Akilesh Gokul, Stuti Misra, Jennifer P. Craig
Department of Ophthalmology, New Zealand National Eye Centre, The University of Auckland, New Zealand

Purpose: Discomfort symptoms within adverse environments such as airplanes are common. This study sought to establish if application of a lipomimetic eye drop in borderline dry eye, prior to exposure to an adverse environment, has prophylactic benefit.

Methods: Prior to exposure to a simulated adverse environment (SAE), a lipid-containing eye drop (LD) (Systane® Balance, Alcon) and a non-lipid containing eye drop (NLD)(Systane® Ultra, Alcon) were administered to the right and left eyes of 30 borderline dry eye participants (aged 21-60 years), in a prospective, double-masked, randomised manner. Lipid layer grade (LLG), non-invasive tear break-up time (NIBUT) and tear meniscus height (TMH) were measured at baseline, following instillation of eye drops, and post SAE exposure.

Results: Exposure to a fan at 1m for 2.5 minutes provided a reliable and repeatable SAE model for inducing tear film instability and discomfort symptoms in individuals with borderline dry eye. LD instillation resulted in increased NIBUT and LLG ($p<0.001$) that did not fall below baseline levels following SAE exposure ($p=0.698$ and $p=0.153$, respectively). The NLD, however, although capable of increasing NIBUT ($p<0.001$) and preventing its decline below baseline ($p=0.100$), did not increase LLG following instillation ($p=0.134$) and allowed LLG to drop significantly below baseline levels, post-SAE exposure ($p=0.012$). TMH following SAE exposure remained above baseline levels with the NLD ($p<0.001$) but not the LD ($p=0.305$).

Conclusions: Results suggest that the increased NIBUT observed with LD was related to improved LLG, whereas that with NLD reflected reflex tearing, and was therefore likely a more transient phenomenon. Application of a lipid-containing dry eye drop 15 minutes prior to exposure to an adverse environment thus appears to be a viable strategy to reduce the risk of evaporative dry eye by preventing disruption of the lipid layer.

[Akilesh Gokul was supported by a summer student scholarship from the Faculty of Medical and Health Sciences at the University of Auckland]

USE OF NOVEL IL-1 RECEPTOR INHIBITOR (EBI-005) IN THE TREATMENT OF PATIENTS WITH MODERATE TO SEVERE DRY EYE DISEASE.

Michael Goldstein[1,2], Jennifer Agahigian[1], Gregory Zarbis-Papastoitsis[1], Kathryn Golden[1], Joseph Kovalchin[1], Cameron Wheeler[1], Siddhartha Chowdury[1], Abbie Celniker[1], Eric Furfine[1]
[1]Eleven Biotherapeutics, Cambridge, MA, United States,
[2]Ophthalmology, New England Eye Center/Tufts Medical Center, Boston, MA, United States.

Purpose: To describe the results of a recently completed multi-center, double-masked, environmental trial in patients with moderate to severe dry eye disease (DED) using a novel, topically applied, IL-1 receptor inhibitor.

Methods: In a double-masked, placebo-controlled study, 74 subjects were randomized to receive vehicle, EBI-005 5 mg/mL or 20 mg/mL. The study was powered to show a statistical trend (P=0.2). Subjects were dosed 3x/day for six weeks. Safety assessments included: adverse event reporting, complete ophthalmic examination, corneal esthesiometry, corneal pachymetry, ocular surface microbiology, and serum laboratory testing. Assessments of biological activity included: corneal fluorescein staining (CFS), OSDI, SANDE, patient individual symptom assessments, global assessments (patients and investigators) and rescue tear use. Exploratory biomarker assessments included impression cytology and tear collection.

Results: Topical EBI-005 was safe and well tolerated. There were no patient drop-outs and no serious ocular or non-ocular adverse events. EBI-005 significantly improved signs and symptoms of DED compared to baseline at week six by up to 30% ($p<0.001$) and 36% ($p<0.001$) respectively. In addition, there was a statistical trend in improvement of signs (CFS) and symptoms (OSDI, individual patient symptom assessments, investigator global assessments) in the EBI-005 treated group compared to the vehicle control. Subjects who received vehicle control used significantly more rescue tears than those receiving EBI-005 ($p=0.032$).

Conclusions : Topical EBI-005 treatment is a promising therapy for patients with moderate to severe dry eye disease. These results further validate the importance of IL-1 blockade in DED and support continued development of the drug in a planned 12-week study designed to further characterize the safety and efficacy of EBI-005 in patients with DED.

Commercial Relationships: Dr. Goldstein is a consultant to Eleven Biotherapeutics. Mr. Chowdury is a contractor to Eleven Biotherapeutics. All other authors are employees of Eleven Biotherapeutics.

SURGERY-INDUCED DRY EYE DISEASE (I.E. REFRACTIVE, CATARACT, PK, PLASTIC)

José AP Gomes, MD PhD. Cornea/External Disease Service and Advanced Ocular Surface Center (CASO), Department of Ophthalmology, Federal University of São Paulo (UNFESP/EPM), Brazil.

Surgery-induced dry eye disease has been described after a variety of ocular procedures as refractive surgery (LASIK and PRK), cataract surgery (EECC and phacoemulsification), corneal transplants, oculoplastic surgery, and glaucoma surgery.

Potential causes include pre-existing ocular surface disease that is exacerbated by surgery, toxicity secondary to postoperative medications and their preservatives, and surgical damage of the corneal afferent nerves leading to postoperative hypoesthesia and disruption of the ocular-surface-lacrimal gland functional unit. Hyperosmolarity, inflammation and epithelial cell apoptosis may play an important role in the physiopathology.

Limited epidemiologic data are available, but its prevalence seems to be much more common than what it was believed ranging from .25 to 48% in LASIK patients. This condition may compromise wound healing and has been associated with an increased risk of postoperative complications. Current treatment includes the use of topical lubricants and immunomodulators for at least a few weeks after the surgery.

A critical review of new physiopathological findings and the most important directions for future research, including the value of pretreatment strategies to reduce the incidence and duration of surgery–induced ocular surface disease, will be presented.

Supported in part by Fapesp.

BIOMIMETIC AND COMPOSITE CORNEAS FOR HIGH RISK TRANSPLANTATION

May Griffith[1], Chyan-Jang Lee[1], Ranjithkumar Ravichandran[1], Vijayalakshmi Rajendran[2], John Forrester[2], Lucia Kuffova[2]. [1]Integrative Regenerative Medicine Centre, Linköping University, Linköping, Sweden; [2]Section of Immunology and Infection (Ocular Immunology), Division of Applied Medicine, School of Medicine and Dentistry, University of Aberdeen, Aberdeen, Scotland

Purpose: We have shown that corneal implants made from crosslinked biomimetic human recombinant collagen are immune compatible and can act as regeneration templates when implanted into patients as substitutes of donor allografts in keratoconus and central scarring patients. However, newer, stronger implants and nano-composites are needed for high risk transplantation such as Herpes Simplex Keratitis (HSK), where the immune privilege seen in normal corneas is disrupted.

Methods: We developed biomaterials based on interpenetrating networks (IPNs) of recombinant human collagen and a synthetic phosporylcholine. These have now been tested in corneas with more severe pathology such as chemical burn or HSK. We have also explored the option of combination of virus-resistant stem cells as composite grafts.

Results: We showed that the new IPNs are significantly stronger and more enzyme resistant but retain the optical clarity and biocompatibility of the clinically tested older implants. These IPNs are better tolerated in HSK mice than allografts. We are able to release drugs such as Acyclovir both *in vitro* and in HSK mice. The Acyclovir can be substituted with innate anti-viral peptides, e.g. LL37. Gene transfer of LL37 DNA into corneal cells for subsequent expression and secretion was able to block HSV-1 activity *in vitro*. Cells and implants can be combined as composite grafts.

Conclusions: We have shown that biomimetic corneas may be possible afternatives to donor allografts for high risk transplantation cases such as HSK in mice. In the future, with more optimization, they may be suitable for human use.

(The authors thank the EU Nanomedicine ERA-net programme for funding I-CARE; Swedish Research Council and LInköping University and the County Council of Östergötland for funding our research.)

INTRINSIC LIPID CIRCUITS: KEY REGULATORS OF OCULAR SURFACE INNATE IMMUNE AND REPARATIVE RESPONSES

Karsten Gronert. Vision Science Program, School of Optometry, University of California Berkeley, CA

Inflammation, even in the ocular surface, by design is a frequent, self-resolving and healthy response. The caveat is that healthy inflammation depends on the highly controlled and precarious activation of primary effector cells such as neutrophils (PMN), which drive macrophage and lymphocyte functional programs. Distinct classes of lipoxygenase- and cyclooxygenase-derived lipid mediators (LM) are the earliest response signals to injury, stress or infection and key regulators of innate and adaptive immune responses; more importantly the enzymes and specific LM receptors are highly expressed in the eye. Contrary to existing paradigms PMN are present in healthy lacrimal glands, the limbus and nocturnal tears and PMN dramatically increase in the lacrimal gland and cornea during ocular surface stress, infection and injury. To ensure controlled PMN activation and resolution of routine innate immune responses the ocular surface is endowed with conserved protective mechanisms. We have discovered that the ocular surface highly expresses intrinsic lipid circuits that control innate immune response, leukocyte function, wound healing and angiogenesis. These protective lipid circuits are amplified in the cornea and retina by dietary omega-3 PUFA, providing a much-needed molecular mechanism for the beneficial actions of fish oils. Even though sex-specific prevalence of ocular surface immune diseases such as dry eye are striking, the mechanism for these differences and potential sex-specific differences in the pathogenesis are not well understood. Our ongoing work has uncovered that estrogen receptors regulate protective LM circuits in the ocular surface as well as PMN and macrophage function. This correlates with sex-specific and estrogen-driven differences in corneal inflammatory/reparative responses and ocular surface innate immune responses triggered by desiccating stress. Hence, estrogen's selective regulation of intrinsic protective lipid circuits may provide novel insights into the pathogenesis of sex-specific ocular surface diseases. The lecture will present our current understanding of the regulation, mechanism of action and therapeutic potential of intrinsic lipid circuits in the ocular surface.

BEYOND NON INVASIVE BREAK UP TIME: A NOVEL METHOD TO QUANTIFY CONTACT LENS ON EYE WETTING KINETICS OVER THE FULL INTERBLINK PERIOD

Michel Guillon[1,2], Cécile Maissa[1,2], [1]OTG Research & Consultancy, London, UK; [2] School of Life and Health Science, Aston University, Birmingham, UK.

Purpose: The measurement of the pre-contact lens non-invasive break up time is the clinical marker of contact lens on eye wettability. The technique measures the length of time with full tear coverage but does not give any indication of the severity the break at the blink. The latter highly influences friction between the contact lens and the upper lid during the blink, a major contributor to contact lens dry eye. The objective was to develop a new clinical methodology that quantifies the tear film kinetics during the whole interblink period.

Methods: The pre-contact lens tear film was recorded during the full interblink period using the Tearscope and a digital video recording system. The tear film kinetics was characterised by four parameters: Non-Invasive Break Up Time (NIBUT), Protected Surface Area, Maximum Surface Exposed Area, Speed of surface dehydration.

Results: The NIBUT is not an indicator of the status of the contact lens surface at the time of the blink: a short NIBUT can be associated with minimal exposed area at the time of the blink, whereas it is not infrequent that have a long NIBUT and large surface exposure at the time of the blink. The natural blink occurs most commonly when the exposed area is less than 11%, but in a significant minority of cases the

exposed area is between 60% and 100% before a blink occurs; The presence of large exposed areas prior to a blink is a contact lens induced phenomenon very rarely observed for the pre-ocular tear film.
Conclusion: The novel technique to quantify the full interblink tear film kinetics suggests that the measurement of the break up time is insufficient to characterise on eye contact lens wettability and that a more critical parameter is the amount of surface exposure at the blink.

AUTOMATIC QUANTIFICATION OF OPTICAL COHERENCE TOMOGRAPHY IMAGES FOR EVALUATION OF THE OCULAR SURFACE SHAPE BEYOND THE LIMBUS.

Tina Hakimi,[1,2] Arthur Ho,[1,2,3], Fabian Conrad[1,2], Brien Holden,[1,2]
[1.] Brien Holden Vision Institute; [2.] School of Optometry and Vision Science University of New South Wales, Sydney, NSW, Australia; [3.] University of Miami, Millers School of Medicine, Miami, Florida, USA

Purpose: Contact lens fitting that incorporates the ocular surface shape beyond the limbus may provide positive benefits to lens fit[1] and comfort. Optical coherence tomography (OCT) images could provide a practical and powerful means for the incorporation of ocular shape data in contact lens fitting if the data can be readily and easily obtained. We demonstrate a novel technique to automatically extract quantitative ocular shape data from OCT images.
Methods: Extended-width (18 mm) anterior segment images were acquired using an OCT (Bioptigen Envisu S4300) and submitted to an algorithm comprising three major steps: 1) establishment of transverse linearity, 2) image processing (equalization, median, Gaussian and Canny filters), 3) fitting of surface shape to high-order polynomial. Software implementing the algorithm was developed in MATLAB (with Image Processing Toolbox). Technique was evaluated against a model bi-sphere measured using a profilometer.
Results Quantification of technique performance has been validated on OCT images of subjects with a range of corneal topographic parameter values including limbal diameter and corneal curvature. It was determined that 10^{th}-order polynomials provide an adequate fit for the ocular surface data. Typical standard error of estimate of polynomial fitting is around 12 m. Parameters relevant to contact lens design and fitting were calculated from the fitted polynomials including: apical/local curvatures, corneal sagittal height, limbal diameter and corneoscleral junction angle.
Conclusions: The present algorithm provides a fully-automatic method that quantifies the anterior ocular surface to produce clinically pertinent fitting parameters.
[1] Hall *et al* (2011) IOVS, 52: 6801-06.

IS THERE A RELATIONSHIP BETWEEN SERUM LEVELS OF 3 -DIOL-G AND DHEA-S AND DRY EYE SYMPTOMS IN POST-MENOPAUSAL WOMEN?

Ulrike Hampel[1, 2], Blanka Golebiowski[1], Noor Badarudin[1], Fiona Stapleton[1]. [1]School of Optometry and Vision Science, University of New South Wales, Sydney, NSW, Australia. [2]Department of Anatomy 2, Friedrich Alexander University Erlangen Nürnberg, Erlangen, Germany.

Purpose: The incidence of dry eye is increased post-menopause and it is thought that androgen levels may play a role. This study investigated the relationship between serum levels of 3 -Androstanediol-Glucuronide (3 -diol-G) and Dehydroepiandrosterone sulfate (DHEA-S) and dry eye symptoms and signs in post-menopausal women.
Methods: The study involved 42 post-menopausal women previously diagnosed with dry eye (mean age 65±5 years, range 56-84). Subjects completed the Ocular Comfort Index and Ocular Surface Disease Index . Corneal and conjunctival sensitivity, staining, non-invasive tear break-up time, Meibomian gland assessment, tear osmolarity (TearLab corporation) and tear volume (Phenol Red Thread and Schirmer tests) were assessed. Venous blood was collected and serum concentrations of 3 -diol-G and DHEA-S were determined using specific Enzyme-linked immunosorbent assay. Associations were examined using Pearson's or Spearman's correlations as appropriate.
Results: Mean DHEA-S concentration was 0.78±0.54µg/ml; 3 -diol-G concentration was measured to be 2.10±2.85ng/ml. Higher DHEA-S levels correlated with higher 3 -diol-G serum levels (=0.45, p=0.004). No evidence of a relationship between 3 -diol-G levels and ocular symptoms or tear parameters was apparent (p>0.05). Likewise, no association was found between the investigated parameters and DHEA-S levels (p>0.05). There was no association between year post-menopause and any of the investigated variables (p>0.05).
Conclusions: Circulating levels of the androgen precursor DHEA-S and the androgen metabolite 3 -diol-G appear to not predict symptoms of dry eye in post-menopausal females. However, measurements of androgen levels in tears or in ocular surface tissue might be useful parameters for future evaluation.
Funding support: Science Faculty Interdisciplinary Grants Program, University of New South Wales

CORNEAL NERVES MAY MEDIATE CORNEAL DENDRITIC CELL TRAFFICKING–AN INTRAVITAL MULTIPHOTON MICROSCOPY STUDY

Pedram Hamrah[1,2], Takefumi Yamaguchi[1,2], Kai Hu[1,2], Deshea Harris[2], [1]Cornea Service, and Schepens Eye Research Institute, Massachusetts Eye & Ear Infirmary, Department of Ophthalmology and [2]Harvard Medical School, Boston, MA

Purpose: The role of dendritic antigen presenting cells in corneal transplantation has been firmly established. While their functional characterization has thus far mainly relied on the analysis of ex vivo studies, there remains a clear need to investigate their behavior in the context of intact tissues in real-time. Here we evaluate *in vivo* kinetics of dendritic cell (DC) in the cornea and submandibular draining lymph nodes (dLN) after corneal transplantation and trigeminal axotomy as a control for nerve damage model
Methods: Corneal buttons from BALB/c (allogeneic) and C57BL/6 (syngeneic) mice were orthotopically grafted onto CD11c-GFP-DTR (C57BL/6 background) recipients. CD11c-GFP DCs were imaged in the graft, peripheral recipient cornea and dLN under anesthesia using multiphoton microscopy 2 weeks after corneal transplantation and trigeminal axotomy. The density, kinetics and speed of DCs were calculated and 3D movies rendered using high performance 4D imaging software (IMARIS).
Results: The density of CD11c-GFP$^+$ cells in grafts and recipient corneas significantly increased after corneal transplantation and trigeminal axotomy compared to controls ($p< 0.01$). CD11c-GFP$^+$ cells velocity in grafts significantly increased from 0.58 mm/min (normal, central cornea) and 1.26 (axotomy) to 1.30 (syngeneic), and 1.80 (allogeneic, $P<0.001$). Velocity of CD11c-GFP$^+$ cells in peripheral recipient cornea significantly increased from 0.94 mm/min (normal, peripheral cornea) and 1.22 (axotomy) to 2.31 (syngeneic), and 2.36 (allogeneic, $P<0.001$). Velocity of CD11c-GFP$^+$ cells in dLN significantly increased from 2.01mm/min to 2.92 after allogeneic corneal transplantation ($P<0.001$). The meandering index, an index for directionality, significantly increased in corneal allografts ($p<0.01$) compared to isografts or axotomy.
Conclusions: These studies are the first to demonstrate long-term migratory kinetics of corneal DCs after corneal transplantation in both the cornea and submandibular dLNs through high-resolution intravital multi-photon microscopy and demonstrate a potential role of corneal nerves in corneal cell trafficking.
Grant Support: NIH K08-EY020575 (PH), Research to Prevent Blindness Career Development Award (PH),

OPHTHALMIC FORMULATION OF LIPOSOMES AND AN OMEGA-3 FATTY ACID FOR THE TREATMENT OF DRY EYE DISEASE.

Rocío Herrero-Vanrell[1], Marta Vicario-de-la-Torre[1], Omar Avelino-Cruz[1], Maria Caballo-Gonzalez[1], Beatriz de-las-Heras[2], Jose M. Benitez-del-Castillo[3], Irene T. Molina-Martínez.[1] Department of Pharmaceutical Technology, [1]Department of Pharmacology, [2] Faculty of Pharmacy, Complutense University, [3] Department of Ophthalmology, Hospital Clínico San Carlos, Madrid, Spain.

Purpose: Omega-3 fatty acids have shown beneficial properties for the underlying inflammation in dry eye therapy. Liposomes made of phospholipids can resemble the lipid layer of the preocular tear film. Fatty acids can be included in liposomes. The objective of this work was the design and evaluation of a new artificial tear based on liposomes and alfa-linolenic acid (omega-3 fatty acid).

Methods: Liposomes (phosphatidylcholine, cholesterol and vitamin E) were prepared according to the thin-layer hydration method. Alfa-linolenic acid (ALA) was incorporated in the lipid bilayer in a ratio 10:0.6 (Phosphatidylcholine:ALA). The lipid vesicles were suspended in a hypotonic solution of hyaluronic acid and trehalose.

Once prepared, the unpreserved ophthalmic formulation was characterized by particle size of liposomes (dynamic light scattering), pH and osmolarity. In-vitro tolerance was studied in Human Corneal Inmortalized- Limbal Epithelial Cells; HCLE (Donated by Ilene Gypson, Schepens Eye Research Institute, Harvard Medical School, Boston, MA), at short (15 min) and long term (1h and 4h) exposure times.

Results: The liposomal formulation showed a neutral pH (7.3±0.6) with an osmolarity value of 207.4 ±1.3 mOsm/L. The particle size of liposomes was 115.1 ±1.3 nm. Cellular viability resulted higher than 80% after short and long term exposure times.

Conclusions: The preservative free ophthalmic formulation composed by liposomes and alfa-linolenic acid resulted suitable for topical administration in terms of particle size, pH, osmolarity and in-vitro tolerance. This research was supported by grants FIS PI10/00645, FIS PI10/00993, RETICS RD12/0034 and Complutense Research Group 920415.

DAMAGE IN CORNEA AND LACRIMAL GLANDS OF SMOKING RAT.

Akihiro Higuchi,[1] Murat Dogru,[1] Masanori Kitamura,[2] Tetsuya Kawakita,[1] Yoko Ogawa,[1] Kazuo Tsubota.[1] Department of Ophthalmology, Keio University School of Medicine, Tokyo,[1] Department of Molecular Signaling, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi[2], Japan

Purpose: Smoking is one of the serious public health problems around the world, which is a risk factor in many diseases, such as lung cancer, COPD, and many eye diseases. Cytochrome P450s (CYPs) are xenobiotic metabolizing enzymes, and are distributed in corneas, protecting ocular surface against chemical compounds from environment. While CYPs are principal detoxification enzymes, CYP1A1 and CYP2A6 are known to participate in inducing lung cancer by smoking. We study participation of CYPs in corneal dysfunctions caused by smoking using smoker rat model.

Methods: Male 6-week-old SD rats were exposed to main cigarette smoke (MCS). The rats were placed in an experimental smoking chamber. Three hundred mL of MCS was injected into the smoking chamber every 30 minutes for a total of 6 times/3 hours during the exposure period. The fluorescein score and tear volume for each rat were measured before MCS treatment and after 3 and 5 days of treatment. After 5 days of treatment, the corneas and lacrimal glands were collected to use RNA isolation and immunohistochemical analysis.

Results: MCS treatment caused corneal damage and lacrimal glands dysfunction. Immunohistochemical analysis revealed that CYP1A1 expression was induced in corneal epitherium and duct in lacrimal glands accompanied with increase of production in reactive oxygen species (ROS). Increase of 8-OHdG, which is a marker of oxidative DNA damage, was only detected in the expression area of CYP1A1, while HEL adduct, which is an initial marker for oxidative damage of phospholipids, did not increase. MCS treatment damages corneas and lacrimal glands probably through DNA oxidation by ROS produced by CYP1A1.

Conclusions: Although influence of other components in MCS remains unclear, CYPs, especially CYP1A1, probably participate in corneal damage and lacrimal glands dysfunction by smoking.[This work was supported in part by a grant from the Smoking Research Foundation.]

PREDICTORS OF TEAR FILM-ASSOCIATED OPTICAL DEGRADATION

Holly Hindman, Michael Chen, Christine Callan, Ranjini Kottaiyan, Gheorghe Salahura, Geunyoung Yoon, James Zavislan, & James Aquavella. University of Rochester, Rochester, NY.

Purpose: to determine tear-film factors associated with optical degradation.

Methods: Data were collected from 248 unique encounters from 36 eyes. Eyes were classified on the basis of clinical testing as normal, Sjögren's Sydrome (SS), aqueous deficient dry eye (ADDE), and meibomian gland dysfunction (MGD). A quantitative lipid tear scope and infrared ocular surface camera were used for non-invasive tear imaging, and wavefront data were captured with a Hartmann-Shack wavefront sensor. Videographic images were recorded and dynamic changes in the tear film over a 5 second post-blink interval obtained. Lipid thickness and uniformity were quantified over the central one third of the cornea (excluding pupil), and ocular surface temperature (OST) was measured over the central 9 mm. Tear film parameters studied included mean lipid thickness (MLT), difference in lipid thickness (DLT), mean lipid heterogeneity (MLH), difference in lipid heterogeneity (DLH), and difference in OST (DOST). The RMS wavefront error was measured over a 3 mm pupil and the baseline RMS wavefront error was subtracted from all subsequent RMS values, and the mean difference (MDRMS) was used as a measure of dynamic aberration changes induced by tear film instability. Multivariate regression analysis was performed to assess predictors associated with MDRMS.

Results: Of the 8 proposed factors in the conceptual framework for predicting MDRMS, clinical classification was a significant predictor ($p<0.01$, ADDE 42.7%, MGD 45.5%, and SS 92.9% increase above normals) of MDRMS while remaining variables in the model were held constant. Overall, the model accounted for 18.5% of the variance in the outcome. Addition of tear film parameters (MLT, DLT, MLH, DLH, and DOST) to the clinical classification, age, and race, improved the performance of the model by 27%.

Conclusions: Inclusion of objectively measured tear film parameters improved the predictability of our model. A larger sample size may demonstrate these parameters to also be independent predictors of optical degradation.

Disclosures: None. Support: HBH K23EY019353, RPB

INFLUENCES OF RADIOTHERAPY ON MEIBOMIAN GLANDS AND TEAR FILM.

Takahiro Hiraoka, Yasuaki Ito, Tetsuro Oshika. Department of Ophthalmology, Faculty of Medicine, University of Tsukuba; Ibaraki, Japan

Purpose: To prospectively investigate morphologic changes in meibomian glands in patients undergoing radiation treatment and to assess the relationship between the meibomian glands' changes and tear film function.

Methods: Nine eyes of 7 patients (4 men, 3 women) (63.1 ± 12.7 years, mean ± standard deviation) who were diagnosed with ocular adnexal lymphoma and underwent unilateral or bilateral radiotherapy were included. The following tests were performed before and 1 year

after radiotherapy; grading of meibomian gland morphologic changes (meiboscore) assessed with a noncontact meibography, measurement of tear film breakup time (BUT), tear production by the Schirmer I test, and tear film stability with the tear stability analysis system (TSAS). Relationships among these parameters were also assessed.
Results: Mean meiboscore at baseline and 1 year after radiotherapy was 2.7 ± 0.8 and 3.9 ± 1.2, respectively. Mean BUT was 4.8 ± 2.5 sec and 3.1 ± 1.7 sec, respectively. Mean value of Schirmer test was 14.1 ± 10.8 mm and 15.6 ± 11.4 mm, respectively. Mean value of TSAS was 3.1 ± 2.1 sec and 1.2 ± 1.4 sec, respectively. There were significant differences in meiboscore, BUT, and TSAS results between the pre- and post-treatment values ($P<0.05$), but not in Schirmer test. No significant correlations were observed between meiboscore and other parameters.
Conclusion. Radiotherapy caused morphologic changes in meibomian glands such as constriction, distortion, and shortening. It also induced instability of tear film. Although no apparent relationship between the meibomian glands' changes and tear film function was observed in the present study, there is a possibility that functional reduction in meibomian glands after radiotherapy are associated with the development and exacerbation of dry eye disease. Further studies should be conducted to clarify the exact mechanism underlying the development of dry eye disease after radiotherapy.
[Financial Disclosure, All authors have no commercial relationships and grant supports regarding this study.]

A NEURAL BASIS OF COLD-INDUCED DRY EYE PAIN: SENSITIZATIONS OF DRY-SENSITIVE CORNEAL AFFERENTS PRODUCED BY HYPEROSMOLAR TEARS VS. MENTHOL.

Harumitsu Hirata, Department of Neurology, Thomas Jefferson University, Philadelphia, PA, USA
Purpose: Dry eye pain or discomfort reveals as multiple symptoms ranging from dryness to burning. The purpose of the present study was to determine if the putative corneal nociceptors that are excited by corneal cooling of >4°C could carry a sensation often described by dry eye patients as sensitivity to a cold or dry environment. However, in a normal environment, cooling of this magnitude is seldom encountered. At the same time, dry eye disease is characterized by a ubiquitous presence of hyperosmolar tears. Thus, we hypothesized that these nociceptors would be excited by much milder cooling after sensitization by hyperosmolar tears, giving rise to painful conditions.
Methods: Single corneal neurons excited by drying of the cornea and cold stimuli (4°C to greater than 12°C changes) were recorded extracellularly *in vivo* from rat trigeminal ganglia. Response of the neurons to corneal cooling was monitored in the presence of the hyperosmolar tears prepared with mannitol and NaCl applied to the eye as well as during the stimulation by a TRPM8 agonist, menthol, and potassium channel blocker, 4-AP.
Results: Both hyperosmolar tears, menthol, and 4-AP all produced an increase in responses to corneal cooling such that the neurons that were normally excited by cold stimuli of greater than 4°C changes (cold nociceptors) were now responsive to a mere 1-2°C cooling after sensitization. The increase in (or more precisely the appearance of) activity in response to 2°C cooling was particularly striking after hyperosmolar tears-induced sensitization. For the neurons that displayed no temperature sensitivity down to 10°C (cold-insensitive), the sensitization to cooling was still evident, suggesting the involvement of non-TRPM8 mechanisms.
Conclusions: The excitability increases in cold-induced responses of dry-sensitive corneal afferents after hyperosmolar tears may explain the pain and discomfort of dry eye patients in a mild cooling environment. The mechanisms underlying these sensory changes may be related to the excitability changes in TRPM8 and/or potassium channels. Supported by grant EY-020667

FUNCTIONAL MOUSE LACRIMAL GLAND REGENRATION IN VIVO

Masatoshi Hirayama[1], Miho Ogawa[2], Masamitsu Oshima[3], Shigeto Shimmura[1], Tetsuya Kawakita[1], Takashi Tsuji[2,3] & Kazuo Tsubota[1]
Purpose: Tear secreted from lacrimal gland plays a multifaceted role to maintain a homeostatic microenvironment for ocular surface. Dry eye is an important public health problem, and it is expected to develop a novel therapeutic treatment for the restoration of the lacrimal gland functions. Here, we report a successful fully functioning lacrimal gland replacement achieved though the transplantation of bioengineered lacrimal gland germ in adult mouse.
Methods: The care and handling of animals were performed in accordance with NIH guidelines. Protocols were approved by the Animal Care and Use Committee. We regenerated the bioengineered lacrimal gland germ and harderian gland germ by our developed organ germ method, and transplantd them to adult lacrimal gland defect model mouse. The development of bioengineered glands, histological structure including expression of aquapolin-5 (AQP5), lactoferrin and lipids, tear secretion ability and ocular surface protection effect were analyzed.
Results: The bioengineered lacrimal gland germ and harderian gland germ successfully achieved correct gland structure including acini, myoepithelial cells and nerve, followed by successful duct integration. AThe GFP-labelled bioengineered lacrimal glands were regenerated and could be observed in the engrafted area of the adult mice.QP5 and lactoferrin in the bioengineered lacrimal gland, and lipids in the bioengineered harderian gland were histologically detected. The bioengineered lacrimal gland received appropriate neural control and had a secretion ability equivalent to that of natural lacrimal gland. Tear from the bioengineered glands had appropriate tear components such as lactoferrin. The ocular surface status including fluorescein staining and corneal epithelial thickness was significantly improved in the bioengineered lacrimal gland transplantation mouse compared with that in the lacrimal gland defect model mouse.
Conclusions: We demonstrated that bioengineered lacrimal gland and harderian gland, which had the correct gland structure, could produce the tear followed by successful duct integration and restore the lacrimal gland physiological functions in response to nervous stimulations, and could protect the ocular surface. This study thus represents a substantial advance and demonstrates the possibility of novel therapeutic approach for dry eye as a future organ replacement regenerative therapy.

EVALUATION OF AGE-RELATED AND GENDER-RELATED CHANGES IN NON-INVASIVE TEAR BREAK-UP TIME WITH A NEWLY DEVELOPED KERATOGRAPH.

Jiaxu Hong,[1] Zuguo Liu,[2] Xinghuai Sun,[1] Jianjiang Xu.[1]
1 Department of Ophthalmology and Visual Science , Eye, Ear, Nose, and Throat Hospital, School of Shanghai Medicine, Fudan University, 83 Fenyang Road, Shanghai 200031, China; 2 Eye Institute of Xiamen University, Fujian 361005, China
Purpose: To investigate age-related and gender-related changes non-invasive tear break-up time with a Newly Developed Keratograph.
Methods: Forty normal volunteers [male:female, 13:27; mean age (range), 33.3 ± 15.9 y (17-74) y] were recruited from the community. The clinical and demographic data, including age, gender, fluorescein tear film break-up time (FBUT), and Schirmer I test values were collected from the subjects. Non-invasive tear film break-up time (NI-BUT) was measured using a new method based on a corneal topographer equipped with modified scan software. The correlations between the NI-BUT and age and gender were determined.
Results: In total, a significant difference between the NI-BUT and the FBUT was found (4.4 ± 2.2 seconds versus 7.2 ± 3.6 seconds; $P < 0.001$). No statistically significant difference in the NI-BUT was observed between the male and female subjects (4.9 ± 2.6 seconds versus 4.2 ± 2.3 seconds; $P = 0.363$). In addtion, no significant

correlation was detected between the NI-BUT and age.
Conclusions: The NI-BUT values found in this study are much less than previous reports. This phenomenon should be noted in the clinical assessment of tear film stability. The results indicate that tear physiology of the local population in China may not be the same as in Western populations.
This work was supported by grants from National Natural Science Foundation of China (81170817, 81200658.

OCULAR SAFETY OF CATIONIC EMULSION OF CYCLOSPORINE IN IN VITRO AND ANIMAL MODELS.

Liang Hong[1,2], Christophe Baudouin[1,2], Philippe Daull[3], Jean-Sébastien Garrigue[3], Françoise Brignole-Baudouin[2,4]. [1]Quinze-Vingts National OphthalmologyHospital, Paris, France; [2]Vision Institute, Paris, France; [3]Santen France, Novagali Innovation Center, Evry, France;[4]Paris Descartes University, Faculty of Pharmaceutical and Biological Sciences, ToxicologyDept., Paris, France.
Purpose: The present study goals were to assess the safety profile of a cationic emulsion (CEm) of cyclosporine (CsA) both *in vitro* with a dynamic corneal wound healing assay using human corneal epithelial (HCE) cells, and *in vivo* with a rabbit acutetoxicity model of repeated instillations.
Methods: The CEm (0.05%CsA) was compared *in vitro* and *in vivo* to a 0.05%CsA anionic emulsion (CsA-AEm, Restasis®), and a 0.05%CsA-Oil solution (hospital preparation), with phosphate buffered saline (PBS) and a 0.02% benzalkonium chloride (BAK) solution as positive and toxic controls, respectively. *In vitro*, a confluent HCE cell monolayerscraped in a standardized manner was exposed 30 min to 1/10 dilutions of the different formulations. Cell migration, and proliferation were evaluatedat days 1, 2, and 3 to assessthe recovery rate. *In vivo*, 15 instillationsof the different eye drops were applied at 5-min intervals onto rabbit eyes. Clinical observations (Draize test) and *in vivo* confocal microscopy (IVCM) were used for detailedexamination of corneal epithelium, stroma, limbus, and conjunctiva-associated lymphoid tissue (CALT) structures.
Results: *In vitro*, the three CsAformulationspresented a normal healing rate with a behavior similar to PBS. In the rabbit, 0.02%BAK showed important toxicity signs: redness, chemosis with damaged corneal epithelium, and inflammatory cell infiltrations. CsA-AEm and CsA-Oil induced moderate infiltrations of inflammatory cells around the CALT.CsA-CEmpresented the lowest signs of toxicity with patterns similar to PBS.
Conclusions: While CsA-AEm, CsA-CEm,and CsA-Oil are generally well tolerated *in vitro* and *in vivo*, only CsA-CEm was able to maintain both HCE cells'normal healing rate *in vitro* and low levels of inflammation *in vivo*. Hence, the cationic emulsion appears to be a very good vehicle for the ocular delivery of CsA.

DEMODEX INFESTATION IN PATIENTS WITH OCULAR DISCOMFORT

Jutta Horwath-Winter,[1] Dieter Franz Rabensteiner,[1] Gerold Schwantzer,[2] Manuela Fischl,[1]
Christa Wachswender,[1] Haleh Aminfar,[1] Ingrid Boldin.[1] Department of Ophthalmology,[1] Institute for Medical Informatics, Statistics and Documentation,[2] Medical University Graz, Austria
Purpose: The presence of Demodex species can be associated with blepharitis. To date, the pathogenic potential of these mites in Meibomian Gland Dysfunction (MGD) remains unclear. The purpose of this study was to determine the prevalence of Demodex in eyelashes of patients with ocular discomfort and to evaluate associated changes of the lid margins and the meibomian glands.
Methods: Consecutive patients with ocular discomfort (n=229) from the dry eye unit of the Ophthalmological Department, Medical University Graz, Austria, were investigated for the presence of Demodex mites on sampled eyelashes.
Lid margins were evaluated according to scales, vascularisation, Marx line, expressibility and quality of meibomian gland secretion. Non-contact IR-Meibography was performed and loss of meibomian glands was scored according to the meiboscore of Arita and colleagues.
Results: Demodex species were found in 40.2% of patients with ocular discomfort. Compared to the non-infested, patients with Demodex mites had significantly more scales formed as sleeves, a higher Marx line score and vascularisation of the lid margins and a lower quality of the meibomian gland secretion. No significant association was observed with the expressibility of meibomian glands and the meibography drop out score.
Conclusions: Demodex mites are associated with changes of the anterior and posterior lid margin in patients with ocular discomfort and may therefore play a pathogenic role in blepharitis and MGD.

THERAPEUTIC EFFECTS OF REBAMIPIDE EYE DROPS FOR SHORT-BUT TYPE DRY EYE.

Hisashi Hosotani, Hiroyuki Shirasaki. Department of Ophthalmology, Shakaihoken Kobe Central Hospital, Kobe, Japan.
Purpose: Dry eye patients with short tear-film breakup time (BUT) are considered to lack secreted mucin in the tear and membrane-bounded mucin on the surface of corneal epithelial cells. Rebamipide eye drops are designed to increase the number of conjunctival goblet cells and therefore increase the amount of both type of mucin. In this study, the therapeutic effect of rebamipide eye drops for short-BUT type dry eye patients was prospectively evaluated.
Methods: The subjects are 12 patients (1 male, 11 females) diagnosed as short-BUT type dry eye. The mean age is 67.2 year old. The study was started with rebamipide eye drops 4 times a day in both eyes. Schirmer test 1, BUT and corneal fluorescein stain score were measured at the beginning and 2, 4 weeks after. Subjective symptoms (11 items) of the patients and the degree of bitterness after dropping that is one of the characteristics of the rebamipide eye drops were evaluated using the visual analogue scale before the study and 2, 4 weeks after the start. Artificial tear was appropriately used between the study.
Results: At the end of the study the value of Schirmer test 1 was prolonged from 8.9±8.7mm to 10.7±8.8mm (p=0.0296) and also that of BUT was significantly extended from 2.8±1.7seconds to 4.6±2.2seconds (p=0.0011). There was no significant change of fluorescein stain score. About the subjective symptoms, at the two weeks after the beginning of the study the items like foreign body sensation, dryness and tiredness were significantly ameliorated compared to the before. And at the four weeks after the start adding to these symptoms the items like eye heaviness, photophobia and eye discomfort were significantly ameliorated. And the degree of bitterness varied widely and there was no significant change.
Conclusions: Rebamipide eye drops are very effective to prolong the tear-film breakup time and to ameliorate the symptoms of the short-BUT type dry eye patients. [No commercial and financial support]

THE EFFECT OF BOTULINUM TOXIN TYPE A TREATMENT FOR BLEPHAROSPASMIC PATIENTS ON TEAR FILM PROPERTY

Yuka Hosotani,Yumiko Nakamura,Osamu Mimura. Department of Ophthalmology, Hyogo College of Medicine, JAPAN
Purpose: To evaluate the effect of Botulinum toxin type A (BTX-A) therapy on the patients of blepharospasm by monitoring their ocular surface condition, including tear film before and after the treatment.
Methods: We evaluated 12 eyes of 6 patients (all female, age 57-72 years old(mean:64.7)) diagnosed as blepharospasm at Department of Ophthalmology, Hyogo College of Medicine. We measured the tear film break-up time (BUT), corneal and conjunctival staining score with fluorescein which resulted in a full mark of 9 points. In addition, we

tracked 12 subjective symptoms using the VAS scale. All patients were treated with BTX-A injection the first time without rebamipide or diquafosol sodium eyedrops.
Results: Before the BTX-A injection, BUT was 1.72±1.42 seconds, and corneal and conjunctival staining score was 0.42±0.90. After BTX-A injection, BUT was 3.47±2.47 seconds (P<0.01) and corneal and conjunctival staining score was 0.25±0.62. The shortening of BUT was observed in patients with blepharospasm before treatment but after treatment BUT was significantly elongated. Corneal and conjunctival staining score was decreased after treatment but not significantly. Regarding the subjective symptoms, only the foreign-body sensation was significantly improved, (P<0.05) but the other subjects showed only the tendency of improvement with nothing significant to note.
Conclusions: Blepharospasm patients showed shortened BUT, but it was extended with BTX-A treatment. We estimate that the improved tear film condition is one factor for subjective symptoms being improved with BTX-A treatment.

EFFECT OF BOTULINUM TOXIN TYPE A INJECTION FOR LATERAL CANTHAL RHYTIDES ON TEAR FILM STABILITY AND TEAR PRODUCTION.
Yi-Ting Hsieh,[1,2] Min-Chieh Ho,[1] Wei-Cherng Hsu,[1,2]. Department of Ophthalmology, Buddhist Tzu Chi General Hospital, Taipei Branch, New Taipei, Taiwan,[1] School of Medicine, Tzu Chi University, Hualien, Taiwan.[2]
Purpose: To investigate the effect of botulinum toxin type A on tear film stability and tear production after treatment for lateral canthal rhytides.
Methods: Fifty-eight women 30 to 60 years of age with lateral canthal rhytides were prospectively recruited one regional hospital. Botulinum toxin type A was injected at the lateral canthal areas; one eye of each subject was randomly chosen for dose A (3 injections, 2 U in 0.05 mL per injection), and the other eye received dose B (3 injections, 4 U in 0.05 mL per injection). Tear film break-up time (TBUT) and Schirmer's tests without and with anesthesia were measured at baseline and 1 week, 1 month, 3 months and 6 months after treatment. The results of TBUT and Schirmer's tests were compared between different time periods and between different doses.
Results: TBUT decreased significantly at 1 week after botulinum injection (P=0.003), and the effect persisted at 1 month and 3 months after treatment (P=0.01 and 0.02, respectively). Younger subjects had their TBUT recovered faster than older ones did. Schirmer's tests without and with anesthesia decreased gradually with significant reduction at 1 month after treatment (P=0.05 and 0.02, respectively) and then recovered gradually. Such effect didn't differ significantly between different doses.
Conclusions: Tear film stability decreased as soon as 1 week after botulinum toxin treatment for lateral canthal rhytides, and the effect persisted for more than 3 months. Tear production decreased to the trough at 1 month after treatment and then recovered gradually.

DISRUPTED INDUCTION OF INDUCED REGULATORY T CELLS IN THE DRY EYE HOST EXACERBATES CORNEAL ALLOGRAFT REJECTION
Jing Hua, William Stevenson, Yihe Chen, Thomas Dohlman, Takenori Inomata, Hyun Soo Lee, Sunil Chauhan, Reza Dana
Immunology Laboratories, Schepens Eye Research Institute, Massachusetts Eye and Ear Infirmary, Harvard Medical School
Purpose: Anecdotal clinical observations suggest that recipient dry eye disease (DED) undermines corneal graft survival. However, the underlying mechanisms are not well understood. This study investigated the survival rate and the adaptive immune response following corneal allotransplantation in DED host mice.
Methods: DED was induced in female Balb/c mice using the controlled environment chamber with subcutaneous scopolamine injections over two weeks. C57Bl/6 donor corneas were then grafted onto Balb/c mice with DED. Normal Balb/c hosts residing in room air (RA) served as controls. Additional DED hosts received adoptive transfer of sorted Treg or non-Treg $CD4^+$ cells from naïve Balb/c. Corneal graft survival was observed over 8 weeks. Flow cytometry of lymph nodes (LN) and blood cells, LN explant culture, and T cell suppression assay were carried out 10 days following transplantation.
Results: Kaplan-Meier survival curves indicated that significantly fewer corneal grafts survived in DED hosts (22.2%, n=9) after eight weeks, compared to RA controls (50%, n=10, Wilcoxon p=0.0084). Tregs from LN of DED hosts showed significantly lower suppressive ability in the T cell suppression assay by ANOVA analysis (2.6±9.6% in DED, 43.9±2.9% in RA, and 85.1±0.3% in naïve control, n=3). In addition, although the frequencies of $CD4^+CD25^+Foxp3^+$ Treg were comparable between all groups, frequency of $CD4^+CD25^+Foxp3^+Nrp\text{-}1^-$ induced Tregs (iTregs) in both LN and blood samples were significantly lower in DED hosts. LN explant culture demonstrated that in DED hosts there were significantly increased levels of secreted IL-6 and IL-23. Finally, adoptive transfer of naïve Balb/c Tregs to DED hosts significantly increased graft survival to 44.4% (n=9) with improved iTreg frequencies (LN and blood), compared to 10% survival in the non-Treg CD4+-transferred hosts (n=10, p<0.01).
Conclusions: Disrupted iTreg induction due to increased IL-6/23 in the DLN may be a key mechanism underlying the exacerbation of corneal allograft rejection in DED hosts.
NIH EY12963 and EY20889 (RD) and EBAA (JH).

EFFECT OF PHOTOTOXICITY ON THE OCULAR SURFACE AND TEAR FILM BY AN OPERATING MICROSCOPE.
Hyung Bin Hwang, M.D., M.S., Hyun Seung Kim, M.D., Ph.D.
Department of Ophthalmology, St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea
Purpose: We evaluated light exposure-induced dry eye syndrome by investigating the phototoxic effects of an operating microscope on the ocular surface and tear film in rabbits.
Methods: Sixty eyes of 30 rabbits were divided into three groups according to the intensity of light exposure by an operating microscope: Control group, no exposure to light; Group A, 40,000-lux intensity for 30 min; and Group B, 100,000-lux intensity for 30 min. To evaluate the potential damage to the ocular surface and tear film, Schirmer's tests, rose bengal staining, and conjunctival impression cytology were performed before the light exposure and 1, 3, and 5 days afterward. In addition, the expression of interleukin 1-beta (IL-1ß) was analyzed in tear samples. The expression of mucin 5AC was evaluated using immunofluorescence staining, and periodic acid-Schiff staining was conducted on conjunctival tissues. Corneal and conjunctival tissues were observed by electron microscopy.
Results: Potential damage to the ocular surface and tear film was revealed in the light-exposed groups as evidenced by: 1) decreased aqueous tear production, 2) devitalized corneal and conjunctival epithelial cells, 3) squamous metaplasia of conjunctival epithelial cells, 4) decreased conjunctival goblet cell density, 5) decreased expression of mucin 5AC, 6) ultrastructural cellular damage to corneal and conjunctival tissues, and 7) increased IL-1ß expression in tears. This damage was more noticeable in Group B than in Group A ($p < 0.05$).
Conclusions: Light exposure from an operating microscope had a phototoxic effect on the ocular surface and tear film in this *in vivo* experiment. These changes seemed to intensify as the luminous intensity increased. Therefore, excessive light exposure during ophthalmic procedures could be a pathogenic factor of dry eye syndrome after surgery.
[The authors acknowledge funding from St. Mary's Hospital, College of Medicine, Catholic University of Korea. This project was funded by the Institute of Clinical Medicine Research, St. Mary's Hospital.]

GENETIC VARIANTS IN IL6 AND IL6R GENES ARE STRONG ASSOCIATED WITH KOREAN DRY EYE DISEASE.

Kyu Yeon Hwang, Kyung-Sun Na, Jee-Won Mok, Choun-Ki Joo
Department of Ophthalmology and Visual Science, Catholic university of Korea, Catholic Institute of Visual Science, Seoul, Republic of Korea.

Purpose: To determine whether variations altering the function or expression of IL6 and IL6R, contribute to the pathogenesis of dry eye disease.

Methods: Genomic DNA was extracted from blood samples of unrelated dry eye disease patients;non-Sjogren's syndrome(non-SS) patients(n=521) and Sjogren's syndrome(SS) patients(n=166). PCR and direct sequencing were used to screen variations in promoter region, 5 untranslated region and exons of IL6 and IL6R genes. 150 control individuals without corneal disease were selected from the general population.

Results: We investigated 8 SNPs for IL6 and 5 SNPs of IL6R; rs1800797(-597 a>g), rs1800796(-572 c>g), rs56108454(A8T12), -353 tins, rs1800795(-174 g>c) in promoter and rs34280821(S15L), rs142759801(P31T) and rs2069830(P32S) in exon 2 of IL6, and -100 c>t, -93 g>t and -30 a>t in promoter, rs4845617(EX1 +230 g>a) in 5' untranslated region and rs2228145(D358A) in exon 6 of IL6R. Among them, rs1800796 and rs56108454 of IL6 and rs4845617 and rs2228145 of IL6R were significantly different between patients and control groups. Particularly, *c/*c genotype of the rs1800796(-572 c>g) of IL6 in non-SS and SS patients were significantly increased compared with control subjects(vs. non-SS` O.R. =1.667; vs. SS` O.R. = 2.571). Also *G/*G genotype of the rs4845617 of IL6R in both dry patient groups were significantly increased compared with control subjects(vs. non-SS` O.R. =1.704; vs. SS' O.R. = 1.769). The genotype frequencies of rs56108454 of IL6 and rs2228145 of IL6R were showed difference between SS and control groups(O.R. = 0.578 and O.R. = 1.119, respectively).

Conclusions: The present study showed that the genetic variants in IL6 and IL6R, in the rs1800796(-572 c>g) of IL6 and rs4845617(EX1 +230 g>a) of IL6R, are associated with a higher risk of dry eye disease patients. It is suggested that genetic variations of IL6 and IL6R may act as potential susceptibility variants in Korean non-Sjogren and Sjogren DED.

OCULAR SURFACE ALTERATIONS IN BLEPHAROSPASM PATIENTS TREATED WITH BOTULINUM TOXIN-A INJECTIONS

Murat Irkec ,[1] Sibel Kocabeyoglu,[1] Hande Taylan Sekeroglu,[1] Mehmet C. Mocan,[1] Ali Sefik Sanac.[1] Hacettepe University School of Medicine,[1], Ankara, Turkey

Purpose: To evaluate ocular surface changes induced by periocular botulinum toxin-A injection in patients with essential blepharospasm.

Methods: Thirteen eyes of 13 patients with essential blepharospasm were included in this prospective study. Botox® (Allergan, USA) injection was performed in the periocular area depending on the region of contractions as 4 IU/injection. Patients were evaluated prior to and at 2-week and 1-, 3- and 6-month time points following injections. Ocular surface tests were carried out in the order of tear break up time (TBUT), lissamine green (LG) staining and Schirmer I test with anesthesia for all patients. Ocular surface disease index (OSDI, Allergan Inc, Irvine, California) questionnaire was also employed at each visit. Subjects with a history of ocular surgery, trauma, contact lens and topical medication use were excluded. The Friedman's test for non-normally distributed data was used with Conover's post hoc method for statistical comparisons of values at different time points.

Results: The mean age of patients (6M/7F) with blepharospasm was 64.3±11.0 years. The increase in TBUT was significant at 1-month after the injection (8.5±2.1; p=0.018) and decreased below baseline levels (6.4±2.1) at the 6-month visit (5.7±2.0; p=0.018). None of the Schirmer test values at follow-up visits were significantly different as compared to baseline levels (11.3± 5.5) although the 2-week measurement (14.3±5.6) was significantly higher as compared to that at the 6-month follow-up visit (9.6±4.9; p=0.034). There was also a significant decrease in LG staining scores at 2-week (0.6±0.4; p=0.012) and 1-month (0.6±0.4; p=0.012) time points compared to the baseline levels (1.1±0.6). The OSDI scores improved at 2-week (5.4±6.8; p<0.001), 1-month (3.2±5.1; p<0.001), 3-month (2.5±4.4; p<0.001) and 6-month (5.5±5.4; p<0.001) time points as compared to its baseline levels (11.6±8.5).

ASSESSMENT OF LANGERHANS CELL DENSITY IN THE CENTRAL CORNEA IN PATIENTS WITH SEVERE DRY EYE UNDER ANTI-INFLAMMATORY THERAPY WITH CYCLOSPORINE A 0.05% EYE DROPS

Christina Jacobi, Eva Schneider, Friedrich E. Kruse. Department of Ophthalmology, University of Erlangen-Nuremberg, Germany

Purpose: To investigate the density of Langerhans cells in the central cornea of patients with dry eye syndrome under topical anti-inflammatory therapy with cyclosporine A 0.05% eye drops.

Methods: 31 patients (age 21-85 years) with severe dry eye were enrolled in the trial. For Langerhans cell counts, patients were examined in vivo with the Heidelberg Retina Tomograph II and the Rostock Cornea Module prior to and after 3 months of treatment with cyclosporine A 0.05% eye drops twice daily. In addition, tear film osmolarity, break up time (BUT), Schirmer test with anaesthesia and meibomian gland dysfunction were evaluated in each patient. Statistical analyses were performed using Statistica™ software, p-values < 0.05 *, < 0.001** were considered significant.

Results: There was a significant reduction in Langerhans cell density in the central corneal epithelium after anti-inflammatory treatment from 110±12 cells/mm² to 56±10 cells/mm². Tear break up time increased significantly from 6.9 seconds (range 3-12 seconds) to 15.0 seconds (range 10-26 seconds). Tear film osmolarity, Schirmer test with anesthesia and meibomian gland dysfunction did not show significant changes.

Conclusions: Evaluation of Langerhans cell density in the central corneal epithelium by in vivo confocal microscopy could be an effective objective diagnostic feature in monitoring anti-inflammatory therapy in patients with dry eye disease or other ocular surface pathologies.

Conclusions: Botulinum toxin-A injection appears to have a positive but temporary effect on ocular surface parameters in patients with blepharospasm.

THE EFFECTS OF AGE AND EVAPORATIVE STRESS ON MEIBOMIAN GLAND FUNCTION

James V. Jester, Geraint Parfitt, Mikhail Geyfman, Yilu Xie, Donald Brown Gavin Herbert Eye Institute, University of California, Irvine, Irvine, CA.

We have previously shown that both mouse and human meibomian glands (MG) develop age-related meibomian gland atrophy that leads to MG dropout similar to that observed in human meibomian gland dysfunction. In our continuing studies we have used Immunofluorescent Computed Tomography (ICT) and Coherent Raman Scattering (CRS) microscopy to further characterize meibomian gland function with age and evaporative stress associated with experimental dry eye. Our ICT findings suggest that individual MG acini exhibit a range of forms from predominantly cellular/proliferative to lipid synthesizing. With aging meibomian glands showing dramatic acinar dropout similar to what is detected in age-related human MGD. Importantly, immunostaining for cytokeratin expression showed no increase in CK1 ductal staining, suggesting that hyperkeratinization is not involved in mouse age-related MGD. Interestingly, CK1 staining did detect anterior movement of the mucocutaneous junction, similar to what is detected in older humans, suggesting important parallels in the mechanism of mouse and human age-related MGD. CRS analysis also shows a high protein to lipid ratio within acini that uniformly decreases as meibum moves from the acini-ductule-central duct and orifice suggesting lipid maturation. Following evaporative stress there is a marked increase in cell proliferation that is

coupled with abnormal maturation of gland lipid that leads to a higher meibum protein content within the lipid of the central duct. Together these findings suggest that the meibomian gland can respond dynamically to environmental stress and that stress can affect meibum lipid maturation leading to changes in meibum quality.
Supported by: NEI EY0121510, Discovery Eye Foundation, Skirball Program in Molecular Ophthalmology and Research to Prevent Blindness Inc.

THE EVALUATION OF LID WIPER EPITHELIOPATHY IN CONTACT LENS WEARERS IN A CONTROLLED LOW HUMIDITY ENVIRONMENTAL EXPOSURE CHAMBER

Lyndon W. Jones, [1] Jalaiah P. Varikooty, [1] Nancy J. Keir, [1] Fiona Soong, [2] Piyush Patel. [2] CCLR-School of Optometry, University of Waterloo, Waterloo, [1] Inflamax Research, Mississauga, [2] ON, Canada.

Purpose: To measure the clinical grades of lid wiper epitheliopathy (LWE) in contact lens (CL) wearers before and after exposure to conditions of temperature, low humidity and air flow in a controlled low humidity environmental exposure chamber (LH-EEC).

Methods: In this double-masked, feasibility study, 10 symptomatic CL wearers were randomized to contralateral lens wear with narafilcon A and etafilcon A lenses. CL wear was discontinued 48 h prior to assessments and Refresh Plus® artificial tears (ATs) were instilled t.i.d. in both eyes. For LWE measures, the upper (UL) and lower lid (LL) margins were stained with sodium fluorescein and lissamine green dyes using an optimized technique and LWE was graded on a 0-3 scale at Baseline, prior to CL insertion and entry into the LH-EEC. In the LH-EEC, subjects were exposed to controlled temperature of 22±3°C, relative humidity of 10±3% and an air velocity of approx. 5ft/sec for 180 min. Upon exit, CLs were removed and ATs instilled every 15 min for 120 min. LWE was evaluated post-chamber exposure (PC), PC+30min, PC+90min and PC+120min.

Results: After 180 min in the LH-EEC, mean LWE grades in the upper lid increased from Baseline to PC and were 1.25 to 2.23 for narafilcon A and 1.18 to 1.93 for etafilcon A. In the lower lid, it changed from 1.00 to 2.48 for narafilcon A and 0.90 to 2.03 for etafilcon A (Wilcoxon matched pairs, all $p<0.05$). The mean LWE grades did not return to Baseline levels at PC+120mins and were 2.47 and 2.11 for narafilcon A, and 2.42 and 1.89 for etafilcon A in the UL and LL respectively. These LWE responses were similar for the two lenses, despite their markedly different material properties.

Conclusions: LWE increased significantly with CL wear in the UL and LL after 3 hours of exposure to adverse environmental conditions. ATs did not have an effect in LWE reduction during the two hours following exit from the adverse environment. The LH-EEC model may prove a valuable tool to study conditions that result in alterations in LWE.
[Financial support from Inflamax Research.]

INFRA-RED IMAGING OF MEIBOMIAN GLANDS & EVALUATION OF THE LIPID LAYER IN SJOGREN'S SYNDROME PATIENTS

Lyndon Jones, Sruthi Srinivasan, Kara Menzies, CCLR-School of Optometry, University of Waterloo, Waterloo, Ontario. Canada.

Purpose: The purpose of this study was to evaluate meibomian gland (MG) drop-out and lipid layer thickness in patients with and without Sjogren's syndrome (SS).

Methods: Eleven participants with SS (1 male and 10 females; mean age = 56.0±9.1yrs) and 10 non dry eye (NDE) controls (3 males and 7 females; mean age=58.5±4.7yrs) were recruited. Ocular and oral dryness symptoms were assessed using the American-European Consensus Criteria for Sjögren's Syndrome (AECC) in the SS group. All participants completed the Ocular Surface Disease Index (OSDI) questionnaire to assess dryness symptoms. Lipid layer thickness (LLT) was assessed using the Tearscope Plus (Keeler) based on the appearance of the lipid layer. Non-invasive tear break up time (NITBUT) was assessed. The lower lid (LL) and upper lid (UL) of all subjects were everted and the MGs imaged using the infra-red (IR) camera of the Keratograph 4 (OCULUS). A MG drop-out score (MGDS) due to complete or partial gland loss of both lids was obtained via digital analysis of the images using ImageJ software.

Results: 100% of the SS participants reported ocular and oral dryness symptoms in the AECC questionnaire. The SS group recorded a higher OSDI score (40.0±21.0 vs 1.7±1.7; $p<0.001$), lower LLT (20±14nm vs 64±5nm; $p<0.05$) and lower NITBUT (3.6±1.7 vs 11.6±7.6sec; $p<0.001$), compared to the NDE controls. Digital MGDS (% drop out) was significantly higher for the SS group for the UL (OD: 22% vs 3%; $p<0.005$; OS: 22% vs 6%; $p<0.02$), and LL (OD: 31% vs 12%; $p>0.05$; OS: 31% vs 8%; $p<0.05$). MGDS were negatively correlated with NITBUT ($r=-0.71$, $p<0.05$). There was a positive correlation between LLT and NITBUT for both OD ($r= 0.44$; $p<0.05$) and OS ($r= 0.72$; $p<0.05$).

Conclusion: Patients with SS showed higher MGDS and reduced LLT and NITBUT which likely contribute to the severe dry eye symptoms reported by SS subjects.
[Financial support from the Sjogren's Society of Canada]

INTER- AND INTRA-OBSERVER AGREEMENT AND REPEATABILITY OF IMAGING THE MEIBOMIAN GLANDS WITH THE OCULUS KERATOGRAPH 4 AND KERATOGRAPH 5M

Lyndon Jones, William Ngo, Sruthi Srinivasan, Marc Schulze, CCLR-School of Optometry, University of Waterloo, Waterloo, Ontario. Canada.

Purpose: To compare the inter- and intra-observer agreement and repeatability of using a 4-point scale to grade meibography images taken from the OCULUS Keratograph 4 (K4) and OCULUS Keratograph 5M (K5M) instruments.

Methods: The superior and inferior eyelids from a single eye of 40 participants were imaged 3 times on the K4 and K5M. The images were organized into 3 sets each containing 160 images (2 devices x 40 images each for inferior superior eyelid). Arita et al's 4-point grading scale (0 = no dropout, 1=less than 1/3 total area dropout, 2=1/3 to 2/3 total area dropout, 3=>2/3 total area dropout) was used to grade meibomian gland dropout. The first set of images served to train 2 observers, O1 and O2; the second and third sets were presented to both observers (24 hours apart) on a 50" high definition monitor in a darkened room. All images in each set were presented in a random order.

Results: For both devices combined, the mean difference in ratings between O1 and O2 was 0.08±0.55 on day 1 (D1), and 0.13±0.50 on day 2 (D2); the concordance correlation coefficient (CCC) between O1 and O2 was CCC=0.79 on D1 and CCC=0.81 on D2. When looking at both devices individually, the inter-observer mean differences in ratings when using the K4 was -0.01±0.58 on D1 and 0.08±0.52 on D2; while the mean differences when using the K5M was -0.16±0.51 on D1, and 0.18±0.47 on D2. Inter-observer CCCs when using the K5M (D1=0.79, D2=0.81) were slightly higher than with the K4 (D1=0.78, D2=0.80).

When comparing ratings between sessions for each observer, the intra-observer mean difference in ratings for O1 was -0.08±0.49 and -0.01±0.58 for the K5M and K4, respectively; mean difference for O2 was -0.09±0.51 and -0.10±0.68 for the K5M and K4, respectively. The intra-observer CCC for the K5M (O1=0.82, O2=0.80) was higher than for K4 (O1=0.76, O2=0.68).

Conclusion: The repeatability of the K5M was slightly better than the K4 when using a 4-point grading scale. In all cases, both observers graded within -1 to +1 grades of each other and against themselves.

MEMBRANE PERMEABILITY OF *STAPHYLOCOCCUS AUREUS* AGGREGATES EXPOSED TO CONTACT LENS CARE SOLUTIONS

Lyndon Jones, David McCanna.

Centre for Contact Lens Research, School of Optometry & Vision Science, University of Waterloo
Purpose: Microbial keratitis and corneal infiltrates have been associated with the development of bacterial aggregates (biofilms) on the surface of contact lens cases. Contact lens case contamination with biofilms occurs often due to the resistance of the bacterial aggregates to the antimicrobials present in contact lens care solutions. This study investigated the ability of contact lens care solutions to damage the cell membranes of *Staphylococcus aureus* (SA) aggregates.
Methods: *Staphylococcus* aggregates were formed by growing the bacteria on Mueller-Hinton Agar, harvesting with physiological saline and washing using centrifugation (500 x g for 5 minutes). Commercial contact lens solutionsevaluated contained the antimicrobials polyquaternium-1 (PQ1) and polyhexamethylene biguanide (PHMB), PHMB alone, PQ1 and alexidine (ALX), and PQ1 and ALDOX. Each solution was challenged with 5 x 10(7)cfu/ml of SA (ATCC 6538) for 4 hours. After exposure, the bacteria were stained with SYTO 9 and propidium iodide (PI). Using a confocal microscope with a 488nm laser and the appropriate emission filters for these two dyes, the number of cells with damaged cell membranes was determined.
Results: The contact lens solution that caused the greatest damage to the SA cell membranes was the formulation based on PQ1-ALX, with 81% of all cells being permeable to PI. The other formulations caused some of the bacteria to lose membrane integrity (13 - 30%), but did not cause as much damage to the bacteria cell membranes (all $p < 0.05$) as the PQ1-ALX formulation.
Conclusions: One of the five lens care systems tested caused a substantial number of SA bacteria to lose membrane integrity. Although membrane damage is only one of the many mechanisms by which an antimicrobial can kill microorganisms, understanding the ability of contact lens care solutions to damage bacteria cell membranes in an aggregate formation could lead to improved formulations for eradicating biofilms from contact lens cases.
Funding: NSERC Canada

AUTOLOGOUS SERUM EYE DROPS FOR TREATMENT OCULAR SURFACE DISORDER AND POST-OPERATIVE DELAYED CORNEAL EPITHELIAL HEALING

Passara Jongkhajornpong, Kaevalin Lekhanont, Rossanun Sikaringul, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
Purpose: To evaluate the efficacy and the safety of an autologous serum treatment of ocular surface disorder and post-penetrating keratoplasty corneal epithelial defects at Ramathibodi Hospital.
Methods: A retrospective chart review was conducted from March 2009 until June 2011. Demographic data, indications, concentration and frequency of serum eye drops, fluorescein staining, subjective symptoms and adverse effects were recorded at baseline and at follow up. The main outcomes were the success rate of complete corneal epithelialization or decreased fluorescein staining and the safety profile of autologous serum eye drops (ASE).
Results: 246 eyes were included in this study. Twenty percent, 50%, 100% ASE were used (3.66%, 6.91%, 85.37%, respectively). Overall success rate (complete corneal epithelialization) after ASE treatment was 227/246 (92.28%, 95% CI, 0.87-0.96). The median time to complete corneal epithelialization was 7 days (95% CI, 6-10). CL use, indications and frequency of ASE treatment were statistically significant to corneal epithelialization ($P<0.01$). However, in COX regression multivariate analysis, only the indication of ASE has statistically significance influenced on time to complete epithelialization ($P<0.01$). The adverse effects of ASE were eye irritation in 3 eyes (1.22%) and sterile corneal infiltration in 2 eyes (0.81%).
Conclusions: The ASE processed at Ramathibodi Hospital was effective and safe in treating ocular surface disorder and post-operative delayed epithelial defects. No serious adverse events was reported from 100% ASE using.
These authors have no relevant conflicts to report.

ASSOCIATION OF INTERLEUKIN-17A AND -17F GENES SINGLE NUCLEOTIDE POLYMORPHISMS WITH DRY EYE DISEASES

Choun-Ki Joo[1,2,3], Jee-Won Mok[1,], Je-Hyung Hwang[1,2,3], Yong-Eun Lee[1,2,3], Kyung-Sun Na[1,2,3]
1 Catholic Institutes of Visual Science, The Catholic University of Korea, 2 Department of Ophthalmology & Visual Science, College of Medicine, The Catholic University of Korea, 3 Seoul St. Mary's hospital Eye Institute (SSEI)
Purpose: To determine whether polymorphisms altering the function or expression of IL17A and IL17F, which is a potent cytokine and a potential contributor to the etiology of dry disease
Methods: Genomic DNA was extracted from blood samples of unrelated dry eye disease patients (without Sjogren's syndrome) and Sjogren's syndrome patients. PCR and direct sequencing were used to screen variations in promoter region and 5' untranslated region IL17A and IL17F genes. One hundred control individuals without corneal disease were selected from the general population.
Results: We investigated 7 SNPs: 5 in IL17A, namely rs8193036, -307 G>A, rs2275913, rs8193037 and rs3819025, and 2 in IL17F, namely, -1658 T>C and -1436 G>A. Among them, the -307 G>A, rs8193037 and rs2275913 of IL17A gene and -1658 T>C of IL17F gene were significantly different between patients and control groups. The *A allele frequency of the -307 G>A and a allele frequency of rs8193037 of IL17A in dry eye patients were significantly decreased compared with control subjects. The frequency of the *A allele of the rs2275913 SNP was higher in the Sjogren's disease patients than in the controls (OR: 2.32). In IL17F gene, the frequency of the *C allele of the -1658 T>C in both dry eye patient (vs. dry eye O.R. =1.64; vs. Sjogrens' O.R. = 1.63).
Conclusions: The present study showed that the genetic variants in IL17A and IL17F, the -307 G>A, rs8193037 and rs2275913 of IL17A gene and -1658 T>C of IL17F gene, are associated with non-Sjogren and Sjogren DED patients. It is suggested that genetic variations of IL17A and IL17F may act as a potential susceptibility variants in Korean dry eye disease.

INVESTIGATION OF SCREENING STANDARDS OF DRY-EYE PATIENTS USING FUNCTIONAL VISUAL ACUITY MEASUREMENT SYSTEM

Minako Kaido,[1] Miki Uchino,[1] Yu-ichi Uchino,[1] Norihiko Yokoi,[2] Dogru Murato,[1] Motoko Kawashima,[1] Masaki Fukui,[1] Yoshiyuki Ichihashi,[1] Aoi Komuro,[2] Yukiko Sonomura,[2] Hiroaki Kato,[2] Motoko Yamatsuji,[3] Mitsuko Nishida,[3] Shigeru Kinoshita,[2] Kazuo Tsubota.[1] [1]Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan, [2]Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan, [3]Health care section, IT Products Business Division, AVC Networks Company, Panasonic Corporation, Osaka, Japan
Purpose: Investigation of the screening standards of dry-eye (DE) patients with functional visual acuity (VA) measurement system
Methods: 491 subjects were targeted (Male: 326, Female: 165, Age: 43.3 ± 8.9). The subjects were classified into 2 groups based on the Japanese DE diagnostic criteria: 320 subjects in DE group (both definite and probable DEs), and 171 normal subjects in non-DE group. The functional VA examination and DE questionnaire were conducted. Discriminant analysis was performed and assessed whether it is possible to detect which group, either DE or non-DE group, a subject belongs to. A stepwise variable selection method was used to choose the predictors among the functional VA parameters and the 12 questions of DE questionnaire, and the discriminant equation was calculated. Moreover, for checking the validity of the discriminant equation, it was applied to another cohort with 369 subjects with 218 in DE and 151 in non-DE groups.
Results: The variables selected for the discriminant equation were "visual maintenance ratio", "blinking frequency", "eye fatigue"," eye

discomfort", "dry eyes", and "increased sensitivity to light". When comparing calculated diagnoses with actual diagnoses, sensitivity and specificity were 85.9% and 45.6%, respectively. When applied to another cohort, the sensitivity was 93.1%, with specificity of 64.9%.
Conclusion: The obtained equation may be appropriate for screening for DE patients. The combination of the functional VA measurement and subjective symptoms questionnaire is one promising method to screen DE patients.
[This research was supported by Santen Pharmaceutical Co.,Ltd and AVC Networks Company Panasonic Corporation.]

ARE MICE RELEVANT MODELS FOR HUMAN MEIBOMIAN GLAND RESEARCH?
Wendy R. Kam, Raheleh Rahimi Darabad, Shaohui Liu, David A. Sullivan. Schepens Eye Research Institute, Massachusetts Eye and Ear, Harvard Medical School, Boston, MA, USA
Purpose: One of the most compelling features of dry eye disease (DED) is that it occurs predominantly in women. We hypothesize that this female prevalence is linked to sex-related differences that are known to exist in the anatomy, molecular biology and physiology of the meibomian gland (MG). This tissue plays a critical role in maintaining the tear film, and its dysfunction (MGD) is believed to be the major cause of DED. To better understand the factors that underlie MG sexual dichotomy and promote DED in women, we would like to identify a model for the human MG. Our study examined whether a murine MG gland is such a model. Towards that end, we evaluated whether sex differences in MG gene expression are the same in mice and humans.
Methods: After obtaining IRB or IACUC approval, lid tissues (n = 5 to 36 lids and 3 to 7 samples per sex/species) were collected from age-matched humans and BALB/c mice. MGs were isolated and processed for RNA extraction and the analysis of gene expression by using Illumina BeadChips and bioinformatics.
Results: Our evaluation of the 500 most highly expressed genes from human (10,099 genes) and mouse (18,302 genes) MGs showed that only 23.4% were similar. If ribosomal genes were excluded, only 16.6% of the genes were the same. Our comparison of the 100 genes with the greatest sex-related differences in human (e.g. lysozyme, 18.2-fold, M>F) and mouse (e.g. androgen binding protein zeta, 109-fold, F>M) MG tissues demonstrated that none were the same. Of interest, sex significantly influenced the gene expression of a number of chromosomes, but the nature of this activity was species-specific. In addition, sex exerted a significant impact on numerous biological process, molecular function and cellular component ontologies, as well as many KEGG pathways, but these effects were also primarily species-specific.
Conclusions: Our findings demonstrate that mice are not appropriate models for understanding sex-related differences in gene expression of the human MG. (Supported by NIH grant EY05612 and the Margaret S. Sinon Scholar in Ocular Surface Research fund)

OBSERVATION OF MEIBOMIAN GLAND MORFOLOGY IN ACUTE-PHASE OF STEVENS JOHNSON SYNDROME AND CHEMICAL BURN
Kozue Kasai,[1] Seika Shimazaki-Den,[1] Murat Dogru,[1,2] Jun Shimazaki,[1,2] Tokyo Dental College Ichikawa General Hospital, [1] Chiba, Japan, Keio University School of Medicine, [2] Tokyo, Japan
Purpose: To report time course changes in meibomian gland (MG) morphology and their association with ocular surface changes in acute-phase of Stevens Johnson syndrome SJS and chemical burn (CB).
Methods: We examined time course changes in MG morphology using non-contact infrared meibography (NIM) in the acute phase of SJS (n=2) and CB (n=1). We also investigated the association between MG changes and ocular surface status over time.
Results: Case1: 87y, female, SJS by lenalidomide administered for multiple myeloma. Although blepharitis in both eyes was severe in the acute phase, shortening and drop out of MG were moderate. Total corneal epithelial defects in both eyes were successfully treated in 17 days by topical steroid and hyaluronic acid (HA). Case 2: 19y, male, SJS by **acetaminophen** administered for heatstroke. Severe blepharitis and keratinization around MG orifices were observed in both eyes. Marked shortening of MG ducts and drop out of MG acini were observed by NIM in the acute phase, however, they were improved following continuous application of lid hygiene and warm compress. Case 3: 37y, male, CB by nitric acid. Although total corneal defects in the left eye was successfully treated by topical steroid, HA and bandage contact lens, allogeneic cultivated limbal epithelial sheet transplantation was needed in the right eye due to severe limbal damage. NIM showed marked shortening of MG ducts and drop out of MG acini in the right eye, which was much less severe in the left eye.
Conclusions: NIM was useful for continuous observation of morphological changes in MG in acute SJS and CB. Timely management of MG may be effective for prevention of severe MG dysfunction in severe ocular surface disorders.

LONG-TERM EFFECT OF 3% DIQUAFOSOL SODIUM OPHTHALMIC SOLUTION IN PATIENTS WITH SHORT BREAKUP TIME TYPE DRY EYE WITH "SPOT BREAK"
Hiroaki Kato[1], Norihiko Yokoi[1], Aoi Komuro[1], Yukiko Sonomura[1], Rieko Sakai[1], Minoru Shimoyama[1], Akane Yamaguchi[1], Masaki Baba[1], and Shigeru Kinoshita[1]. Department of Ophthalmology, Kyoto Prefectural University of Medicine[1], Kyoto, Japan
Purpose: Animal studies have shown that diquafosol sodium (DQS) facilitates water and mucin secretion from the conjunctiva and that DQS possibly promotes the expression of membrane-associated mucin related to the wettability of the ocular surface. The purpose of this present study was to investigate the effectiveness of 3% DQS ophthalmic solution for treating patients with short breakup time type dry eye with "spot break" (SBUTDE).
Methods: This study involved 21 female SBUTDE patients (mean age: 58 years) who were unresponsive to treatment with conventional eye drops. Each subject was administered 3% DQS, and tear meniscus radius (TMR, mm), fluorescein BUT (FBUT, seconds), and ocular-surface epithelial damage (OSED) score were evaluated at 1, 3, 6, and 12 months after initiating treatment. At those same intervals, subjects were questioned about dry-eye-related symptoms using the visual analog scale (VAS). The frequency of observed "spot break" when FBUT was measured was also evaluated.
Results: TMR significantly increased at 3 to 12 months (p<0.05). FBUT before instillation and at 1, 3, 6, and 12 months was 1.0±1.1, 1.9±1.5, 2.5±1.9, 2.5±1.5, 3.1±2.0, respectively, and showed significantly greater values from 1 to 12 months (p<0.02). OSED scores significantly decreased at 12 months (p<0.04). "Dryness" and "eye fatigue" were significantly improved from 1 to 12 months (p<0.005). "Foreign-body sensation", "pain" and "sensitivity to light" were significantly improved at 3 to 12 months (p<0.04). "Blurred vision" was significantly improved at 3 months (p<0.005). "Discharge" deteriorated significantly at 3 to 12 months (p<0.006). The frequency of observed "spot break" decreased significantly at 1 to 12 months (p<0.05).
Conclusions: The findings of this study show that 3% DQS is effective for treating patients with SBUTDE.
[Funding support and financial interests: None to report for all authors]

THE RELATIONSHIP BETWEEN CONTACT LENS USE AND SUBTLE MEIBOMIAN GLAND DYSFUNCTION
Costas Katsoulos[1], Anastasios Charonis, MD[1], George Charonis MD[1].[1]Athens Vision eye clinic, Athens, Greece
Purpose: This is a prospective study in order to study the relationship between contact lens (CL) use and subtle meibomian gland dysfunction

(MGD), in young patients attending an ophthalmology clinic in Greece.
Methods: 100 eyes of 50 young patients (younger than 35 years old, mean age 29.2, STD 7.43) were examined in order to look for signs of any type of MGD, even mild. We excluded patients with seborrheic blepharitis, patients with history of microbial or viral conjunctivitis or keratitis, patients who have taken any kind of ocular anti-microbial or anti-inflammatory medication in the previous years, and patients who had history of any type of lid pathology (chalazion, stye etc). They were asked regarding history of contact lens use, the years the CLs were worn, the average hours per day that the lenses were worn, and the type of contact lens worn (daily disposable, monthly disposable, conventional 1-year replacement, or RGP). Patients who have switched from one type of lens modality to another during their years of CL use were excluded from the study.
We used the SPSS v19 statistical package. The null hypothesis that there is no association between contact lens use and Meibomian gland dysfunction was tested with the Fisher's exact test. We additionally tested the association between Meibomian gland dysfunction and total years of contact lens use, between MGD and average hours worn, and between MGD and type of contact lens worn, with the Chi-square test.
Results: The Fisher's exact test yielded a p-value of 0.009 for the association between MGD and CL use. For the associations between MGD and years of contact lens use, hours worn per day, and type of lens worn, the Chi-square test yielded p-values of 0.011, 0.028 and 0.203 respectively. The odds ratio of someone wearing CLs to have MGD was about 5 times higher than the odds ratio of non-CL wearers.
Conclusions: Our study demonstrated that CL use is associated with mild MGD, indicating that CL use is a risk factor for MGD. There was also an association at the 0.05 level for MGD and years of CLs worn, and for MGD and hours per day of CL use, but not at the 0.01 level. On the other hand there was no association between MGD and type of CL worn.

STUDY OF THE SAFETY AND EFFICACY OF D-3-HYDROXYBUTYRATE EYE DROP FOR THE TREATMENT OF DRY EYE

Tetsuya Kawakita,[1] Uchino Miki,[2] Fukagawa Kazumi,[3] Yoshino Kenichi,[4] Shimazaki Seika,[5] Toda Ikuko,[6] Tanaka Mari,[7] Arai Hiroyuki,[8] Sakatani Keiko,[8] Hata Seiichiro,[9] Okano Takashi,[10] Tsubota Kazuo.[1]
Ophthalmology, Keio Univ School of Medicine,[1] Ryogoku Eye Clinic, [2] Iidabashi Eye Clinic,[3] Yoshino Eye Clinic,[4] Shimazaki Eye Clinic,[5] Minamiaoyama Eye Clinic,[6] Tokyo, Japan. Yatsuekimae azisai Eye Clinic,[7] Chiba, Japan. Minatomirai Eye Clinic,[8] Sky building Eye Clinic,[9] Smile Eye Clinic,[10] Kanagawa, Japan.
Purpose: The purpose of this study is to investigate the safety and efficacy of D-3-Hydroxybutyrate (HBA) eye drop in the treatment of dry eye patient.
Methods: Prospective, randomized, multicenter, double–blind placebo–controlled study were performed. Dry eye patients were randomly assigned to placebo and HBA eye drop and patients were received 6 times a day for 4 weeks. Corneal fluorescein staining, cornea and conjunctival rose bengal staining, tear film breakup time (BUT), Schirmer test and subjective symptoms were evaluated.
Results: In the corneal rose bengal score, statistically significant improvement was observed between the placebo and 1% HBA eye drop at 2 and 4 week (n=57, $P < 0.05$). Trend toward a significant improvement between placebo and 1% HBA eye drop at week 4 in the patient with Schirmer ≤ 5 mm and BUT ≤ 5 sec with apparent foreign sensation and dryness. we was subjected clinical trial based on these analysis results and changed from 1.0% to 1.5% HBA. In the efficacy assessment, statistically significant improvement was not observed between placebo and 1.5% HBA drop at 2 and 4 week (n=63, $P > 0.05$). In the eyes that were the criteria on the day of enrollment, there were statistically significant difference between placebo and 1.5% HBA in the corneal fluorescein staining score at 4 week (n=90, $P < 0.05$). In the incidence of adverse effects, statistically significant difference was not observed between placebo, 1.0% and 1.5% HBA.
Conclusions: These results indicate that 1.5% HBA is observed a curative effects for dry eye by extending a treatment period and increasing the number of cases. [Conflict of Interest (COI) of the Principal Presenter No potential COI to disclose]

EVALUATION OF DRY EYE DISEASE AMONG OFFICE WORKERS USING NEW INSTRUMENTS: THE OSAKA STUDY IN MORIGUCHI

Motoko Kawashima,[1] Norihiko Yokoi,[2] Masaki Fukui,[1] Yoshiyuki Ichihashi,[1] Hiroaki Kato, [2] Motoko Yamatsuji,[3] Mitsuko Nishida,[3] Shigeru Kinoshita,[2] Kazuo Tsubota.[1]
[1]Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan, [2]Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan
[3]IT products business unit, AVC Networks Company, Panasonic Corporation, Osaka, Japan
Purpose: To investigate prevalence of dry eye disease (DED) in visual display terminal (VDT) users and to assess new DED evaluation instruments.
Methods: A cross-sectional survey was conducted at a company whose employees were young and middle-aged Japanese engaging in office work with VDT (N=414). The tests used for DED diagnosis were the Schirmer test, fluorescein staining, and the tear film break-up time (TBUT) test. To evaluate DED symptoms, newly developed questionnaire, Dry Eye related Quality of life Score (DEQS), was administered. We also introduced new instruments including strip meniscometry. The prevalence of DED and their DED characteristics were evaluated.
Results: Of the 414 workers, 369 (89.4%; mean age, 44.5 ± 8.5 years; 331 men and 38 women) completed the entire questionnaire and dry eye examinations. The percentage of definite DED was 3.8% (14: 11 men and 3 women) and probable DED was 55.3% (204: 175 men and 29 women). Average values of the Schirmer test and TBUT were 17.6 ± 11.6 mm and 4.5 ± 3.1 s, respectively. DEQS scores were significantly higher in the definite and probable DED than the non-DED (P=0,004, P=0.000, respectively). Average tear strip meniscometry value was 3.9±3.4 mm, which was correlated with TBUT and the Schirmer values.
Conclusions: DED is prevalent among young and middle-aged Japanese VDT users.
The distinctive sign of DED in most of the VDT workers with probable DED was only short TBUT. DEQS were significantly higher in the probable DED as well as in the definite DED than in the non DED. We, ophthalmologists should work on drawing attention to DED for health management of office workers who are considered to be at high risk of DED. New easy and quick DED evaluation tools including DEQS and strip meniscometry are expected to work in practice.
[This research was supported by Santen Pharmaceutical Co.,Ltd and AVC Networks Company Panasonic Corporation.]

A NEW TEARSCOPE (HOSIK'S TEARSCOPE) MADE OF PAPTER FOR LIPID LAYER EVALUATION IN TEARFILM RELATED DISEASE

Man Soo Kim,[1] Kyoung Sook Cho,[2] Ho Sik Hwang.[3]
Catholic University of Korea,[1] Kyoungwon University,[2] Hallym University[3] Korea
Purpose: To introduce a new tearscope (Hosik's Tearscope) made of paper for lipid layer of tearfilm and to present the findings of dry eye syndrome, meibomian gland dysfunction (MGD) and ocular GVHD (Graft versus host disease) patients.
Methods: We made this tearscope with a common copying paper (3 x 6 cm). We made a round hole (diameter 6.0 mm) at the 1/4 end of the paper for observation window. After positioning the tearscope in front of the patient eye (1 cm from the cornea), we found interference

patterns on the cornea through the hole with slit lamp. After adjusting magnification, we captured the images by CCD (charge coupled device) camera connected to the slit lamp. We took photographs of tearfilm of dry eye syndrome, MGD and ocular GVHD patients. Additionally, we measured BUT (tear break up time) and took photographs of upper lid margin and cornea stain patterns.
Results: Colors of lipid layer were grey color (thin, meshwork, wave, amorphous), color (a few color, many color) depending on the thickness and quality of lipid layer. In healthy subject, meshwork, wave or amorphous patterns with grey color were observed. In dry eye syndrome patients, thin patterns with grey color were observed frequently. In MGD or GVHD patients, many colors (red to violet) were found and globus or clumping were found in severe cases. It took less than 10 seconds to take photographs of lipid layer using this tearscope and it could be used easily. Interference patterns of lipid layer could be saved as image files and dynamic changes of tearfilm by blinking could be saved as movie files.
Conclusions: This new tearscope made of paper was easy to use and effective to evaluate the lipid layer in tearfilm related disease.
Financial disclosure: We are applying patent for this Hosik's tearscope.

THE EFFECT OF THE BLINK CYCLE ON THE MULTILAMELLAR MODEL OF THE TEAR FILM LIPID LAYER

P. Ewen King-Smith,[1] Richard J. Braun,[2] Kathleen S. Reuter,[1] Daniel Powell,[1] Heather L. Chandler,[1] Melissa D. Bailey.[1] College of Optometry, Ohio State University, Columbus, OH,[1] Mathematical Sciences Dept., University of Delaware, Newark, DE,[2] U.S.A.
Purpose: A multilamellar model of the tear film lipid layer, TFLL, has recently been proposed (King-Smith et al., 2013, ARVO #6007; The Ocular Surface, in press). The nonpolar lamellae provide the main resistance to evaporation and are 5 nm thick, interdigitated bilayers of cholesteryl and wax esters. We consider how this model would respond to the blink cycle.
Methods: The model was evaluated in relation to color images of the TFLL from 95 subjects (75 women, 42 dry eye, mean age 51±14 yr). High resolution images covered an area of 200 μm diameter (0.5 μm/pixel) and low resolution images covered 6 mm diameter (6 μm/pixel). Stroboscopic illumination was used to eliminate blur from image movement.
Results: Three image features were considered. 1. At the start of the downstroke, wrinkling of the TFLL was often seen over much of the cornea. 2. A narrow, thick band of lipid accumulates under the lid in the downstroke. 3. During the upstoke, small "clouds" of lipid may be seen within an apparently bare aqueous surface (King-Smith et al., 2011, The Ocular Surface 9, 197).
Conclusions: The observations can be interpreted in terms of the following proposed properties of the lamellae. A. The lamellae cannot be readily stretched or compressed. B. They can be easily bent. The tapered structure of cholesteryl ester molecules may aid in forming folds. C. The lamellae can readily slip over each other. The three image features can be related to these properties, as follows. 1. The wrinkling of the TFLL may be related to the incompressibility of the lamellae. 2. The three properties permit the TFLL to fold like an accordion, forming the thick band under the lid. 3. Clouds may correspond to tearing of the (inextensible) lamellae during the upstroke. Later, surface tension forces may pull the clouds together to form a continuous TFLL.
This presentation is intended as a basis for discussion, rather than a finalized model.
[Supported by EY017951 (PEK-S) and NSF 1022706 (RJB)]

OCULAR SURFACE COMMENSAL BACTERIA IN HEALTH AND DISEASE AND INNATE IMMUNITY ON THE OCULAR SURFACE

Shigeru Kinoshita, M.D., Ph.D. Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan.
The basic understanding of ocular-surface mucosal innate immunity upon interaction with commensal and/or pathogenic bacteria has been one of the enlightening topics in scientific fields related to the ocular surface. Thus, we extensively examined commensal bacteria species in normal subjects, from newborns through to the elderly. Our findings showed that up until 6 years of age, *Streptococcus pneumoniae* (*S. pneumoniae*) is the dominant commensal bacteria, followed by other *Streptococcus species*, *Propionibacterium acnes* (*P. acnes*), and coagulase-negative *Staphylococcus* (CNS). In children over 7 years of age, *P. acnes* becomes more dominant than *Streptococcus* species. In early and middle-age adults, the dominant commensal bacteria is *P. acnes*, followed by CNS, with a virtual disappearance of *Streptococcus* species. Conversely, the dominant commensal bacteria in elderly subjects are *Staphylococcal* species, including methicillin-resistant *Staphylococcus aureus* (MRSA), followed by CNS and P. acnes. A solid understanding of the principal age-related alterations of commensal bacteria in normal subjects is essential for understanding the ocular surface in health and disease.The ability of cells to recognize pathogen-associated molecular patterns depends upon the expression of a family of toll-like receptors, and RIG-1, MDA5, etc. Immune-competent cells such as macrophages can recognize the various microbial components mentioned above and induce the inflammation, and then exclude the microbes. Ocular-surface epithelial cells also selectively respond to microbial components, inducing limited inflammation due to the unique innate immune response to coexistence with commensal bacteria on the ocular surface.

NONINVASIVE OBSERVATION OF TEAR FILM BREAK-UP WITH NEWLY DEVELOPED KERATOGRAPH

Shizuka Koh,[1] Chikako Ikeda,[1] Yoshihiro Takai,[1] Shinya Watanabe,[1] Hitoshi Watanabe, [1,2] Motokazu Tsujikawa,[1] Naoyuki Maeda, [1] Kohji Nishida[1]
[1] Department of Ophthalmology, Osaka University Graduate School of Medicine, Osaka, Japan, [2] Kansai Rosai Hospital, Amagasaki, Japan
Purpose: To observe tear film break-up and meibomian glands noninvasively and investigate the relationship between noninvasive tear film break-up time (NIBUT) and meibomian glands.
Methods: Enrolled were 34 eyes of 34 patients with aqueous-deficient dry eye and 29 eyes of 29 normal subjects. Newly developed Keratograph 5M (Oculus, Wetzlar, Germany) were used for NIBUT measurement and observation of meibomian glands. Location of first tear film break-up point were recorded and classified into 4 quadrants; superior nasal (SN), superior temporal (ST), inferior nasal (IN), and inferior temporal (IT). Partial or complete loss of the meibomian glands was scored for each eyelid using 4 grades (meiboscores): grade 0 (no loss of meibomian glands) through grade 3 (the lost area was more than two thirds of the total meibomian gland area). The meiboscores for the upper and lower eyelids were summed for each subject.
Results: NIBUT values of dry eye (4.7±1.3 seconds) were significantly less than those of normal eye (9.8±6.8 seconds) ($p<0.001$). In dry eye, first tear film break-up points found in SN, ST, IN, and IT were 8.8, 11.8, 50.0, and 29.4 , respectively. The occurrence ratio in IN was significantly greater than those in others ($p=0.002$). In normal eyes, first tear film break-up points found in SN, ST, IN, and IT were 13.8, 27.6, 31.0, and 20.7 , respectively. There was no significant difference in the occurrence ratio among the location. Meiboscore of dry eye (1.6±1.4) were significantly greater than those of normal eye (0.5±0.8) ($p<0.001$). There were weak negative correlation between NIBUT and meiboscore. ($R= -0.26$, $p=0.03$)
Conclusion: In dry eye, the first tear film break-up point occurred most often in the inferior temporal quadrant, while there was no distinctive pattern in normal eyes. In addition, dropout of meibomian glands may have a role in NIBUT.
[The authors have no commercial relationship.]

EVALUATION OF P2Y2 RECEPTOR EXPRESSION IN THE SOD1 KNOCK OUT MICE

Takashi Kojima[1,2], Taeko Nagata[2,3], Ayako Igarashi,[3],Murat Dogru[2,3], Kazuo Tsubota[2], Jun Shimazaki[3]
[1]Gifu Red Cross Hospital, Gifu, Japan [2]Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan [3]Department of Ophthalmology, Tokyo Dental College, Chiba, Japan

Purpose: We previously reported that the ocular surface phenotype of the Sod1 knockout (KO) mice is similar to age related dry eye disease in humans. Currently, topical diquafosol, a P2Y2 receptor agonist, is targeted to treat dry eye disease by stimulating secretion of water and ocular surface mucins. Several reports have proved the clinical efficacy of topical diquafosol eye drops for the treatment of dry eye diseases. In the current study, time wise changes of P2Y2 receptor mRNA expression were evaluated in the Sod1 KO and the wild type mice.

Methods: Fifty and 10 week old C57/B6 wild type (wt:6 male mice in each group) and *Sod1*$^{(-/-)}$ mice (6 age and sex matched mice) underwent tear function tests including break up time, vital stainings and phenol red test. The same mice were also used for evaluations of P2Y2 receptor mRNA expressions. IHC of conjunctival specimens using P2Y2 receptor antibodies was also carried out after sacrifice (AbNova). SYBR Green–based quantitative real-time PCR was performed using StepOnePlus system (Applied Biosystems). Mouse glyceraldehyde-3-phosphate (GAPDH) primers and P2Y2 receptor primers were used. Data were normalized to GAPDH. A t-test was used for statistical analyses with statistical significance set at 5%. ARVO Statement for the Use of Animals in Ophthalmic and Vision Research was adhered to in this study.

Results: BUT and vital staining scores deteriorated with aging in both mice. The staining intensity of P2Y2 receptor antibodies increased significantly with aging in both mice. In both the wt and *Sod1*$^{(-/-)}$ mice, the P2Y2 receptor mRNA expression in 50 week old mice was significantly higher than that in the 10 week old mice (10 weeks wt mice: 0.44±0.25; 50 week wt mice: 2.25±0.55; 10 weeks *Sod1*$^{(-/-)}$ mice: 1.03±0.15; 50 weeks *Sod1*$^{(-/-)}$ mice: 4.16±2.1 (%RNA GAPDH).)

Conclusions: Increased mRNA expression of P2Y2 receptors was confirmed in the aging *Sod1*$^{(-/-)}$ mice. Targeting the P2Y2 receptors with P2Y2 agonists could rescue the tear functions and the ocular surface from the age related dry eye disease in the *Sod1*$^{(-/-)}$ mice.

DIQUAFOSOL SODIUM OPHTHALMIC SOLUTION INCREASES TEAR-FLUID SECRETION IN DRY-EYE PATIENTS WITH SJÖGREN'S SYNDROME

Aoi Komuro, Norihiko Yokoi, Hiroaki Kato, Yukiko Sonomura, Shigeru Kinoshita. Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan

Purpose: Animal studies have shown that diquafosol sodium (DQS) ophthalmic solution that contains the $P2Y_2$ receptor facilitates water secretion from the conjunctiva. The purpose of this present study was to evaluate the increase in tear fluid volume induced by 3% DQS in dry eye patients with Sjögren's syndrome (SS).

Methods: This study involved 17 dry-eye patients with SS (1 male and 16 females; mean age: 66.4±10.1SD years) who underwent topical instillation of two ophthalmic solutions; artificial tears (AT) in 1 eye and DQS in the fellow eye, in a masked manner. After evaluation of corneal and conjunctival staining, the radius of the lower central tear meniscus radius (TMR, mm) before (baseline) and at 15 minutes after instillation were measured using a video meniscometer to evaluate changes in tear fluid volume. Simultaneously, those 17 patients self-evaluated symptoms of wetness via the use of a visual analog scale (VAS).

Results: In all patients, the optical instillation of DQS increased the TMR at 15 minutes post instillation (0.21 ± 0.08) compared to at baseline (0.16 ± 0.07) (P<0.001, paired t-test). In the eyes treated with AT, there was no significant difference between at baseline (0.18 ± 0.09) and at 15 minutes post instillation (0.18 ± 0.09). The VAS score for wetness (baseline; 15-minutes post instillation) in the DQS- and AT-treated eye groups were 12.9±14.2; 36.9±19.0 and 11.6±11.1; 36.1±23.2, respectively. Significant increases were observed in both groups (P=0.0001 and P=0.0003, respectively). In the DQS-treated eyes, the difference of TMR between at baseline and at 15 minutes post-instillation was not correlated to the baseline value of the Schirmer test, corneal staining score, or conjunctival staining score.

Conclusion: The findings of this study show that DQS increases tear-fluid secretion on the ocular surface in dry eye patients with SS, yet its action might not be reflected upon the primary lacrimal gland function.

DEBRIDEMENT-SCALING OF THE LINE OF MARX AND KERATINIZED LOWER LID MARGIN IS EFFECTIVE IN INCREASING MEIBOMIAN GLAND FUNCTION AND PATIENT COMFORT

Donald Korb,[1,2] Caroline Blackie.[1,2] Korb Associates,[1] Boston, MA, TearScience Inc, Morrisville, NC.[2]

Purpose: To evaluate whether mechanical debridement-scaling of the Line of Marx (LOM) and keratinized lower lid margin would improve meibomian gland (MG) function and reduce dry eye symptoms.

Methods: 16 symptomatic subjects undergoing treatment for evaporative dry eye (age range: 26-81 yrs, mean: 55.9 +/- 15.0 yrs) who also evidenced anteroplacement and a thickened LOM, were enrolled. Exclusion criteria included active ocular infection/inflammation, ocular surgery within the previous 6 months, lid abnormalities other than those related to MGD and/or normal age related lid margin changes. Prior to debridement-scaling the LOM was stained with lissamine green (Odyssey Medical, TN). Debridement of the stained LOM using a stainless steel, golf club spud (Akorn Ophthalmics, IL) was followed by scaling of the entire width of the keratinized lower lid margin. MG function and symptoms were assessed pre and approximately 1-month post lid margin debridement-scaling. No new or additional treatment was permitted during the 1-month post debridement period. MG function was evaluated along the full length of the lower lid margin using the standardized MG evaluator. Symptoms were evaluated with the SPEED questionnaire (max. score = 28). Only data for the right eye is reported.

Results: There was a significant decrease in symptoms and increase in the number of functional MGs as a result of the debridement-scaling. Symptoms: baseline mean pre-debridement-scaling = 13.4 +/- 4.6 and 1 month post-debridement-scaling = 10.5 +/- 3.7 ($p < 0.0001$, paired t-test). Number of functional MGs: baseline mean pre-debridement-scaling = 2.5 +/- 1.2 and 1 month post-debridement-scaling = 3.7 +/- 1.4 ($p = 0.0007$, paired t-test).

Conclusion: The results indicate that debridement-scaling of the LOM and keratinized lower lid margin provides significant symptom relief and improves MG function. Thus, lid margin debridement-scaling should be given further consideration in the context of age related lid margin changes, and for the prevention and management of MGD and dry eye symptoms.

MEIBOMIAN GLAND FUNCTION CANNOT BE PREDICTED BY MEIBOGRAPHY UNLESS THERE IS TOTAL MEIBOMIAN GLAND DROP OUT IN PATIENTS WITH MGD

Donald Korb,[1,2] Caroline Blackie[1,2], Heiko Pult PhD.[3] Korb Associates, Boston, MA,[1] TearScience Inc, Morrisville, NC,[2] Cardiff University, UK.[3]

Purpose: To determine if meibography results could predict meibomian gland function in patients with meibomian gland dysfunction (MGD).

Methods: Patients (n=20) diagnosed with MGD who met the inclusion criteria for the study were enrolled. Inclusion criteria: over 18 years, no lid abnormalities, no current ocular inflammation/disease, no ocular surgery within the last 6 months, no history of lid surgery.

Symptoms were scored using the SPEED questionnaire. MG function was performed prior to meibography. Meibography was performed using the Modi Topographer and analyzed using the Phoenix software provided. Lower lid MG function was evaluated using the standardized MG evaluator and MG drop out was analyzed in three equal sections: nasal (N), central (C) and temporal (T). MG Drop out was defined as level 3 loss (>75%), level 2 loss (74-50%), level 1 loss (49-25%) and level zero loss (24-0%)

Results: Only data for right eyes are presented. The mean age and symptom score of the patients was 50.7±17.7 years (7 males; 13 females) and 12.4±5.4 respectively. The average number of functional glands per lid section was: N=1.6±1.5, C=1.3±1.4, T=0.2±0.5. The N and C lid sections had significantly more functional MGs relative to the T section ($p < 0.005$). Conversely the N sections had a significantly higher frequency (55%) of level 3 MG drop out (>75%) relative to the C(25%) and T(25%) sections. Similarly the N sections had much lower frequency (10%) of level 1 drop out (24-0%) compared with the C(45%) and T(55%) sections.

Conclusion: While the nasal sections of the lower lid consistently evidence higher numbers of functional MGs relative to the central and temporal sections of the lid, the opposite is true for the level of MG drop out as determined by meibography. These counterintuitive results strongly indicate that meibography cannot be used to predict MG function except in the case of total gland drop out, when the glands are completely absent.

THE EFFECT OF SYSTANE® BALANCE ARTIFICIAL TEARS ON CORNEAL SENSITIVITY AND TEAR FILM STABILITY

Illés Kovács[1], Lóránt Dienes[1], Huba Kiss[1], János Németh[1], Mari-Carmen Acosta[2], Juana Gallar[2]. [1]Department of Ophthalmology, Faculty of Medicine, Semmelweis University, Budapest, Hungary. [2]Instituto de Neurociencias, Universidad Miguel Hernandez-CSIC, San Juan de Alicante, Spain.

Purpose: To investigate the short term effects of Systane® Balance (SB) artificial tear on corneal sensitivity and tear film stability.

Method: We measured with a 0-10 visual analogue scale (VAS) the irritation sensation evoked by selective mechanical, chemical, and thermal stimuli applied on the central cornea of 10 healthy volunteers (age: 32.1±3 year) using the original Belmonte gas esthesiometer. Suprathreshold moderate and intense mechanical (170 and 260 ml/min), chemical (55% and 75% CO2 in air), and cold (-1.5 and -3.9 ºC corneal temperature change) stimuli were used. We did the measurements before and 5 min after topical application of SB. The non-invasive tear film break up time (NIBUT) was also measured in one eye with Keeler Tearscope Plus, before and after SB.

Results: Irritation sensation VAS values were significantly lower for moderate stimulation than for intense stimulation ($p<0.01$), except for cold stimulation ($p>0.05$). After one drop of SB, no significant changes were observed in the VAS response to moderate stimuli (mechanical: 3.1±0.9 vs. 3.1±0.9 VAS units before and after, respectively; chemical: 4.2±0.9 vs. 2.6±0.6; cold: 1.1±0.4 vs. 0.8±0.3; $p>0.05$). VAS values for selective intense mechanical, chemical and cold stimulation of the cornea decreased significantly after SB application (mechanical: 5.3±0.8 vs. 2.3±0.8 VAS units before and after, respectively, $p<0.01$; chemical: 6.7±0.5 vs. 5.0±0.5, $p<0.01$; cold: 2.4±0.7 vs. 0.9±0.2; $p<0.05$). After one drop of SB the NIBUT increased significantly from 11.1±2.4 s to 14.15±2.4 s ($p<0.05$).

Conclusion: Shortly after one drop of Systane® Balance, the tear film break up time increased, and the irritation sensation caused by intense suprathreshold selective stimulation of corneal sensory nerves were decreased. Decreasing of the irritative sensation is probably caused by a thicker tear film, and a better tear film structure which protects the cornea against environmental stimuli.

CR: None. Support: SAF2011-22500 and CSD2007-00023 (JG, MCA); OTKA-NN-106649 (LD, IK).

RESPONSES OF THE OCULAR SURFACE TEMPERATURE AND LIPID LAYER THICKNESS TO STRESSED ENVIRONMENTAL CONDITIONS IN NORMAL AND DRY EYES

Ranjini Kottaiyan[1]; Holly Hindman[1, 2]; Gheorghe Salahura[1, 2]; James M. Zavislan[2, 1]; Geunyoung Yoon[1, 2, 3]; James V. Aquavella[1] 1.Flaum Eye Institute, University of Rochester 2. Center for Vision Science 3.The Institute of Optics, Rochester, NY, USA

Purpose: To study the tear film effects by stressing the ocular surface in low relative humidity (RH) and high temperature conditions and to elicit the different responses between normal and dry eye subjects using thermal imaging and tearscope, quantifying two-dimensional distribution of temperature and lipid thickness, respectively.

Methods: A Thermal camera and a quantitative tearscope were used to measure a total of 14 eyes comprising 5 normal and 9 aqueous deficiency (ADDE) dry eyes before (baseline) and after 30 minutes of acclimation to three different environmental conditions; 40% RH and 75 °F (C1), 20% RH and 75 °F (C2), and 40% RH and 85 °F (C3),in our controlled chamber. Thermal data was analyzed to calculate the average ocular surface temperature (OST) in the central 9 mm of the cornea, while the average lipid thickness (LT) and uniformity were calculated from the central one-third of the cornea. Changes in OST and LT were compared between the two groups.

Results: The average OST/LT at baseline was 34.9 ± 0.2°C/40.4 ±8.3 nm and 33.8 ± 0.3°C/57.9 ± 30 nm in normal and ADDE subjects, respectively. After 30 min of acclimation, normal subjects did not show a significant change in OST under any condition. ADDE, however, showed a significant change in OST in C1 (mean difference = -0.65°C, P= 0.022) and C3 (mean difference = -0.63°C, P= 0.003). On the lipid thickness, ADDE showed an increase by 8.77 ± 10.95 nm in C1 while a decrease of 14.47 ± 29.04 nm, in C3.

Conclusions: Normal subjects were able to maintain their OST in response to environmental changes, while the ADDE was sensitive to environmental changes and changed more in response to temperature than humidity challenges. This maybe, because of the greater evaporation occurring in the temperature challenge than the humidity challenge in the ADDE patients.

None of the authors have any commercial relationships. Supported by an unrestricted grant from Research to Prevent Blindness.

IMAGING INFLAMMATORY PROCESSES IN VIVO

Paul Kubes

It is clear that sterile injury does engage the innate immune system to help in resolution. Using a very simple model of sterile injury and various imaging modalities, we can observe the role of various blood borne cells in repair. Initially platelets arrive within the first few seconds after perturbation helping to recruit neutrophils to the site of injury. Chemotactic gradients within the vasculature and from dead cells help to recruit the neutrophils. Shortly thereafter, monocytes enter the site first as inflammatory monocytes recruited via CCR2 and then as anti-inflammatory monocytes. In addition, iNKT cells are initially rejected from the site of injury but then they are recruited and help shape the environmental milieu. This leads to very effective healing and disruption of these processes alters resolution. By contrast, in infectious inflammation, the platelets do not appear to bind avidly to endothelium but rather interact with resident macrophage to help encapsulate certain bacteria and also bind to neutrophils to form neutrophil extracellular traps to further ensnare bacteria. The adhesive mechanisms for neutrophils also differs between sterile and infectious inflammation however the monocyte recruitment appears to be similar. Finally, iNKT cells tend to bind to cells that have engulfed bacteria including Kupffer cell and a CD1d dependent antigen presentation ensues. These process can differ from organ to organ.

COMPLEX LIPID MIXTURES THAT RESEMBLE TEAR FLUID LIPID LAYER DO NOT DECREASE EVAPORATION RATE

Pipsa Kulovesi, Antti H. Rantamäki, Juha M. Holopainen. Helsinki Eye Lab, Department of Ophthalmology, University of Helsinki, Helsinki, Finland.

Purpose: The tear film lipid layer stabilizes the tear film, but in addition lipids are believed to prevent evaporation. However, the evidence of the latter property is scarce. We studied the properties of wax esters (behenyl oleate (BO), lignoceryl lignocerate (LL), linolenyl oleate (LI), or lauryl oleate (LA)) mixed with L- -phosphatidyl choline (PC), cholesterol oleate (CO), and triglycerides (TG) and possible evaporation retardation effect of these lipid mixtures. These artificial tear film lipid mixtures mimic tear film lipid layer.

Methods: 12 samples of artificial tear fluid lipid layer mixtures were made by mixing PC, CO, and TG with wax esters (BO, LL, LI, or LA). These mixtures were analyzed using a Langmuir-trough with two moving barriers for compression studies and a custom built system was used for the evaporation studies at 35 °C. Lipid films were viewed using Brewster angle microscopy (BAM).

Results: Surprisingly none of the lipid mixtures decreased the evaporation rate. All lipid mixtures had similar compression isotherms regardless of the wax ester species or its concentration. This suggests that the wax esters are most likely not at the air-water interface, but rather are constructed on top of the polar lipids with other non-polar lipids. BAM images revealed structural differences between the samples: samples with LI and LA formed rather uniform films whereas LL and BO formed more condensed regions.

Conclusions: Complex lipid mixtures that resemble tear fluid lipid layer are organized in a layered manner so that amphiphilic lipids are adjacent to the aqueous phase and the non-polar lipids are layered on top of these. Yet, this layering of lipids does not decelerate evaporation. The current study suggests that the main function of lipids is not to prevent evaporation of tear fluid.

AUTOMATED MEASUREMENT OF MEIBOMIAN GLAND DROPOUT

Carolina M.E. Kunnen[1,2,3], **Percy Lazon de la Jara[1,2,] Brien A. Holden[1,2,3], Eric B. Papas[1,2,3]**

[1]Brien Holden Vision Institute, Sydney, NSW, Australia; [2]School of Optometry and Vision Science, University of New South Wales, Sydney, NSW, Australia; [3]Vision CRC, Sydney, NSW, Australia;

Purpose: To establish objectively measurable characteristics of the palpebral meibomian glands that correspond with judgments of meibomian gland dropout made by human observers.

Methods: Images of the upper eyelid from 21 individuals were captured by using non-invasive infra-red photography and digitally analysed with custom made image processing software (in MATLAB). The following objective meibomian gland parameters were compared to a subjective grading scale: proportion relative to eyelid surface area, maximum width, mean width and the total number of glands. Two experienced observers graded the same images for meibomian gland dropout using a 0 to 4 scale.

Results: Correlation analysis indicated significant correlations ($p < 0.05$) between the average subjective gradings by the observers and objective analyses in the meibomian gland proportion coverage, the number and maximum width of the glands. However the proportion of the meibomian gland coverage has a stronger correlation with the grading score ($R^2 = 0.75$, $p < 0.001$) than the number of glands ($R^2 = 0.53$, $p < 0.001$) and the maximum width ($R^2 = 0.24$, $p = 0.024$). The mean width of the glands ($p = 0.98$) showed no correlation. 95% limits of agreement between observers were -1.56 to 0.92 grade units.

Conclusion. Subjective meibomian gland dropout grading was well represented by objective measurement of the proportion of the eyelid covered by meibomian glands. Objective, automatic assessment of meibomian gland morphology is a viable alternative to subjective grading.

TEAR SECRETION IMPAIRMENT AS A FUNCTION OF SEVERITY OF HERPETIC KERATITIS

M Labetoulle (1,2), M M'Garrech (1), A Rousseau A (1,2), G Kaswin (1), A Sauer (3), T Bourcier T (3), E Barreau E(1).

1) Ophthalmology Department, Hôpital de Bicêtre, Assistance Publique-Hôpitaux de Paris, 94275 Le Kremlin-Bicêtre Cedex, FRANCE

2) Laboratoire de Virologie Moléculaire et Structurale, CNRS, UPR 3296, Centre Nationale de la Recherche Scientifique, 91198 Gif-sur-Yvette

3) Ophthalmology Department, Nouvel hôpital Civil, hôpitaux universitaires de Strasbourg, BP 426, 67091 Strasbourg, France

Purpose: To assess the quality of tear secretion in eyes of patients with a history of unilateral and recurrent herpetic keratitis

Methods: Prospective non-interventional study including 33 patients with a history of recurrent herpetic keratitis (either archipelago keratitis, KA, or kerato-uveitis, KU, or neurotrophic keratitis, KN). They were compared with 33 normal subjects. A complete ophthalmologic examination was performed, including tear osmolarimetry, tears break-up time (TBUT), Schirmer I test, and corneal sensitivity.

Patients with other potential causes of abnormal tears were excluded, and all tests were performed at least 3 months after the last relapse of keratitis. Controls were selected among asymptomatic patients, and were matched for age and gender with patients.

Results: The patients group (19 men, 14 women, aged 52 ± 7 years) included 16 patients with KA, 13 with KU, and 6 with KN. In the control group, all tests were symmetrical between the two eyes. In the 3 groups of patients, tear osmolarimetry was significantly greater in affected eyes than in controls, as well as TBUT was significantly reduced. In contrast, Schirmer I test was reduced only in eyes of patients with a history of KU or KN. Finally, only KN eyes were statistically less sensitive than healthy eyes.

Conclusions: Recurrent herpes keratitis induced changes in lacrimal secretion, even when the disease is apparently quiescent, and the abnormalities are more important as the corneal disease is progressing In the context of our study, tear hyperosmolarity appeared to be a particularly sensitive test to detect impairment of tear secretion.

Financial disclosure: No

LACRITIN SURVIVAL SIGNALING RESTORES CORNEAL EPITHELIAL HEALTH BY SEVERAL MECHANISMS AND IS REDUCEABLE TO A SYNTHETIC MIMETIC.

Gordon W. Laurie,[1]Ningning Wang,[1] Jeffrey Romano,[1] Robert L. McKown,[2] University of Virginia,[1] Charlottesville, VA; James Madison University,[2] Harrisonburg, VA, USA

Purpose: Topical lacritin negates lissamine green staining of eyes of aqueous deficient Aire$^{-/-}$ mice, and restores health of cultured corneal epithelial cells stressed with the inflammatory cytokines interferon gamma (INFG) and tumor necrosis factor (TNF). Normal human tears are also protective but not normal tears immunodepleted of lacritin, suggesting that lacritin may be a master protector. Here we further explore this prosurvival activity, and ask whether it is reduceable to a synthetic peptide.

Methods: FOXO1-ATG7 autophagic signaling was monitored in cultured human corneal epithelial cells stressed with INFG and TNF. Stressed cells were incubated with lacritin or lacritin truncation C-25 in the absence or presence of AKT inhibitor AKTVIII without or with purified recombinant lacritin or C-terminal truncation mutant C-25. FOXO1 immunoprecipitates were blotted for ATG7 or acetyl lysine. Phosphorylation of p62 (captures ubiquitinated proteins into autophagy) was monitored. FOXO3 translocation in INFG/TNF stressed cells served as a measure of cell survival after treatment with lacritin, C-25, or with lacritin peptides N-94 or N-94/C-6.

Results: Lacritin stimulated ligation of FOXO1 with ATG7 was

inhibitable with AKTVIII. AKTVIII also blocked FOXO1 acetylation and inhibits lacritin survival activity. Lacritin stimulated the phosphorylation of p62 to enhance binding of ubiquitinated proteins. 10 nM N-94 or N-94/C-6 were equally effective as 10 nM lacritin in promoting corneal epithelial cell survival.
Conclusions: Lacritin dependent phosphorylation of FOXO1 is necessary for binding autophagic mediator ATG7. Lacritin stimulates the removal of ubiquitinated proteins from stressed cells by promoting the phosphorylation of p62. Lacritin survival activity requires only the 19 amino acid sequence KQFIENGSEFAQKLLKKFS (N-94/C-6).
[This research was supported by EY018222 and EY013143 to GWL; McKown: F, EyeRx. G.W. Laurie: E, TearSolutions LLC. P, UVa Licensing & Ventures Group].

OCULAR SURFACE IN SJOGREN'S SYNDROME

Lazreg Sihem Cabinet d'ophtalmologie Blida Algeria
Introduction: Sjogren's syndrome is a systemic autoimmune disease characterized by impairment of exocrine glands, particularly salivary and lachrymal glands, the consequence is a severe dry eye with corneal damage.
Patients and method: prospective study of 78 patients with dry eye syndrome , examined between September 2010 and November 2012, they all received a complete ophthalmologic examination, Schirmer, Fluorescein and lissamine green, immune serum assessment and salivary gland biopsy, in addition to the treatment prescribed by a rheumatologist, they all treated with tear drops and punctual plugs.
Results: 75 females, mean age 45.4+/- 12.6, and association with rheumatoid arthritis in 42% of cases, Oxford score on D0 was 6+/- 1.8 for both eyes, OSDI score on DO was 76.2+/- 10.2, wih a Schirmer average of 4.5+/-1.5, 40cases of filamentous keratitis, the SSA and SSB antibodies were positive in 85 and 89%, punctual plugs reduced the frequency of use of drops on 50% of cases.
Comments: Sjogren's syndrome is a serious disease, with an impact on the quality of life , corneal involvement is often very severe and sometimes irreversible, systemic treatment (plaquenil, corticosteroids, pilocarpine) do not have any impact on ocular damage.
Conclusion: dry eye in Sjogren's disease is perceived by the patient as the most difficult to live, multidisciplinary care is needed.

VERNAL KERATOCONJUNCTIVITIS CLINICAL AND THERAPEUTIC ASPECTS.

Lazreg Sihem, Cabinet d'ophtalmologie Blida
Purpose: to evaluate clinical aspects, specific sensitization, epidemiological and therapeutic characteristics of vernal keratoconjunctivitis (VKC)
Methods: Prospective clinical case series included 350 VKC patients,between May 2008 and May 2013, data included patients and family histories, results of ocular surface exams, allergic tests and response to corticosteroids assocated to mast stablizers (NAAGA) treatment.
Results: The great majority of VKC patients were male(87%), 36% of limbal forms, 56% of corneal complications, 24 keratoconus associated, a skin prick tests and specific serum IgE was positive in 58% and 64% of patients respectively. Therapeutic results were good in 73% cases, satisfactory with frequent relapses treated with corticosteroids in 21% cases, 5% of steroid dependent and 1% of several unresponsive forms.
Conclusion: vernal keratoconjunctivitis, very common in our climate remains a severe form by they corneal complications and diffficult managment.

EXPERIMENTAL DRY EYE-ASSOCIATED NEUROINFLAMMATORY CHANGES IN TRIGEMINAL GANGLIA

Hyun Soo Lee[1,2]; Kishore Reddy Katikireddy[1]; Thomas Dohlman[1]; William Stevenson[1]; Sunil K. Chauhan[1]; Reza Dana[1]
1. Schepens Eye Research Institute and Massachusetts Eye & Ear Infirmary, Harvard Medical School, Boston, Massachusetts, USA
2. Department of Ophthalmology&Visual Science, Seoul St. Mary's hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea
Purpose: To determine whether Dry Eye Disease (DED) induces neuroinflammatory changes in trigeminal ganglion (TG), we evaluated the expression of proinflammatory cytokines and neuropeptides in TG of a murine DED model.
Methods: Seven to 8 week old female C57BL/6 mice underwent main lacrimal gland excision, and were housed in a controlled environment chamber to induce experimental DED. Mice housed in room air following surgery served as controls. Expression of proinflammatory cytokines (IL-1 , IL-6, TNF) and neuropeptides (Substance-P, calcitonin gene related peptide (CGRP), vasoactive intestinal peptide (VIP), neuropeptide-Y (NPY), and nerve growth factor (NGF)) in the cornea and TG were evaluated using real-time polymerase chain reaction (PCR). In addition, glial fibrillary acidic protein (GFAP) expression and immune cell infiltration of TG were assessed by real-time PCR and flow cytometry.
Results: DED significantly increased mRNA expression of IL-6, TNF, and substance P in cornea and TG at day 5 and day 14, and CGRP and NGF mRNA expression increased in cornea but not TG. In the DED group, TG showed increased expression of GFAP and infiltration of CD4+ T cells, but not CD11b+ cells.
Conclusions: These findings suggest that DED-induced neuroinflammatory changes occur at the level of the trigeminal ganglion, and are not only restricted to the ocular surface.
This research was supported by NIH-R01/EY20889.

GENETIC VARIANTS IN THE TNFA ARE ASSOCIATED WITH KOREAN DRY EYE DISEASE

Hyun Soo Lee,[1,2,3] Chang Rhe Rho,[1,2,4] Kyung-Sun Na,[1,2,3] Jee-Won Mok,[1] Choun-Ki Joo[1,2,3]
Catholic Institutes of Visual Science,[1] Department of Ophthalmology & Visual Science,[2] College of Medicine, The Catholic University of Korea, Seoul St. Mary's hospital Eye Institute (SSEI),[3] Seoul, Korea, Daejeon St. Mary's Hospital,[4] Daejeon, Korea
Purpose: To determine whether variations altering the function or expression of TNFA, contribute to the pathogenesis of dry eye disease.
Methods: Genomic DNA was extracted from blood samples of unrelated dry eye disease patients (non-Sjogren's syndrome patients (n=200) and Sjogren's syndrome patients (n=100). Polymerase chain reaction and direct sequencing were used to screen variations in promoter region of TNFA gene. One hundred fifty control individuals without corneal disease were selected from the general population.
Results: We investigated 6 SNPs of TNFA; -1196 C>T, -1031 T>C, -863 C>A, -857C>T, -308 G>A (rs1800629) and -238 A>G (rs361525) in promoter. Among them, -1196 C>T, -857 C>T, rs1800629 and rs361525 were different between patient groups and control groups. The *A allele frequency of rs361525 in dry eye patients (2.6%) and Sjogrens' patients (2.5%) were decreased compared with control subjects (5.1%). The *A allele frequency of rs1800629 was lower in Sjogrens' patients (2.5%) than in the controls (4.8%). In -1196C>T variation, *T allele of both patient groups was decreased compared with control subjects. Whereas, the * T Allele frequency of -857 C>T was higher in the Sjogrens' patients (30.0%) than in the controls (16.8%). The genotype distributions of all polymorphisms of TNFA among the control subjects and the affected individuals were in Hardy-Weinberg equilibrium.
Conclusions: Our results suggested that the genetic variations of TNFA gene seem to be associated with dry eye predisposition in a Korean.

GLUCOSE METABOLITES CHANGES AND THEIR IMPACT ON THE CHANGES IN DRY EYE INDUCED MOUSE LACRIMAL GLAND

Hyung Keun Lee,[1,2] Yong Woo Ji,[1] Hye Mi Noh,[1] Eun Ae Jung[1]
1. Institute of Vision Research, Department of Ophthalmology, Yonsei University College of Medicine, Seoul, Korea., 2. Severance Institute for Vascular and Metabolic Research, Yonsei University College of Medicine, Seoul, Korea

Purpose: Lacrimal gland (LG) is one of the major target organs for dry eye disease (DED) and consisted with many different types of cells. Although the LG is responsible for the tear production, which may be required high energy, the cellular and molecular mechanisms responsible for the impairing LG secretion in DED are still poorly understood. The purpose of this study is to investigate the change of glucose metabolites in mouse LG in DED and determine their role in the change of LGs.

Methods: Six to 8-week-old female (C57BL/6) mice were used to induce DE by controlled environment chamber and subcutaneous injections of 0.1mL of scopolamine hydrobromide, 5mg/mL (Sigma-Adlrich). Then, LGs were secured from the mouse and preserved -80°C until metabolite analysis. By using 80% methanol extraction method, polar metabolites were obtained and metabolic profiles were acquired by Seahorse XF or LC-MS.

Results: Firstly, we determined concentration of lactate and alanine, as a marker for glycolysis, and found to significantly increase during the DE stressed LG. The concentration of lactate from dry eye induced LG is 25.6 times higher than normal from one week after induction. Then, by using LC-MS, concentration of glycolysis and Kreb cycle intermediates were measured. Interestingly, succinate, gamma amino butyric acid (GABA), citrate concentration was significantly elevated by DE induction with time dependent manner. In contrast, isocitrate and glutamate concentration was significantly decreased in DE induced LG.

Conclusions: DE induction causes derangement of glucose metabolism. The elevated glucose metabolites may induce inflammatory cytokines and responsible for further damage of LG by DE.

This research was supported by Advanced Science Research Program (grant no.: 2012R1A2A2A02009081) through the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Science, and Technology, Korea.

CULTIVATED TRIGEMINAL NEURONS FROM DRY EYE MICE INDUCE HIGHER EXPRESSION OF MATURATION MARKERS BY BONE MARROW-DERIVED DENDRITIC CELLS.

Sang-Mok Lee,[1] William Stevenson,[1] Kishore Reddy Katikireddy,[1] Hyun Soo Lee,[1] Thomas Dohlman,[1] Sunil Chauhan,[1] Reza Dana[1]
Schepens Eye Research Institute, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA, USA[1]

Purpose: The expression of maturation markers by antigen-presenting cells are known to increase in dry eye disease (DED), and their priming of T_H1 and T_H17 cells has been suggested as a potential mechanism for the pathogenesis of autoimmunity in DED. Though the cornea is the most densely innervated tissue, the role of corneal nerves - a branch of trigeminal neurons (TGNs) - in the immunopathogenesis of DED remains unclear. This study compared the effect of cultivated TGNs from DED on the maturation of cultivated bone marrow-derived dendritic cells (BMDCs) to that from control mice.

Methods: Bone marrow cells were harvested from C57BL/6 mice of 6-8 weeks age, and cultured in the presence of granulocyte/macrophage colony-stimulating factor (GM-CSF, 20ng/ml) for 6 days to proliferate immature DCs. Trigeminal ganglions were harvested from 8-10 week old mice 14 days after lacrimal gland excision (LGE) or sham operation. Neurons selected with density gradient were cultured for 3 days and loosely-adherent immature DCs were collected from the BMDC culture and added to the primary cultured TGN for 18 hours in the presence of IFN- (10ng/ml) to induce maturation. The expression level of MHC class II (mouse I_A/I_E) and CD86 on $CD11c^+$ DCs was analyzed using flow cytometry.

Results: The cultured TGN from LGE mice induced higher expression of MHC class II by IFN- -stimulated BMDC compared with TGN from sham operated mice (MFI: 709.15 ± 31.01 for LGE group and MFI: 639.71 ± 49.36 for sham operated group, $P \leq 0.05$, Mann-Whitney U test). The cell-surface expression of CD86, a co-stimulatory molecule, demonstrated similar changes (MFI: 23.65 ± 0.18 for LGE group and MFI: 22.86 ± 0.74 for sham operated group, $P \leq 0.05$).

Conclusion. Cultivated TGN from DED mice can induce BMDC maturation, suggesting that corneal nerves may directly contribute to DED pathogenesis by enhancing antigen presentation.

[This research was supported by NIH grant R01 EY20889]

THE ROLE OF NUTRITION AND EXERCISE IN DECELERATING THE DEGENERATIVE DISEASES OF AGING.

Christiaan Leeuwenburgh, University of Florida, Institute on Aging, Department of Aging and Geriatric Research, Gainesville, FL, USA

Exercise is likely the most potent "medicine" to counter almost all diseases. Both acute and chronic exercise increase oxygen flux in the systemic circulation and cellular microenvironment, generally improving adaptive responses. Researchers are continually discovering more about the biological mechanisms of exercise. Many of the benefits likely are tied directly to oxygen itself and the reactive oxygen species produced during heightened metabolic demands. Oxygen, which is a potent di-radical, produces powerful adaptive responses and increases antioxidant enzymes, angiogenesis, and mitochondrial biogenesis. Mitochondrial biogenesis proteins such as PGC-1 and TFAM lose their ability to function properly with age. Exercise upregulates these critical proteins and improves mitochondrial biogenesis, even at old age. Muscle is especially adaptive to increases in oxygen demands. In fact, we have shown marked increases in antioxidant enzymes following exercise training. Superoxide dismutase, glutathione peroxidase, and catalase all increase following chronic exercise training. These adaptive responses reduce basal oxidant production and prevent oxidative damage and genome instability (mutations and deletions in nuclear and mitochondrial DNA)—driving forces that cause aging. In contrast, recent studies produced troublesome observations that select oral antioxidant supplementation blunts these beneficial adaptions. We will present examples of the benefits of exercise on subjects with peripheral artery disease (PAD) and of the long-term effects on patients who underwent heart thoracic surgery while intermittently "exercising" the diaphragm muscle by receiving electrical stimulation. Exercise and several pharmaceutical compounds can upregulate the master biogenesis protein PGC-1 . We will show enhanced effects if a particular drug is combined with exercise. For example, studies combining exercise with testosterone, exercise with moderate caloric intake, and exercise with resveratrol have shown enhanced response on major parameters of biological and physical health compared to either regimen alone. Our goal is to provide additional validation to findings that exercise combined with natural and/or pharmaceutical compounds have the most powerful hormetic response to achieve optimal health.

This research was supported by grants from National Institute on Health.

OSTEOPONTIN AND ASSOCIATED INTEGRIN AND CD44 RECEPTOR EXPRESSION IN HUMAN CORNEA EPITHEL CELLS UNDER NORMAL AND WOUND HEALING IN VITRO CONDITIONS

Julia Lehmann[1], Maximilian Schmalfuss[1], Kathrin Hoffmann[1], Fabian Garreis[1], Friedrich Paulsen[1], Michael Scholz[1]. [1]Department of Anatomy II, Friedrich Alexander University Erlangen Nuremberg, Erlangen, Germany

Purpose: The phosphorylated glycoprotein Osteopontin (OPN) is considered to play an important role in tissue preservation and regeneration. OPN is upregulated in a variety of cells during inflammation, wound healing, autoimmune diseases and tumor pathogenesis. We aimed to characterize the basal expression of OPN and its receptors as well as the influences of OPN on wound healing mechanism at the ocular surface *in vitro*.
Methods: OPN, the non-integrin OPN receptor CD44 and the subunits of the most prevalent integrin-type OPN receptors, a 4, 5, 6, v and b 1, 3, 5, 8 were investigated in cultivated human conjunctival (HCjE-Gi) and corneal epithel cell (HCE) lines by means of RT-PCR and immunohistochemistry. Regulation of OPN receptor expression after stress induction with H_2O_2 (150 µM) was analyzed by real-time PCR. The metabolic cell activity (MTS) of OPN treated HCE cells were determined by using a CellTiter 96 aqueous MTS assay. We analyzed the effects of recombinant OPN on cell migration and wound healing by Electric Cell- Subtrate Impedance Sensing (ECIS) and in conventional scratch assays.
Results: Basal expression of OPN, the most prevalent integrin receptors as well as the non-integrin receptor CD44 was detected in HCE and HCjE-Gi cells under normal culture conditions. Neither HCE nor HCjE-Gi cells expressed the av protein. MTS assays revealed that OPN regulated its receptors.
Conclusion: Here we show that OPN and its most relevant receptors are basaly expression in HCE and HCjE-Gi cell lines. In addition, OPN regulates the receptor expression and thus seems to have an effect on corneal wound healing mechanism at the ocular surface. OPN shows an regulatory effect in scratch assays and in ECIS experiments. Real-time PCR results shows that OPN has an influence of receptor regulation after stress induction.

TOLL-LIKE RECEPTOR AGONISTS INCREASE IN VIVO AND IN VITRO EXPRESSION OF MATRIX METALLOPROTEINASES.
Carolina Lema, Rachel Redfern. University of Houston, College of Optometry, Houston, TX, USA
Purpose: Dry eye is a multifactorial disease that results in an increase in proinflammatory cytokines and matrix metalloproteinases (MMPs) leading into epithelial cell death and risk for ulceration. We have previously shown that topical application of toll-like receptor (TLR) agonists cocktail results in corneal ulceration in mice with experimental dry eye; therefore we sought to determine if TLR agonists increase MMP expression in the mouse cornea *in vivo* and in human ocular surface cells *in vitro*.
Methods: Telomerase-immortalized human corneal epithelial cells (hTCepi) were cultured with either TLR1-9 agonist, IL-1beta (10ng/ml) or media alone for 24hrs. The culture media was then collected and MMP-1, MMP-2, MMP-7, MMP-9 and MMP-10 levels were analyzed by Luminex assay (n=3). In C57BL/6 mice (n=3), TLR agonists cocktail or the vehicle control was topically applied to the right and left eye respectively following corneal wounding. After 24 hrs, corneal *in vivo* imaging by Heidelberg Retinal Tomography (HRT) was performed. The eyes were then removed, sectioned and MMP-9 expression was visualized and quantitated by immunostaining.
Results: *In vitro* treatment of hTCepi cells increased MMP-1 and MMP-9 in the culture media of cells treated with TLR2 (HKLM), TLR3 (Poly I:C), TLR5 (Flagellin) and TLR6/2 (FSL-1) agonists. In addition, TLR5 and TLR6/2 were also able to increase MMP-10 levels. Topical application of the TLR agonists cocktail increased immune cells recruitment into the anterior stroma of the cornea compared the vehicle control. Moreover, MMP-9 expression was increased in the corneal epithelium and stroma in response to the TLR agonists cocktail compared to the vehicle treated control eye.
Conclusions: Our results suggest that TLR activation may lead to ocular surface damage by increasing the production of MMPs on the mouse cornea and in human corneal epithelial cells.
There are no commercial relationships with the Authors.
This research was supported by NIH/NEI K08EY018113 (RR) and NIH P30EY07551 (UHCO core grant).

SHORT BUT DRY EYE IS NOT A SPECIAL TYPE OF DRY EYE
Michael A. Lemp, M.D.
Over the last few years Japanese researchers have reported on a new type of dry eye disease (DED) -Short BUT Dry Eye. This is in the context of the prior development of, a consensus definition of DED in Japan, which requires the following characteristics: decreased tear secretion, tear instability, positive ocular surface staining, and more recently positive symptoms. At the same time Western clinical investigators have reported that many patients with clear objective evidence of DED do not have ocular surface staining which is more characteristic of later stage disease development. Other newer findings include that many patients with DED do not report symptoms. These clinical findings suggest that the patients described as short BUT dry eye are identical to what is regarded in the West as an earlier stage of DED. In addition, the Japanese criteria suggest they are referring to aqueous tear deficient dry eye only as dry eye and not including evaporative dry eye the most common form of which is meibomian gland dysfunction. It would seem both systems are describing similar clinical findings and the differences are largely definitional. Our Japanese colleagues have called our attention to an important group of younger patients with significant implications for contact lens wear and refractive surgery.

COMPARISON OF TWO LACRIMAL SUBSTITUTES IN PATIENTS AFTER LASIK: HYLABAK (PRESERVATIVE FREE) VERSUS SYSTANE (PRESERVED).
Alla B. Lisochkina, Yuri S. ASTAKHOV, Sergei Y. Astakhov.
Department of Ophthalmology, Saint-Petersburg I.P. Pavlov State Medical University, 6-8 Lev Tolstoy str., Saint-Petersburg, 197022, Russia.
Purpose: To compare dry eye signs, symptoms and ocular tolerability after LASIK in patients treated with Hylabak® or Systane® for 3 months.
Methods: In this single centre, investigator-masked, prospective, non-inferiority, parallel group clinical study, patients undergoing bilateral LASIK were randomised to receive 0.15% sodium hyaluronate preservative free eye drops (Hylabak® in ABAK® dispenser system, Laboratoires Thea) or comparator (Systane®, Alcon) with polyethylene glycol 400, propylene glycol, 0.001% polidronium chloride as preservative; one drop in each eye four times daily for 3 months.
Results: 54 patients were enrolled. The mean fluorescein test scores (primary criterion) were similar on day 1 post surgery for Hylabak® and Systane® (0.26, 95% CI 0.11-0.41 and 0.28, 95% CI 0.12-0.44) respectively. At day 84, these scores improved to 0.11 (95% CI 0.01-0.22) and 0.04 (95% CI -0.03-0.11) respectively showing the non-inferiority of Hylabak®, while there was a trend (p=0.0571) for a more rapid improvement for Hylabak®group. An amelioration in ocular symptoms, LIPCOF test and BUT were observed in both groups at day 84. Investigators rated the global efficacy as 66.67% of 'Very Satisfactory' for Hylabak® treated patients at day 28 and 28% for Systane®; at 84 days, these figures increased for both products, however the difference was not statistically significant. Both treatments were well tolerated by the subjects and a majority of them reported the study treatments to be comfortable. Three AEs consisted in corneal oedema (related to LASIK surgery); no other systemic adverse events, serious AEs or deaths, no discontinuations or withdrawals due to the treatment adverse events were noted.
Conclusions: The efficacy of Hylabak® appears to be non-inferior to Systane®, so far as the primary efficacy parameter is concerned; both treatments were well tolerated in this post-LASIK surgery population

even on the first day after surgery.
Disclosure: Sponsored by Laboratoires Théa.

INFLUENCE OF AZITHROMYCIN ON HUMAN MEIBOMIAN GLAND EPITHELIAL CELLS

Yang Liu, Wendy R. Kam, Juan Ding and David A. Sullivan. Schepens Eye Research Institute, Massachusetts Eye and Ear, Harvard Medical School, Boston, MA, USA.

Purpose: The most common treatment for meibomian gland dysfunction (MGD) in the USA is the off-label use of topical azithromycin. This macrolide antibiotic is thought to be effective because of its anti-inflammatory and anti-bacterial actions, which may suppress the MGD-associated posterior blepharitis and growth of lid bacteria. We hypothesize that azithromycin can also act directly on meibomian gland epithelial cells to promote their differentiation. Our objective was to test this hypothesis.

Methods: Immortalized human meibomian gland epithelial cells were cultured in the presence or absence of azithromycin (10 μg/ml) for varying time periods. Cells were evaluated for morphology, acidic organelles (e.g. lysosomes; LysoTracker), cholesterol (Filipin) and neutral lipid (LipidTox) staining, and matrix metallopeptidase-9 (MMP-9) generation.

Results: Our findings demonstrate that azithromycin induces a marked, time-dependent accumulation of lipid in human meibomian gland epithelial cells. Within several days of azithromycin exposure, the number, size and staining intensity of intracellular cholesterol- and neutral lipid-containing organelles had clearly increased, as compared to those of vehicle-treated control cells. This azithromycin effect on lipids coincided with an enhanced MMP-9 production in these cells. Morphological examination indicated that azithromycin may promote terminal maturation of human meibomian gland epithelial cells, given that vesicle accumulation was often followed by a cell break-up and vesicle release. In contrast to these actions, azithromycin reduced cellular proliferation.

Conclusions: Our results support our hypothesis that azithromycin stimulates the differentiation, and possibly holocrine-like secretion, of human meibomian gland epithelial cells. (Supported by NIH grant EY05612, the Margaret S. Sinon Scholar in Ocular Surface Research fund, and the Guoxing Yao & Yang Liu Research Fund).

THE HUMAN MICROBIOME - A NEW FRONTIER IN HUMAN HEALTH.

Susan V. Lynch.
Division of Gastroenterology, Department of Medicine, University of California, San Francisco, 513 Parnassus Ave., San Francisco, CA 94143.

Culture-based assays, the workhorse of clinical microbiology for decades, provide a poor representation of the true microbial diversity associated with the human host. The recent application of culture-independent approaches to human samples has dramatically increased our ability to detect a greater diversity of species present in these communities and initiated the rapidly evolving field of human microbiome research. Application of high-throughput technologies to interrogate microbial consortia associated with the human superorganism has revealed the presence of highly functional communities of organisms that represent up to 90% of all cells in the human body and encode as much as 99% of the total functional capacity of the human holobiont. Though the field is still nascent, microbiome studies, particularly those focused on disease states have begun to identify microbial driven processes that play a pivotal role in defining host health status. Given the inherently microbial colonized state of the human system and the close association between human disease, microbiome perturbation and chronic inflammation, novel therapeutic strategies arising from this field will likely involve microbial restoration ecology approaches to restore ecosystem homeostasis in the human host.

PROTEIN POLYMER MEDIATED DELIVERY OF LACRITIN THERAPEUTICS

J. Andrew MacKay, Wan Wang, Gordon W. Laurie, Sarah Hamm-Alvarez

The delivery of protein therapeutics to the anterior segment is hindered by convective and proteolytic clearance of the ocular surface. To address this fundamental obstacle, we explore genetically engineered protein polymers derived from human tropoelastin. Protein polymers are repetitive amino acid sequences that we express in bacteria, seamlessly fuse to functional proteins, and tune to assemble micro and nanostructures on the ocular surface. A class of biocompatible protein polymers called Elastin-like polypeptides (ELPs) undergoes a thermally reversible phase separation above an adjustable transition temperature. These ELPs reversibly form depots, assemble nanoparticles, and even adhere to contact lenses. To explore the potential of ocular ELP therapeutics we selected a potent prosecretory mitogen called lacritin. Integral to the homeostasis of the ocular surface system, lacritin has emerged with potential therapeutic activity related to tear secretion and corneal epithelial cell regeneration. As a peptide therapeutic, lacritin must be dosed frequently to elicit responses, which may hinder its success as a pharmaceutical. To influence the bioavailability of lacritin on the ocular surface, we fused it with a small library of ELPs with a range of assembly properties. Lac-ELP fusions were expressed in E. coli and purified using ELP-mediated phase separation and size exclusion chromatography. Phase behavior and nanoparticle assembly were characterized by optical density, Dynamic Light Scattering (DLS), TEM and Cryo-TEM. In vitro activities were tested using rabbit lacrimal gland acinar cells (LGACs), SV40-immortalized human corneal epithelial cells (HCE-T), and in vivo activity was evaluated in non-obese diabetic mice. The Lacritin-ELP fusion proteins imparted thermo-sensitive assembly of either micron-sized coacervates or 130-140nm diameter micelles depending on the sequence of their ELP tag. Exogenous Lac-ELPs promoted β-hexosaminidase secretion from rabbit LGACs. Also, they were taken up by and evoked Ca2+ wave propagation in both LGACs and HCE-T cells. Supporting their potential for in vivo activity, Lac-ELPs were shown to induce tear secretion in the NOD murine model of dacryoadenitis. As a novel mechanism for loading contact lenses with protein, ELPs were tuned to attach onto contact lenses. Their total retention time on lenses was confirmed to be both temperature and Tt dependent. Exploration of thermo-responsive Lac-ELPs may provide an alternative approach to adjust their ocular retention and interaction with target cellular receptors. Moreover, biocompatible elastin-like polypeptides (ELPs) appear to provide a reversible, temperature dependent bridge between potential ocular therapeutics and contact lenses above the ocular surface.
This work was supported by the USC School of Pharmacy, the USC Whittier foundation, and the USAMRAA/TATRC VRP HAD 11262019.

LENS CARE INFLUENCE ON OCULAR COMFORT IN SILICONE HYDROGEL DAILY WEARERS.

Cécile Maissa[1,2], Michel Guillon[1,2], Renee Garofalo[3], Jami Kern[3]. [1]OTG Research & Consultancy, London, UK; [2] School of Life and Health Science, Aston University, Birmingham, UK, [3]Alcon Research. Ltd., Fort Worth, TX

Purpose: AOSept® Plus® used with two silicone hydrogel contact lenses has been shown to produce significant increase in the Non Invasive Break Up Time after 3 months of use and a decrease in eyelid hyperaemia and papillae, compared with the same contact lenses used with PHMB care systems. The objective of this retrospective analysis was to evaluate the effect of improved tear film stability and eyelid tissue status on comfort.

Methods: Thirty-seven symptomatic contact lens wearers, wearing either *ACUVUE® OASYS®*, or PureVision™, used AOSept® Plus® for three months. Baseline measurements with the subjects' habitual PHMB care systems and measurements after three months of AOSept® Plus® use were analyzed. The subjective comfort was recorded for three conditions (overall, daytime & evening); at both visits, ACUVUE® OASYS® contact lenses were ≥11 days old and PureVision™ contact lenses ≥25 days old.
Results: Comfort was significantly better ($p \leq 0.001$) at follow-up than at enrolment (Mean increase 4.9 to 8.6 points on 0-100 scale). At the 3 month visit, a negative correlation between comfort and papillae grading was recorded (r= -0.241 to -0.290 p= 0.012 to 0.039); further, the subgroup with Grade 0 papillae achieved a significantly higher comfort than the subgroup with Grade 3 papillae ($p<0.05$ 61.5 vs. 83.0). An inverse relationship was observed between NIBUT and comfort: the subgroup with a Median NIBUT ≥ 6.8s (Top 25%) had a higher daytime comfort than the rest of the population. Chi Square Automated Interaction Detector (CHAID) algorithm identified that the papillae grading was the primary factor (p=0.0351) influencing overall comfort.
Conclusion: The use of AOSept® Plus® in conjunction with *ACUVUE® OASYS®* and PureVision™ was shown to produce a significant increase in comfort. Associations between improved comfort and improved eyelid tissue status and pre-lens tear film stability were also demonstrated.
[The study was sponsored by Alcon Research Ltd]

CORNEAL STAINING IS A RELEVANT COMPLICATION OF USING PRESERVED CARE SYSTEMS.

Maria Markoulli[1] School of Optometry and Vision Science, The University of New South Wales, Sydney, Australia[1]
Solution-induced corneal staining (SICS) is a transient condition and is characterised by superficial punctate staining of the epithelium associated with the use of preserved multi-purpose contact lens solutions (MPS). It typically presents as diffuse corneal staining in at least four of the five corneal regions and may or may not be associated with symptoms. While it has been suggested that SICS is a result of contact lens-MPS incompatibility and that it is associated with infiltrative events, recent suggestions are that SICS is in fact preservative-associated transient hyperfluorescence (PATH), whereby preservative components, PHMB or PQ-1, complex with epithelial cells which then bind to fluorescein, giving the appearance of corneal staining. This theory suggests that SICS is in fact an artefact. Several counter-arguments have been raised, including the concept that SICS is observable without the presence of fluorescein. Additionally, *in vitro* studies suggest that staining with fluorescein indicates the presence of damaged or apoptotic cells, while studies using human corneal epithelial cells report that long-term exposure to diluted MPS might interfere with cell metabolism as well as the epithelium's barrier function. This part of the debate will present the current evidence supporting the view that SICS is a relevant complication of using preserved care systems.
[The author thanks the Tear Film and Ocular Surface society for conference attendance support; There are no commercial relationships to declare.]

DIURNAL CHANGES OF LIPID INFLAMMATORY MEDIATORS IN HUMAN TEARS WITH AND WITHOUT CONTACT LENSES

Simin Masoudi, PhD Candidate, [1, 2] Fiona Stapleton, PhD, Professor, [1, 2] Mark Willcox, PhD, Professor.[2] [1]Brien Holden Vision Institute, Sydney, Australia [2]University of New South Wales, Sydney, Australia
Purpose: Contact lenses are associated with discomfort during wear. This may be the result of stimulation of the ocular surface and production of pro-inflammatory mediators which are then released into the tears. These mediators may be metabolites of arachidonic acid or eicosapentaenoic acid which can have inflammatory or anti-inflammatory effects. As discomfort during lens wear increases towards the end of the day, we hypothesized that the concentration of these mediators changes in tears and variation on concentration is related to end of day contact lens-induced discomfort. This study examined changes in the concentration in tears of leukotriene B_4 (LTB_4) prostaglandins (PG - Screening test for PGE_1, PGE_2 and PGF_1) and resolvin-D1 (RSD1) during contact lens wear (CL) and with no contact lens wear (no CL).
Method: The study was a prospective, open-labeled daily wear, single group two-staged investigation. A total of 30 experienced contact lens wearers collected their own basal tears from each eye twice a day (morning and evening or when symptomatic) using a non-invasive method without (stage 1) and with Etafilcon A contact lenses (stage 2) worn on a daily disposable basis for 7-10 days. Tears were then analysed using commercial immunoassay-based kits according to manufacturers' protocols. Statistical analysis was performed using Linear Mixed Model test.
Results: The LTB_4 levels were higher during contact lens wear (CL 43.4±12.6pg/ml *vs* no CL 39.4±13.4pg/ml; $p<0.034$). The concentration of LTB_4 dropped by the end of the day in both groups (CL 46.3±1302 AM *vs* 40.6±11.5 PM, no CL 43.5±15.8 AM vs 35.4±8.8 PM) ($p<0.001$). The total concentration of PGE_1, PGE_2 and PGF_2 (10.7±10.8 ng/ml) and RSD1(1.6±0.5ng/ml) levels did not change during the day ($p>0.05$) or during contact lens wear.
Conclusions: LTB_4 showed diurnal changes with and without lens wear. The level of LTB_4 drops at the end of day but since its level remains higher in CL wear it may be associated with ocular discomfort at the end of the day.

SIGNIFICANT OCULAR FINDINGS IN PATIENTS WITH PRIMARY SJÖGREN'S SYNDROME

Priya M. Mathews, MPH[1]; Sarah Hahn, MD[1]; Alan N. Baer, MD[2]; Esen K. Akpek, MD[1,2]
[1]Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland; [2]Johns Hopkins Jerome L. Greene Sjögren's Syndrome Center, Baltimore, Maryland
Purpose: To report the occurrence and frequency of extraglandular ocular manifestations in patients with primary Sjogren's syndrome (pSS) and determine their relationship with extraglandular systemic findings of pSS.
Methods: Medical records of 183 consecutive patients with pSS evaluated at a tertiary center between 1999-2013. All patients fulfilled the 2002 American-European criteria for pSS classification.
Results: All patients had clinically significant dry eye for an average of 10.4 years. One or more extraglandular ocular findings were present in 58 (32%) patients, and vision-threatening disease in 23 (13%). Nine patients had significant corneal involvement, classified as corneal ulcer/infiltration (n=2) and melt/perforation (n=7). Other serious ocular findings included cicatrizing conjunctivitis (n=4), uveitis (n=5), optic neuritis (n=5), episcleritis/scleritis (n=3), and retinal vasculitis (n=1). Twelve (52%) of the patients with a significant ocular finding did not carry a diagnosis of pSS at the time of presentation. Patients who tested positive for anti-cyclic citrullinated peptide antibodies seemed to be more likely to have serious ocular findings from pSS (OR=3.9; 95% CI=0.55-27.7; p=0.17). Overall, patients with vision-threatening ocular findings were 2.46 times more likely to have extraglandular systemic manifestations of pSS (95% CI=1.0-6.0; p=0.046). Patients with significant ocular findings were also more likely to have peripheral neuropathy, interstitial nephritis, and vasculitis compared to those without ($p<0.05$ for all three).
Conclusions: These results emphasize that pSS is associated with serious ocular and systemic complications. The eye is a sensitive indicator of morbidity from pSS-related extraglandular organ involvement.

[Drs. Akpek and Baer are supported in part by Jerome L. Greene Sjögren's Syndrome Center.]

ESC(1-21) A NOVEL ANTIMICROBIAL PEPTIDE FOR MICROBIAL KERATITIS?

AM McDermott[1], SS Kolar[1], G. Mannino[2], V. Luca[2], ML Mangoni[2]
[1]The Ocular Surface Institute, University of Houston, College of Optometry, Houston TX, USA; [2]Sapienza Universita di Roma, Italy

Purpose: To investigate the antimicrobial efficacy of a novel amphibian antimicrobial peptide, Esculentin 1-21 (Esc 1-21) in vitro.

Methods: Microbroth dilution assays were used to determine the minimal inhibitory concentration (MIC) of Esc(1-21) against PA strain ATCC 27853 and the effects of salt and tears (basal and reflex) on peptide antimicrobial activity. Esc(1-21) activity on a pre-formed biofilm was also tested using the Calgary Biofilm device and evaluated by analysis of re-growth, viable cell (CFU counts and MTT assay) and biomass.

Results: The MIC for Esc(1-21) was 4uM (n=3). Esc(1-21), 1uM, retained the ability to kill 100% of ATCC 27853, within 20 min, in 150mM NaCl (n=3). When tested in the presence of 50 and 70% v/v reflex human tears, killing of ATCC 27853 by 20uM Esc(1-21) was 100% and 94% respectively, after 90 min of peptide treatment. When tested in the presence of 50 and 70% v/v basal human tears, killing was 98% and 70% respectively. The minimum biofilm eradication concentration (concentration inhibiting re-growth of bacteria from peptide treated biofilm) was 6uM (n=4) and the minimum bactericidal concentration (concentration required to reduce the number of viable biofilm cells by ≥3 $\log_{10}$) was 12uM (n=3). Biofilm biomass, evaluated by crystal violet staining, was 15% to 32% for 48-12uM peptide.

Conclusions: Esc(1-21) is effective against both the free-living and sessile forms of PA in vitro and importantly retains significant bactericidal activity in the presence of human tears. As antimicrobial peptides are recognized to induce minimal pathogen resistance Esc(1-21) is a very promising candidate for a novel therapeutic for the treatment of microbial keratitis.

Support: Sapienza Universita di Roma and the Italian Foundation for Cystic Fibrosis, Project FFC#14/2011, (MLM). EY13175 (AMM) and 007551 UHCO CORE grant.

NEW INNOVATIONS IN THE SURGICAL TREATMENT OF OCULAR SURFACE DISEASE

Jodhbir S. Mehta. Cornea and External Eye Disease Service, Singapore National Eye Centre, Singapore

Corneal surgery has changed over the last decade. There has been an increase in the number of lamellar procedure performed worldwide both anterior lamellar kertoplasty procedures and endothelial keratoplasty procedures. The driving factor for lamellar surgery has been improved clinical outcomes with respect to BSCVA, graft rejection and long term graft survival compared to conventional penetrating keratoplasty. There has also been an increase in the number of keratoprosthesis surgery performed. Likewise the driving factor for keratoprosthesis surgery has been the poor outcomes following conventional corneal graft surgery in this group of patients. However, the latter surgical procedures are still associated with many complications. Further improvement in keratoprosthesis surgery outcomes is envisaged with improved functionalization of the artificial surfaces and better integration of the biomaterials to improve host/material interactions.

RAB3D AND RAB27 PLAY DISTINCT ROLES IN REGULATING TEAR PROTEIN SECRETION FROM LACRIMAL GLAND ACINAR CELLS (LGACS).

Zhen Meng[1], Maria Edman-Woolcott[1], Yu Zhou[2], Ebrahim Zandi[2], Sarah Hamm-Alvarez[1]. School of Pharmacy[1], Keck School of Medicine[2], University of Southern California, Los Angeles, CA, USA

Purpose: Rab3 and Rab27 are the only two Rab GTPases identified so far which modulate regulated secretion in many cell types. In this study, we aim to distinguish the functions of Rab3D and Rab27 in regulating the secretion of tear proteins crucial for maintaining the ocular surface integrity. Intriguingly, our recent work has suggested that these two Rab proteins label discrete secretory vesicles (SVs).

Methods: Tear fluid was collected from Rab27 single and doulbe knockout (KO), Rab3D KO and C57BL/6 mice by stimulating the lacrimal gland in situ with carbachol. Quantitative proteomics studies were conducted. LGACs from each mouse strain were isolated, cultured and stimulated with secretagogues. The activities of several secretory enzymes and total protein concentration were measured and compared in fresh mouse tears and between cultures.

Results: Compared with C57BL/6 mice, tears from all Rab27 single and double KO mice had lower Cathepsin S (CtsS), higher -hexosaminidase (hex) and higher carbonic anhydrase VI (CarVI). However, tears from Rab3D KO mice showed the opposite pattern: CtsS was increase while hex and CarVI were decreased. The total protein amount decreased to a greater extent in Rab3D KO mice tears relative to Rab27 KO mice.

Conclusions: Rab3D and Rab27 both participate in regulated secretion and exist in various populations of SV in LGACs. The proteins assayed in this study are all secreted though both Rab27- and Rab3D-labeled SVs. However, our data suggest that hex and CarVI are secreted mainly through Rab3D-enriched SVs and CtsS is secreted mainly through Rab27-enriched SVs. When exocytosis of one set of SV is impaired, proteins may be secreted from the other subset of SV through compensatory mechanisms. Since the spectrum of secretory proteins changes in disease, our model has implications for the resolution of disease mechanisms which may specifically affect one arm of the secretory pathway, and for therapies to restore function.

This research was supported by NIH grant EY011386 and EY016985.

ANALYSIS OF SPREAD MEIBOMIAN FILMS ALONE AND SEEDED WITH DEUTERATED WAX ESTERS OR (OMEGA-ACYL)-HYDROXY-FATTY ACIDS USING NEUTRON REFLECTIVITY

Thomas J Millar, Shiwani R Raju, Burkhardt Schuett. School of Science and Health, University of Western Sydney, Sydney, NSW Australia.

Purpose: Meibomian lipids spread on the surface of tears enable formation of a stable film. Our previous in vitro studies indicate that meibum forms duplex films that have physical properties not emulated by its major components: cholesterol and wax esters (CE, WE); and the surfactant (omega-acyl)-hydroxy-fatty acids (OAHFAs). Our X-ray reflectivity experiments also indicated that meibomian films have a 3-phase structure at low concentrations and Bragg peaks occur at high surface pressures indicating repeating layers.

Methods: We have now investigated the arrangement of individual components in spread films by seeding them with a deuterated WE or a deuterated OAHFA. Films of meibum or meibum seeded with deuterated WE or deuterated OAHFA were spread on D2O, air contrast matched water, or high contrast D2O/water in a Langmuir trough. Neutron reflectivity was carried out on these films at two angles and three different surface pressures.

Results: These initial studies, showed the presence of Bragg peaks in the reflectivity measurements from OAHFAs and not from the WEs. They also indicate that the WEs are in the bulk of the film whereas the OAHFAs are at the aqueous bulk interface, and form stable repetitive layers at high surface pressures.

Conclusions: These results suggest a surprising outcome in that the lipid layer of the tear film is a duplex film stabilised by repetitive

lamination of the OAHFAs rather than being a bilayer with multiple layers of non-polar lipids (WEs and CEs) riding on a surfactant layer (OAHFAs and other polar lipids).
[This research was supported by Allergan and VisionCRC]

INTRASUBJECT TEAR OSMOLARITY AND TEAR MENISCUS HEIGHT CHANGES WITH TWO TYPES OF EYE DROPS.
Giancarlo Montani,[1] Michele Carullo,[2] Università del Salento,[1] Ambulatorio di 3° livello Ospedale di Conversano ASL/BA,[2] Italy
Purpose: To measure tear osmolarity (TO) and tear meniscus height (TMH) over time after instillation of two commercially available eye drops in patients with moderate dry eye.
Methods: This prospective, non-randomized, simultaneous, comparative, open-label, unmasked study included 40 eyes of 20 consecutive subjects (36.4±12.4 years) with a diagnosis of moderate dry eye. Subjects were instructed to administer controlled lubricants to the right eye (RE, Blink Intensive Tears PLUS (BITP), Abbott Medical Optics) and left eye (LE, SYSTANE GEL DROPS ™ (SGD), Alcon) thrice daily for 21 days. The impact of the drops on TO (TearLab Osmolarity System, TearLab Corp.) and TMH (RTVue OCT, Oculus Technologies) was measured immediately prior to and 30, 60, 90, 120, 180 and 210 minutes after instillation of the first set of drops. Follow-up measurements were performed 22 days later, one day after the subjects stopped using the drops.
Results: In RE, TO values were significantly reduced at all time points after instillation ($p<0.01$), except at 210 minutes when the values returned to baseline. In LE, TO was significantly reduced from baseline at 30 minutes up to 90 minutes ($p<0.01$), but returned to baseline at 120 minutes. The highest average reduction from baseline was 90 minutes after instillation for RE (25.2±8.5 mOsm/L) and after 30 minutes for the LE (14.5 ± 10.5 mOsm/L). Both groups showed significantly lower TO at 21 days compared to baseline (RE: 310.6±13.6 vs. 322±12.3 mOsm/L, $p<0.01$, LE: 314.6±10.3 vs. 321.5±10.6 mOsm/L, $p<0.01$). TMH values were significantly higher 30 minutes after instillation ($p<0.01$) in both eyes (RE: 0.15±0.02 vs. 0.19±0.02 mm, $p<0.01$, LE: 0.15±0.02 vs. 0.18±0.03 mm, $p<0.01$). No significant differences were found after 21 days.
Conclusion: Our results show that both eye drops reduce TO, with BITP providing a greater and more sustained reduction than SGD. Both eye drops had a similar effect on TMH, which increased only at the 30 minute time-point. At 22 days after treatment TO was significantly lower than the baseline values in both groups whereas TMH was not significantly different.

TEAR VOLUME, OSMOLARITY AND OPTICAL QUALITY IN SILICONE HYDROGEL CONTACT LENS WEARERS.
Giancarlo Montani,[1] Sudhir Patel,[2] Università del Salento Italy,[1] NHS National Services, Edinburgh, Scotland, UK[2]
Purpose: To determine the effect of silicone hydrogel contact lens (CL) wear on tear volume, osmolarity, and optical quality.
Methods: 30 participants (15 experienced CL wearers and 15 neophytes, M:F ratio 1, age range 18–40 years) with a spherical refractive error between -1.00 D and -6.00 D and astigmatism <0.75 D were enrolled. Each patient was fitted with a silicone hydrogel CL made of balafilcon A (PureVision 2HD, Bausch & Lomb). The following tests were performed in the right eye of each subject before fitting, 10 minutes after fitting, and after 7 hours of wear: tear volume, estimated by checking the tear meniscus height (TMH) at the middle of the lower lid by a slit lamp-adapted Fourier-Domain OCT (SL-SCAN 1, Topcon), tear osmolarity (TO) (TearLab Osmolarity System, TearLab Corporation), and modification of higher order aberrations (HOA) measured in the range of 10 seconds using a Hartmann-Shack aberrometer (KR-1W Wavefront Analyzer, Topcon). Subjects were also asked to assess the comfort of their CL on a 10-point scale (10=no discomfort at all, 0=very uncomfortable) after fitting and after 7 hours of wear.
Results: TO before CL fitting was 303±8.83 mOsm/L, TMH was 0.26±0.06 mm and HOA for a pupillary diameter of 4 mm was 0.092±0.032 m 1 second after blinking and remained stable at 10 seconds. Ten minutes from the fitting, none of the measurements were significantly different from baseline ($p>0.05$). TO was 303±18.74 mOsm/L, TMH was 0.28±0.05 mm, HOA was 0.122±0.032 m at 1 second and increased slightly by 10 seconds (0.147±0.046 m). The comfort score was 7.8±1. After 7 hours of CL wear TO (321±23.12 mOsm/L) was significantly higher than at baseline ($p<0.05$), TMH (0.20±0.03 mm) was significantly lower than at baseline ($p<0.05$) and the comfort score was 7.3±1. HOA was significantly higher than at baseline and was 0.134±0.046 m 1 second after blinking and increased slightly at 10 seconds (0.164±0.084 m).
Conclusion: After 7 hours of wearing a silicone hydrogel CL, TO and HOA increased and TMH decreased compared to baseline. These results may explain the reduction of comfort with longer wear time.

TEAR VOLUME AND OSMOLARITY IN KERATOCONUS PATIENTS FITTED WITH RIGID GAS PERMEABLE LENSES.
Giancarlo Montani,[1] Sudhir Patel,[2] Università del Salento Italy,[1] NHS National Services, Edinburgh, Scotland, UK[2]
Purpose: To determine the effects of rigid gas permeable (RGP) contact lenses (CLs) on tear volume and tear osmolarity in patients with keratoconus.
Methods: This study was conducted on 24 eyes of 12 keratoconic patients fitted with RGP CLs made of Boston XO material (Rose K2, David Thomas, UK). Each CL was fitted according to the manufacturer's guidelines. Patients with systemic or ocular disease, or previous history of dry eye were excluded from the study. Selected patients were instructed to wear their best fitted RGP CLs for at least 4 weeks before examination. After 4 weeks the following measurements were taken in both eyes before fitting, 20 minutes after fitting, and after 7 hours of wear: tear volume, estimated by checking the tear meniscus height (TMH) at the middle of the lower lid by a slit lamp-adapted Fourier-Domain OCT (SL-SCAN 1, Topcon) and tear osmolarity (TearLab Osmolarity System, TearLab Corporation).
Results: Tear osmolarity before lens fitting was 295 ± 10.84 mOsm/L and the TMH was 0.30 ± 0.07 mm. Twenty minutes from the fitting of the CLs all measurements were significantly different from the baseline values ($p < 0.05$), with a decrease in tear osmolarity (281 ± 7.07 mOsm/L) and an increase in TMH (0.42 ± 0.10 mm). After 7 hours of CL wear none of the measurements were significantly different from the baseline values ($p > 0.05$). Tear osmolarity was 298 ± 5.86 mOsm/L and TMH was 0.33 ± 0.10 mm.
Conclusions: The initial increase in TMH and decrease in tear film osmolality immediately after CL insertion was followed by a return towards baseline values with a reduction of the TMH and an increase in tear osmolarity. Our results may be explained by tear fluid hypersecretion due to irritation from CL insertion. This may have induced an increase of tear volume initially and a subsequent reduction in tear film osmolarity. The return of TMH and osmolarity to baseline after 7 hours of wear indicates RGP lenses as well tolerated by keratoconus patients.

PROMINENT DECREASE OF TEAR MENISCUS HEIGHT BY SCL WEARING AND EFFICACY OF EYE DROPS FOR DRY EYE TO PREVENT THE CHANGE.
Yukiko Nagahara[1,2] , Shizuka Koh[2], Hitoshi Watanabe[1,2]
1. Kansai Rosai Hospital 2. Osaka University Graduate School of Medicine
Object: Eighty to Ninety percent of tear film is located in upper & lower tear meniscus(TM) indicating that maintenance of TM height

(TMH) is a kind of keys to prevent dry eye. Previous report has shown that TMH is returned to baseline in 20 minutes of SCL wearing in spite of much higher TMH just after SCL wearing. However, we found that TMH is reduced below baseline in 20 minutes after SCL wearing and reduction rate of TMH depends to water content (WC) rate of SCL. In this study, we investigated the change of TMH non-invasively before and after wearing of different WC SCL by anterior segment optical coherence tomography(OCT). We also explored whether topical application of artificial tear (AT) and anti-dry eye drug are effective to maintain TMH.

Method: The objects are 17 normal volunteers; 14 males and 3females aged from 29 to 51. TMH at the center of lower lid is determined by anterior segment OCT (CASIA®, Tomey) before and after SCL wearing.SCL used in this study are Dailies aqua ® CL (CIBA : WC 69%: 22 eyes, 12cases) , and Air optix EX aqua® CL (CIBA :WC24%: 24eyes, 12cases). We also observed TMH in 5, 15, 30, and 60minutes after topical application of AT(Soft San Tear. Santen) and 2% Diquafosol (Diqaus® Santen) (DQS).

Results: TMH was 2.5±0.5($\times 10^2$ m) before and was significantly reduced to 1.9±0.6($\times 10^2$ m) (76%) after CL wearing in all cases. Among them, in high WC SCL, TMH is significantly reduced from 2.6±0.6($\times 10^2$ m) to 1.6±0.4($\times 10^2$) (61%: $p<0.001$) after CL wearing. In contrast, in low WC SCL, TMH is less reduced from 2.4±0.4 ($\times 10^2$ m) to 2.2±0.6($\times 10^2$ m) (91%) . By topical application of DQS for high WC SCL wearers, TMH was increased from 1.3±0.3 before to 1.4±0.5, 1.5±0.2, and 1.5±0.3($\times 10^2$) in 15, 30, and 60 minutes respectively after application. The value in 15, 30 minutes of DQS treated eyes was significantly increased compared with that of AT treated eyes respectively ($P<0.02$ and <0.05).

Conclusion: Remarkable reduction of TMH was found below baseline after SCL wearing and the decrease rate of TMH depends on SCL WC. DQS eye drop is more effective than AT to maintain TMH while wearing SCL.

DIQUAFOSOL TETRASODIUM STIMULATED LIPID SECRETION IN RABBIT MEIBOMIAN GLANDS.

Masatsugu Nakamura, Asuka Sakamoto, Hidetoshi Mano, Takashi Nagano, Naoto Mori. Santen Pharmaceutical Co., Ltd., Research & Developement Center, Japan

Purpose: Diquafosol tetrasodium (diquafosol) is a dinucleotide derivative that exhibits agonistic effects on $P2Y_2$ receptor, and is used for the treatment of dry eye. The $P2Y_2$ receptor in the ocular surface is expressed in the conjunctival epithelium including goblet cells, corneal epithelium and meibomian glands. The action mechanism of diquafosol contributes to promote water and mucin secretion via the $P2Y_2$ receptor of conjunctival epithelial cells and goblet cells. However, the role of diquafosol on meibomian gland is not fully investigated. For this, we investigated the effect of diquafosol on lipid secretion in rabbit meibomian gland cells, and to examine the distribution to meibomian glands in rabbit following instillation of commercial available eye drops of diquafosol, Diquas ophthalmic solution 3%.

Methods: ***In vitro*** **stimulatory effect on lipid secretion**

Rabbit meibomian glands were removed from tarsal conjunctiva by enzymatic digestion. The secondary cultured meibomian gland cells were plated in 24-well plates, and were cultured until confluence condition. Then the medium was replaced with BH Ringer's solution with or without diquafosol, and the cells were incubated for 1h. The culture supernatant was collected and lipids were extracted by a modification of the Bligh and Dyer procedure. Total lipids were measured by the sulfo-phospho-vanillin reaction.

Distribution to meibomian glands after instillation

Diquas ophthalmic solution 3% was administered to rabbit eyes, and meibomian glands at 5, 15, 30 min, 1, 2 h were collected. The concentration of diquafosol in the meibomian glands was determined by LC-MS/MS.

Results: Diquafosol concentration-dependently increased lipid secretion from rabbit meibomian gland cells and lipid secretion by 100 nM diquafosol was significantly higher than that of vehicle.

After ocular instillation to rabbit, the concentration of diquafosol in meibomian glands reached maximum of 120 ng/g (140 nM) at 5 minutes post-dose.

Conclusions: This study revealed that diquafosol stimulated lipd secretion from meibomian gland cells, and its effective dose distributed to meibomian glands after the instillation of diquafosol. These results suggested that diquafosol might be effective for the treatment of meibomian gland dysfunction.

INTERMITTENT FASTING PREVENTS LACRIMAL HYPOFUNCTION IN RAT VISUAL DISPLAY TERMINAL USERS MODEL : A PIVOTAL ROLE OF ENDOGENOUS D-3-HYDROXYBUTYRATE

Shigeru Nakamura,[1]; Ryuji Hisamura[1]; Toshihiro Imada[1]; Kazuo Tsubota[1]. [1]Keio university, Tokyo, Japan.

Purpose: Calorie restriction extends life span and retards age-related chronic diseases. We previously reported that calorie restriction through intermittent fasting restores lacrimal function in VDT associated dry eye using rat model. (ARVO 2012). In this condition, serum ketone bodies concentrations increases approximately 1000 fold from the normal state. D-3-hydroxybutyrate (3-HB), a major circulating ketone body, has been demonstrated to be effective in neurological disorders such as Alzheimer's, Parkinson's, and found to attenuate corneal disorder in dry eye condition. In the present study, we explored the possible role of 3-HB on the attenuation of lacrimal function by calorie restriction.

Methods: 8-week-old female Sprague-Dawley rats were used for this study. A series of treatments were performed under continuous exposure to low-humidity airflow (25 ± 5%, 2 - 4 m/s). Rats were placed on a swing made of a plastic pipe for 7.5 h/d, and for 16.5 hours, they were placed in individual cages without swing treatment. This series of treatments was repeated for up to 7 days.

Rats were assigned to three groups: AL, ad libitum-fed animals, and IF, intermittent fasting rats, which were provided unlimited access to food every other day, AL+3-HB, 3-HB diluted with saline was injected into rat dorsal skin at a dose of 2000 mg/kg/day. Change in tear secretion was measured by the cotton thread test. Serum and lacrimal glands 3-HB concentrations were measured by enzymatic method.

Results: A significant decrease in tear secretion was observed in the AL compared with the initial value. In the IF and AL+3-HB, slight decreases in the tear secretion were observed, although the differences were not significant compared with the initial values. Change in tear secretion was significantly suppressed in the IF (14.3 ± 0.81, n=9, $p < 0.05$) and AL+3-HB (13.9 ± 0.71, n=10, $p < 0.05$) compare to the AL day 7(10.1 ± 0.79 n=10) Serum and lacrimal glands 3-HB concentrations were significantly increased 15 hours after food deprivation and 0.5 hour to 3 hour after 3-HB injection. The peak 3-HB concentration of serum and lacrimal gland was approximately ten-fold compared to initial value.

WHY PSYCHOLOGICAL COMPLAINTS COMPLICATE THE SYMPTOMS OF DRY EYES

Johannes Nepp. MD. Department of Ophthalmology, Medical University Vienna, Austria, ÖGPPM Austrian Society for psychosomatic and psychotherapeutic medicine

Purpose: Signs and symptoms often are different in dry eyes and it is difficult to find adequate treatment for this patients. Many patients, resistant for any therapy have psychological problems, especially anxiety, depression and vegetative disorders. As behaviors of psychological problems are different, this study was performed to evaluate the cause of dry eyes' complaints by psychological disorders.

Methods: 93 patients with dry eyes were observed. For psychological complaints a questionnaire TRIPS (The Rapid Interactive Psychiatric

Screen) was used to detect anxiety and depression diagnosis of ICD10 by psychic and somatic symptoms. Visual analogue scale (VAS) was used as well as additionally questions for subjective complaints. Dry eyes were observed by slit lamp, Schirmer-test I, break up time, fluorescein, lissamin-green staining, tear meniscus and lipid layer thickness. A score was performed by all equalized results, to find the severity code. The values were 0 (normal) to 1 (supremely severity). Groups were built by the results of the TRIPS-questionnaire: Patients with 1) anxiety, 2) depression 3) vegetative stress, 4) combination of anxiety and depression 5) no classification. Severity of dryness was evaluated for each group, and behavior patterns were observed.
Results: Dry eyes' severity was extreme in patients of group 4 (combination): mean 0.7; anxiety (gr1) 0.54, vegetative stress (gr3) 0.56; depression (gr2) 0.43 and patients without symptoms (gr5) 0.37. Subjective complaints were different: extreme in gr2: 0.9; gr3: 0.83, gr4: 0.8. Gr1: 0.57 and gr5: 0.2. Behavior was very different: patients with anxiety mostly are afraid to do anything with inhibition symptoms. Patients with symptoms of depression mostly have no motivation to use any treatment and believe that there is no help. Therefore they don't feel positive changes. In patients with stress symptoms there is no feeling of symptoms by combined loading. Therefore dryness is always a subordinate problem. Therefore complaints increase if combined symptoms apear.
Conclusion. Various psychiatric disorders aggravate troubles of dry eyes by the psychological behaviors but also by the reduced therapeutic approach. Therefore it is necessary to observe and treat psychological complaints concomitant to the ophthalmological therapy.
(Commercial relationships are disclosed)

A REPORT FROM THE TFOS INTERNATIONAL WORKSHOP ON CONTACT LENS DISCOMFORT

Nichols JJ, Willcox MDP, Craig JP, Dumbleton K, Efron N, Fonn D, Foulks GN, Jones LW, Nelson JD, Nichols KK, Papas EB, Rosenblatt MI, Stapleton F, Sullivan DA, on behalf of the TFOS International Workshop on Contact Lens Discomfort members.
Purpose: Contact lens discomfort (CLD) is the leading cause of patient dissatisfaction with and discontinuation of contact lens wear. Although this condition influences the quality of life of millions of people, there is no global consensus on the definition, classification, epidemiology, etiology, diagnosis or therapy of CLD. To achieve such a consensus, the Tear Film & Ocular Surface Society (TFOS; www.TearFilm.org), a non-profit organization, launched the TFOS International Workshop on Contact Lens Discomfort (www.tearfilm.org/tfoscldreport/index.htm). The mission of the TFOS CLD Workshop was to: (1) conduct an evidence-based evaluation of CLD in health and disease; (2) develop a contemporary understanding of the definition, classification, epidemiology and neurobiology of CLD; (3) examine the role of lens materials, design and care in the etiology of CLD; (4) assess the biocompatibility of contact lenses with the tear film and ocular surface; (5) develop appropriate norms of trial design including outcome measures for CLD; (6) develop recommendations for the management and therapy of CLD; (7) develop recommendations for future innovative research in CLD.
Methods: The Workshop, which required 18 months to complete, finalized its report in September 2013. It involved the efforts of 79 leading clinical and basic research scientists from around the world. These experts, assigned to 11 Subcommittees, reviewed published data and examined the levels of supporting evidence. Subcommittee reports were circulated among all Workshop participants, presented in open forum and discussed in an interactive manner.
Results and Conclusions: This session will present the conclusions and recommendations of the TFOS International CLD Workshop.
[Supported by TFOS, with appreciation for financial assistance from Alcon, Allergan, Bausch+Lomb, Santen, Menicon, Vistakon, Laboratoires Théa, Optima, Oculus and CooperVision)

PROTEIN BIOMARKERS IN POST-MENOPAUSAL DRY EYE DISEASE.

Kelly K. Nichols, Kari B. Green,[1] Sasha Popova-Butler,[1] Liwen Zhang[1], [2] Mass Spectrometry and Proteomics Facility, The Ohio State University, Columbus, OH, USA,[1] College of Optometry, University of Houston, Houston TX, USA[2]
Purpose: Label free quantitative proteomics are used in the analysis of proteins extracted from normal post-menopausal patients and patients with post-menopausal dry eye (DE) to determine quantitative differences in proteins. This data is compared against varying clinical and patient-reported dry eye classification schemes.
Methods: Protein samples were collected from two groups: 12 postmenopausal women with and 12 without DE. Proteins were extracted from Schirmer strips and fractionated using 1D SDS-PAGE, digested with trypsin and analyzed by LC-MS/MS. Scaffolding is used for quantitative analysis and statistical analysis of protein expression between experimental groups was performed with the edgeR algorithm.
Results: In the analysis, 427 proteins were identified with notable differences in protein expression levels between normal patients and DE patients. Eleven proteins are found to be unique to DE and 16 proteins are unique to normal conditions. There is significant overlap with 392 proteins found in both DE and normal conditions. Fold changes of protein these proteins are examined with hierarchical clustering and Principal Component Analysis (PCA). Approximately 40 proteins have a significant fold change (p-value < 0.05 and fold change > 1.5). It is highly interesting to note that the samples do not cluster within their dry eye or normal classification when a symptom-based definition is used. Variation in protein clustering occurs when different dry eye classification schemes are used. Thus, candidate proteins are discussed as biomarker indicators for severe dry eye and for early onset or mild dry eye conditions, aqueous deficient, and evaporative dry eye will be discussed.
Conclusions: PCA shows distinct clustering of the data into two groups, and this clustering can refine a dry eye classification. Interestingly some samples cluster outside the initial symptom-based diagnosis indicating symptoms alone are not adequate in classifying dry eye, and protein profiles may ultimately provide additional insight to the disease.
This work is supported by NIH NEI EY015519.

IDENTIFICATION OF LIPID BIOMARKERS FOR DRY EYE DISEASE IN POST-MENOPAUSAL WOMEN USING SHOT-GUN ELECTROSPRAY MASS SPECTROMETRY.

Kelly K. Nichols,[1] Jianzhong Chen,[2] Kari B. Green.[2] College of Optometry, University of Houston, Houston, Texas, USA;[1] Mass Spectrometry and Proteomics Facility, The Ohio State University[2], Columbus, OH, USA.[2]
Purpose: Shotgun ESI-MS is a rapid method to analyze lipids from meibium to determine quantitative differences in lipids for the identification of lipid biomarkers. This data is compared against our other clinical findings and correlated in subjects with and without self- and clinically diagnosed dry eye.
Methods: Meibum samples were collected from postmenopausal women with dry eye (n = 24), and without dry eye (n = 24). Lipids were extracted using 2:1 chloroform:methanol and infused in a Bruker maXis 4G UHR QTOF. Progenesis Co-Met software was used to quantify differences in lipid profiles. The identity of the lipids showing statistical changes in abundance was confirmed by comparison to our previous results using MS/MS.
Results: We observe marked variation of lipids within dry eye patients compared to normal, which show to be highly reproducible across patients. The dry eye spectra can be described as "high lipid" producing and "low lipid" producing patients. Principal Component Analysis (PCA) results show a set of dry eye patients cluster separately which all coincide with samples described as low lipid producers (few peaks). The 6 dry eye patients categorized as low lipid producers were compared

against a random 6 normal patients. The PCA shows distinct clustering between the two groups. Over 158 lipid species total are found to be up or down regulated between normal and dry eye patients with a p-value of below 0.05 and a fold change greater than 1.5. Lipid identification for these species has been confirmed.
Conclusions: We have identified observable and statistically significant differences in lipids between dry eye and normal patients. PCA shows distinct clustering into two groups. It appears that some samples clinically determined as normal cluster with the dry eye and vice versa. While comparing the results between dry eye samples it is noted that there is very large variation in peak intensities between patients.
This work is supported by NIH NEI EY015519.

PATHOPHYSIOLOGY, DIAGNOSIS, AND TREATMENT OF MEIBOMIAN GLAND DYSFUNCTION.
Kelly K. Nichols, College of Optometry, University of Houston, Texas, USA
Purpose: The purpose of the 2011 MGD workshop was to conduct an evidence-based evaluation of meibomian gland structure and function in health and disease and to assess methods of diagnosis, evaluation, and grading of the severity of meibomian gland dysfunction (MGD). This information was to be disseminated widely. Since the publication of the report, translational and basic researched efforts have grown exponentially, and clinical care of MGD has changed. The impact of the report on the current understanding of MGD pathophysiology, diagnosis, and management will be discussed.
Methods: An evidence-based review of literature published since March 2011 was performed.
Results: Terminal duct obstruction is thought to be the leading cause of obstructive MGD, and meibography is moving the understanding of disease pathophysiology forward. This and other newly available technology for the diagnosis and treatment of MGD have been reported in the literature and are gaining clinical acceptance. The MGD report diagnostic and management algorithms have been used in clinical research and patient care, and evaporative dry eye related to MGD has become accepted terminology.
Conclusions: The last 2.5 years have been very productive in the area of MGD pathophysiology, diagnosis and management. It is accepted that MGD is the most common cause of dry eye, and significant advances are expected to continue as a result of the report.

HUMAN ORAL MUCOSAL FIBROBLASTS SUPPORT THE CULTURE OF EPITHELIAL CELLS FOR CLINICAL USE IN 2D CULTURE AND ON PLASTIC COMPRESSED COLLAGEN (RAFT)
AR O'Callaghan[1], MP Lewis[2], M Dziasko[1] and JT Daniels [1]Department of Ocular Biology and Therapeutics, UCL Institute of Ophthalmology, London, UK.[2]Musculoskeletal Biology Research Group, School of Sport, Exercise and Health Sciences, Loughborough University, UK
Purpose: Limbal epithelial stem cell deficiency (LSCD) can be treated using cultured LESC therapy (CLET) or cultivated oral mucosal epithelial transplantation (COMET). The current gold standard method for culturing LESCs uses an initial expansion step with murine 3T3 feeder cells. Cell therapies should ideally be animal product free. The aims of this study were 1) to investigate whether human oral mucosal fibroblasts (HOMFs) or human limbal fibroblasts (HLFs) could be used as alternative feeder layers to support the culture of limbal epithelial stem cells (LESCs) and 2) how these cells would behave in 3D plastic compressed collagen constructs (RAFT) which could potentially be used as an alternative to the biologically variable amnion currently used for transplantation.
Methods: Human oral mucosal epithelial cells (HOMEs) were isolated from buccal oral mucosal biopsies using Dispase and initially expanded on a growth arrested 3T3 feeder layer. Explant culture was then used to generate HOMFs. Human limbal epithelial cells (HLEs) and human limbal fibroblasts (HLF) were similarly and cultured from limbal rims. Following initial expansion, epithelial cells were split equally onto Mitomycin C growth arrested 3T3, HLF, and HOMF feeder layers and cultured until they stopped proliferating. Population doublings were calculated at each passage. Epithelial cells were analysed by PCR and Western blot for the corneal markers CK12, Pax6, and Mucin 16 and the putative stem cell markers CK15 and p63alpha. 3D organotypic models were set up using compressed collagen gels (RAFT) containing 3T3s, HLFs or HOMFs with epithelial cells (HLEs or HOMEs) on top. Following submerged culture, the gels were airlifted to induce stratification.
Results: HOMFs were equivalent to 3T3s for supporting epithelial cell culture (both HLE and HOME) in 2D in terms of total passage number, the number of population doublings and maintenance of the stem cell markers CK15 and p63alpha. There was no significant difference in Pax6 and Mucin 16 protein expression for HLEs cultured on 3T3s, HLFs, and HOMFs. The number of passages and population doublings was significantly less for HLEs and HOMEs cultured on HLFs compared to 3T3s and HOMFs in 2D ($P<0.05$). When cultured in the 3D models, differences in the number of epithelial cell layers (more cell layers in models containing HOMFs than those with HLFs and 3T3s) and transparency of these constructs were observed with the different epithelialfibroblast combinations.
Conclusions: Results from this study suggest that HOMFs could be used as a feeder layer instead of murine 3T3s for the culture of HLEs and HOMEs for use in CLET or COMET for the treatment of LSCD. This system could improve the safety of these procedures, by utilising an autologous source of feeder cells. 3D models provided additional insight into how these cells might behave once transplanted onto the eye and suggested that cell populated collagen constructs (RAFT) may be useful for the treatment of LSCD.
This work was supported by Fight for Sight, the Special Trustees of Moorfields Eye Hospital and NIHR Moorfields Biomedical Research Centre. No commercial relationships.

INTERNATIONAL CHRONIC OCULAR GRAFT-VS-HOST-DISEASE (GVHD) CONSENSUS GROUP: PROPOSAL OF NEW DIAGNOSTIC CRITERIA FOR CHRONIC GVHD (PART I).
Yoko Ogawa[1], Stella K. Kim[2], Reza Dana[3], Janine Clayton[4], Sandeep Jain[5], Mark I. Rosenblatt[6], Victor L. Perez[7], Hasanain Shikari[3], Anjo Riemens[8], Kazuo Tsubota[1]
1Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan; 2MD Anderson Cancer Center, Houston, TX, USA; 3Massachusetts Eye and Ear Infirmary, Department of Ophthalmology, Harvard Medical School, Boston, USA; 4National Institute of Health, USA; University of Illinois, Chicago, Illinois, USA; 5Weill Cornell Medical College, New York, USA; 6Bascom Palmer Eye Institute, USA; 7University Medical Center Utrecht, the Netherlands.
Purpose: With an objective to describe new diagnostic metrics for chronic ocular graft-versus-host disease (GVHD), the International Chronic Ocular GVHD Consensus Group held 4 working meetings.
Methods: Variables in patients' history and examination that are currently used to determine the presence of chronic ocular GVHD were identified. Subjective and objective factors were considered and scores were assigned to each item to reflect the severity of the disease. Further, consideration was given to the presence of systemic GVHD.
Results: The Consensus Group identified 4 variables to measure subjective and objective findings in patients post-allogeneic hematopoietic stem cell transplantation (HSCT): OSDI, Schirmer's score without anesthesia, corneal staining, and conjunctival injection. Each of these variables were scored 0-3, with a composite score of 11. Based on the composite score, and the presence or absence of systemic GVHD, patients were categorized as NO ocular GVHD, PROBABLE ocular GVHD and DEFINTITE ocular GVHD.

Conclusions: New diagnostic criteria for chronic ocular GVHD are presented by the Consensus Group. Validation studies are needed to identify the best combination of sensitivity and specificity of our proposed metrics.
[This work was supported bySupported by the grant for Global Clinical Research Center Program for Keio University from Japanese Ministry of Health, Labor, and Welfare]

A SERIES OF PILOT STUDIES TO ASSESS DIURNAL VARIATION IN A DRY EYE AND NORMALS UTLIZING CONTINUOUS BLINK CAPTURE.

George Ousler[1], Keith Lane[1], Endri Angjeli[1], Colleen Heckley[1], John Rodriguez[1] Ora, Inc., Andover, MA, USA

Purpose: To conduct preliminary analyses on diurnal variation utilizing continuous blink capture.

Methods: Three pilot studies were conducted utilizing a prototype device designed by Ora for continuous blink monitoring over extended durations. 1. Six normal subjects were tracked for 6 hours. Subjects were also exposed to the Controlled Adverse Environment (CAE) for 30 minutes. 2. One dry eye and one normal subject were tracked for 6 hours. 3. Four normal contact lens wearers were tracked on two separate occasions: a. while wearing glasses, and b. while wearing contacts.

Results: In the first pilot, subjects saw a 29.8% increase in blink rate per minute in the CAE versus pre-CAE. There was also a correlation between blink rate and ocular discomfort, such that when blink rate increased, so did ocular discomfort scores. In the second pilot, dramatic differences were seen in interblink intervals (IBIs) between the dry eye and normal subjects. Low variability was observed in the IBIs of dry eye subjects. In the third pilot, mean daily contact lens wear time was 11.25 hours per day. For all subjects, descriptive symptom profile closely mimicked blink pattern. In two patients, blink pattern, including the variability of time between blinks and overall blink rate, resembled dry eye while wearing contacts and normal while wearing gasses throughout the day, with mean differences in IBIs between two and three seconds.

Conclusions: The results demonstrate dramatic differences between normal and dry eye subjects in terms of blink variability and rate. Utilizing an electrode-based monitoring system to track blink throughout the day allows for blink comparison before and after therapeutic intervention.

All Authors are employees of Ora, Inc. Study was internally funded by Ora, Inc.

COMPARISON OF READING AND BLINK RATES IN DRY EYE AND NORMAL SUBJECTS DURING VISUAL FUNCTION AND READING TASKS.

George Ousler[1], Keith Lane[1], Endri Angjeli[1], Colleen Heckley[1], John Rodriguez[1] Ora, Inc., Andover, MA, USA

Purpose: To determine differences in reading and blink rates between dry eye and normal subjects when completing visual function and reading tasks.

Methods: Fifteen dry eye subjects and ten normal subjects completed a one visit study that consisted of a battery of visual function tests and reading tasks. A total of 6 tests and tasks were incorporated into the protocol including Wilkin's Reading Test and Contrast Sensitivity Reading Test/Time to Blur.

Results: Dry eye subjects had slower reading rates and higher blink rates than normal subjects on all tests. Significance was reached during the Wilkin's Reading test, as demonstrated by slower reading rates in dry eye subjects than normals (128 words/min vs. 161 words/min respectively, p=0.0004) and higher blink rates in dry eye than normal subjects (7.85 blinks/min vs. 5.10 blinks/min respectively p=0.13). Of these dry eye subjects, those who had reflux tearing during the test had significantly slower reading rates compared to those subjects who did not have reflux tearing.

Conclusions: Our results suggest there is a significant difference in reading rate and the time it takes to accomplish the reading task between dry eye subjects and normal subjects. Reflux tearing also seems to be an important parameter because those who reflux tear experience a slower reading rate and time to complete the test. Further research is required to better understand the role of reflux tearing component.

All Authors are employees of Ora, Inc. Study was internally funded by Ora, Inc.

LOCALISATION OF ADULT STEM CELLS IN THE MOUSE MEIBOMIAN GLAND USING STATE-OF-THE-ART STEM CELL MOUSE MODEL

G.J. Parfitt, M. Geyfman, Yilu Xie, D.J. Brown, J.V. Jester. Gavin Herbert Eye Institute, University of California, Irvine, CA, USA.

Purpose: The precise location and number of stem cells in the meibomian gland is currently unknown and has been limited to 2-D analysis that suggests they may exist at the periphery of each acinus, or in the ductal epithelium. Immunoflourescent Computed Tomography (ICT) is a novel method that enables the localisation and quantification of sub-populations of cells in the entire mouse meibomian gland. The purpose of this study was to use ICT to reveal the position of label-retaining cells in meibomian glands of the H2B-GFP transgenic mouse.

Methods: In vivo GFP retention was achieved using the tet-off strategy where H2B-GFP expression is dependent on a doxycycline-controlled transactivator protein (tTA) expressed behind the keratin 5 promoter. When chased with doxycycline, GFP fluorescence in the nucleus is diluted 2-fold with each division. After 4wks and 6wks chase, eyelids were excised, fixed and embedded in butyl methyl methacrylate for ICT reconstruction. Lids were serially sectioned at 2µm for over 1mm and after imaging GFP fluorescence, were sequentially immunolabeled for Ki67 and keratin 6 to identify proliferating cells and ductal epithelium.

Results: After 4wks and 6wks chase, GFP^+ cells are frequently seen in isolation at the entrance of an acini but can occur in clusters of up to 3 in direct proximity. Occasionally, GFP^+ cells are seen at the periphery of an acini. Keratin 6 immunostaining confirms that the vast majority of these GFP^+ cells are part of the ductal epithelium at the ductule or transition zone between ductal and acinar epithelium. Ki67 staining identifies actively cycling cells which are seen frequently in the acinar basal layer and in clusters in the ductule.

Conclusions: The evidence of GFP retention after 4wk and 6wk chase confirms the location of GFP retaining cells thought to be responsible for the renewal of the acinar tissue and maintenance of the meibomian gland. However, additional ICT based reconstructions are required to fully characterise and quantify these cells and their niche.

Support in part by EY021510, RPB Inc. and the Skirball Program.

TREFOIL FAMILY PEPTIDE

Friedrich Paulsen.[1] Department of Anatomy II, Friedrich Alexander University Erlangen Nuremberg, Erlangen, Germany

Purpose: The mammalian Trefoil factor family (TFF) comprises three peptides TFF1-3 that can interact with mucins having an influence on mucus viscosity, promote migration of epithelial cells *in vitro*, are linked to anti-apoptosis, induce cell scattering, participate in immune response, trigger chemotaxis and have further functions. During recent years we could demonstrate that TFF3 plays a pivotal role in corneal wound healing mechanism and also in dry eye disease indicating broad implications for TFF3 as a possible novel therapeutic strategy to treat corneal disease.

Methods: Own and published results from other groups will be summarized.

Results: Recent investigations of our and other groups revealed that TFF expression also occur outside mucosae, i.e. in articular cartilage or in the developing retina and bone. In certain diseases of articular cartilage, i.e. osteoarthritis or inflammatory joint disease TFF3 seems

to support catabolic functions by activating matrix metalloproteinases and enhancing apoptosis of articular cartilage chondrocytes. Therapeutic effects of the TFF have been shown in several animal models of gastrointestinal damage and also in human. Many of the multiple biologic functions of TFFs are thought to be triggered by receptor activation. So far, a putative TFF receptor has not yet been identified. However, by using X-ray based modeling and blocking experiments we were able to demonstrate a dependence of TFF3 activity on the chemokine receptors CXCR4 and CXCR7 which both are present at the ocular surface.
Conclusions: Our data support the hypothesis that TFF3 might be a peptide involved in pro-resolving approaches of the body and rebalancing rather than to just suppress immune and inflammatory responses during the treatment of autoimmune and chronic inflammatory diseases. All these data underline a potential role for TFF3 as a candidate therapeutic for ocular surface diseases.

DEVELOPMENT AND USE OF A NOVEL INEXPENSIVE MEIBOMIAN GLAND TRANSILLUMINATION DEVICE.
E. Ian Pearce, Michelle Snowball, Anna Green, Melissa McKinnon, Elaine F. Wilson. Vision Sciences, Glasgow Caledonian University, Glasgow, Scotland
Purpose: To develop an effective, easy to use meibomian gland transillumination device. To then use the device to examine gland tortuosity, percentage gland drop out and location of drop out in a range of subjects.
Methods: Following a number of iterations an effective device was developed. It comprises a PMMA rod that is illuminated at one end using a single IR LED. The rod diameter was chosen so that the lid naturally wraps around the rod when slight pressure is applied. The rod and illumination system were then mounted on a modified pen torch to give a "wand" like device. Images were initially captured using a webcam modified to be sensitive in the IR. To obtain improved images a high resolution CMOS camera was modified and this was then used throughout. The meibomian glands of the lower lid in 51 normal subjects (22 M and 29 F) were observed. Gland tortuosity (arc-chord ratio), percentage gland drop out and location were measured using image analysis software. OSDI was used to measure dry eye symptoms.
Results: The mean gland drop out was found to be 2.2 ± 4.3% (range 0 to 27.9%). No difference was observed between sexes ($p > 0.05$). Interestingly, a significant difference was observed between right (2.79 ± 4.40%) and left eyes (1.69 ± 4.17%) $p=0.029$. However, drop out was significantly correlated between left and right eyes (rho = 0.488, $p=0.000$). No correlation was found between age and gland drop out, or between OSDI and drop out ($p > 0.05$). Central, temporal and nasal areas did not show significantly differences ($p > 0.05$). Gland tortuosity was 1.07 ± 0.05 (arc-chord ratio). No correlation or difference was found between gland tortuosity and sex, age or OSDI score.
Conclusions: The wand is an easy and effective tool to rapidly measure the anatomical characteristics of the meibomian glands. Its widespread use could better inform the diagnosis and treatment of dry eye disease. Furthermore, it is hoped that it will allow the study of the natural history of meibomian gland atrophy.
[This research was part funded with a grant to MS from Coopervision, UK]

BIOLOGICAL PARAMETERS DETERMINING THE CLINICAL OUTCOME OF AUTOLOGOUS CULTURES OF LIMBAL STEM CELLS.
G. Pellegrini[1], M. De Luca[1]. [1] Centre for Regenerative Medicine S. Ferrari, University of Modena and Reggio Emilia.
Limbal cultures restore the corneal epithelium in patients with ocular burns. We investigate biological parameters instrumental for their clinical success.We report a long-term multicenter prospective study on 152 patients, carrying severe burn-dependent corneal destruction, treated with autologous limbal cells cultured on fibrin and clinical-grade 3T3-J2 feeder cells. Clinical results were statistically evaluated both by parametric and non-parametric methods. Clinical outcomes were scored as full success, partial success and failure in 66.05%, 19.14%, and 14.81% of eyes, respectively. Total number of clonogenic cells, colony size, growth rate and presence of conjunctival cells could not predict clinical Results: Instead, clinical data provided conclusive evidence that graft quality and likelihood of a successful outcome rely on an accurate evaluation of the number of stem cells detected before transplantation as holoclones expressing high levels of the p63 transcription factor. No adverse effects related to the feeder-layer has been observed and the regenerated epithelium was completely devoid of any 3T3 contamination. Cultures of limbal stem cells can be safely used to successfully treat massive destruction of the human cornea. We emphasize the importance of a discipline for defining the suitability and the quality of cultured epithelial grafts, which are relevant to the future clinical use of any cultured cell type.

UNTANGLING THE MYSTERIES OF THE TEAR FILM NEUTROPHIL.
Cameron Postnikoff,[1] Elena Kreinin,[1] Robert Pintwala,[1] Maud Gorbet.[1,2] [1]Systems Design Engineering, [2]School of Optometry, University of Waterloo, Waterloo, ON, Canada
Purpose: Neutrophils accumulate on the ocular surface after prolonged eyelid closure. These tear film neutrophils exhibit a different response to inflammatory stimuli when compared to neutrophils isolated from blood. To develop better *in vitro* models and gain an understanding of mechanisms involved in ocular inflammation, this investigation is focused on inducing a change in blood-isolated neutrophils *in vitro* such that they respond to stimulation similarly to tear film neutrophils.
Methods: Neutrophils were isolated from blood using density gradient centrifugation. Cells were either resuspended in medium containing 10% FBS or in an Artificial Tear Solution (ATS) containing albumin, IgG, lactoferrin, and lysozyme. Cells were incubated for 6 hours in either hypoxic (2% oxygen) or normoxic conditions on a monolayer of human corneal epithelial cells (HCEC) or in a sterile polypropylene tube. Post-incubation, expression of ICAM-1, Mac-1, CD66b (degranulation), and CD45 (PAN leukocyte marker) was investigated by flow cytometry. Cell death was assessed using markers for apoptosis and necrosis. Neutrophils were incubated with PMA, fMLP, or LPS in order to probe the cell's ability to respond to inflammatory stimuli following exposure to various incubation conditions.
Results: A hypoxic versus normoxic environment induced no differential effect on cellular activity. In all experiments, incubation of blood-isolated neutrophils with ATS led to a reduced expression of Mac-1, ICAM-1, and CD66b when compared to incubation with FBS. However, ATS-incubated cells showed an upregulation of these receptors in response to stimulation. Incubation of blood-isolated neutrophils in the presence of HCEC resulted in an upregulation of all receptors and an increase in caspase activation.
Conclusions: Reproduction of the phenotype of tear film neutrophils *in vitro* continues to elude us, though parameters such as incubation media and exposure to HCEC appear to have a significant effect on activation.
The authors thank our phlebotomist, Miriam Heynen, and the NSERC 20/20 Ophthalmic Materials Research Network for funding.

EFFECT OF HPMC/DEXTRAN 0.3% AND SODIUM HYALURONATE 0.18% IN THE TREATMENT OF OCULAR SURFACE DISEASE IN GLAUCOMA PATIENTS.
Pinnita Prabhasawat, Ngamkae Ruangvaravate, Maneerat Thewthong. Department of Ophthalmology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
Purpose: To compare the efficacy of HPMC/dextran 0.3% and sodium

hyaluronate (SH) 0.18% in the treatment of ocular surface disease in glaucoma patients.
Methods: Prospective, randomized, controlled study. Glaucoma patients with ocular surface disease index (OSDI) score >20 points and/or ocular signs complying with the inclusion criteria were randomized to receive either HPMC/dextran 0.3% (group A) or SH 0.18% (group B). Treatment was 1-2 drops of assigned product as required for 28 days. Number of drops used per day was recorded. Efficacy was assessed at days 0, 7 and 28.
Main outcome measures: OSDI score, lid margin inflammation, meibomian gland expressibility, conjunctival injection, corneal and interpalpebral dye staining, fluorescein tear break-up time (FBUT) and Schirmer I test. All adverse events were recorded.
Results: 70 patients (70 eyes) were randomized into 2 groups (A and B), each of 35 patients. There were no significant differences between groups at baseline. At day 28, mean OSDI score, lid skin and lid margin inflammation, conjunctival injection, meibomian gland secretions and expression, corneal staining score, FBUT and Schirmer I test significantly improved ($P < 0.05$) in both groups. However, mean OSDI score, lid margin inflammation and conjunctival injection improved to a greater extent in group B versus group A at days 7 and 28. FBUT and Schirmer I test improved significantly in group B at day 28 ($P<0.05$). No serious adverse event occurred.
Conclusions: HPMC/dextran 0.3% and SH 0.18% significantly improved ocular surface disease in glaucoma patients versus baseline. However, SH 0.18% significantly improved ocular signs compared to HPMC/dextran 0.3%. Ocular surface problems in glaucoma patients should be considered and evaluated at each follow up visit to improve patient compliance and quality of life. Eyedrops, especially SH 0.18%, may be useful in relieving patient discomfort, improving ocular surface health and ocular tolerance to glaucoma medications, particularly those containing preservatives.

THE EFFECT OF THREE DIFFERENT PHOSPHOLIPID-CONTAINING EYE SPRAYS ON TEAR FILM AND SYMPTOMS

Heiko Pult,[1,3] Rhiad Khaireddin,[2] Britta H. Riede-Pult.[1,3]
[1]Dr Heiko Pult – Optometry & Vision Research, Weinheim, DE; [2]Clinical practice in JosefCarrée, Bochum, DE; [3]Cardiff University, School of Optometry & Vision Sciences, Cardiff, UK
Purpose: To investigate the effect of 3 different phospholipid-containing eye sprays on tear film stability (NIBUT), lipid layer (LL) and patients symptoms (COM).
Methods: In this multicentre, randomized, double-masked and co-lateral, prospective study TearsAgain (TA; Optima Pharma, DE) based on phospholipid-liposomes was applied to one randomly selected closed eye of 57 patients (mean age=47.41 ±16.49 SD) while Opticalm (OC; Medena AG, CH) or OmniTears (OT; OmniVision GmbH, DE) both based on a oil-in-water emulsion was applied to the co-lateral closed eye in accordance of the instructions for use. NIBUT and LL were observed before application and 10min, 30min, 60min, and 90min after application of the sprays by Tearscope (Keeler Ltd, UK). Dry eye status was evaluated by the ocular surface disease index (OSDI) and COM by a visual-analogue scale for each single eye before and after every time point. Data were analysed by multilevel models adjusting for the nested data structure.
Results: Mean OSDI scores were 19.92 ±17.84. TA showed a significant positive effect on NIBUT (effect=.071, SE=.024, df=73, t=2.991, p=.0038) and (COM) (effect=.330, SE=.056, df=73, t=5.870, p<.001) lasting up to 60min. This was not shown by OT (effect=.000, SE=.028, df=27, t=.012, p=.991; effect=-.099, SE=.089, df=27, t=-1.105, p=.279, respectively) or OC (effect=.022, SE=.026, df=27, t=.840, p=.408; effect=.040, SE=.088, df=27, t=.454, p=.654, respectively). In OT and OC, LL was not significantly changing (effect=-.001, SE=.004, df=27, t=-.211, p=.834; effect=.002, SE=.004, df=27, t=.583, p=.5649). TA showed a significant temporary improvement of LL (effect=.013, SE=.003, df=73, t=4.799, p<.001). TA peeked in a 1.4x improvement of NIBUT, 1.5x improvement of LL and 1.3x improvement of COM within 60min.
Conclusions: The application of TA significantly improved all observed parameters. Such consistent effects were not observed when applying OT or OC.
Financial Disclosure: Granted by Optima Pharma, DE

DISTRIBUTION OF MEIBOMIAN GLAND LOSS ALONG THE NASAL, CENTRAL AND TEMPORAL SEGMENTS OF THE LOWER EYE LID

Heiko Pult,[1,2] Donald Korb,[3] Caroline Blackie,[3] Britta H. Riede-Pult.[1,2]
[1]Dr Heiko Pult – Optometry & Vision Research, Weinheim, DE; [2]Cardiff University, School of Optometry & Vision Sciences, Cardiff, UK; [2]*Korb Associates and TearScience Inc., USA*
Purpose: To observe local meibomian gland loss (MGL) characteristics of the nasal, central and temporal lower lid using non-contact infra-red meibography.
Methods: Over-all MGL on image of 137 everted lower lids were analysed using Phoenix Meibography Software (PMS, bon, Lübeck, Germany). The first 30 images were classified in two different sessions (SI and SII) by two observers (OI and OII) to analyse repeatability of PMS. For all 137 images the location of the largest and second largest areas of MGL (nasal, central or temporal) was noted. Repeatability of PMS was analysed by Bland Altman plots, paired t-test and 95% confidence interval (CI). Location of the most MGL and of the second most MGL was analysed in its relation to percentage of over-all MGL by descriptive statistics.
Results: Measurements between sessions and observers were not significantly different. Intra-observer agreement (95% CI=8.0% MGL, p=0.231) was better than inter-observer agreement (95% CI=15.9% MGL, p=0.662). Mean over-all MGL score was 35.36% ±19.78% (range 0-93.2%). Except in an over-all MGL of less than 5% (n=8) maximum local MGL was more frequently seen at the nasal third of the eye lid (nasal: 79%, central: 5%, temporal: 6%, no trend: 10.9%). In the second largest area of MGL no further trend between the segments of the eye lid was detected (nasal: 5%, central: 7%, temporal: 23%, no trend: 65%). These trends were irrespective of over-all MGL scores.
Conclusions: The PMS grading appears to be repeatable. MGL detected using non-contact infrared meibography shows that the largest area of loss is most frequently in the nasal segment of the lower lid.
Financial Disclosure: none

CENTRAL LID MARGIN POSITION AND TEAR FILM SPREADING DURING 'LID CLOSURE' IN SPONTANEOUS BLINKING

Heiko Pult,[1,2] Donald Korb,[3] Paul Murphy,[4] Caroline Blackie,[3] Britta H. Riede-Pult.[1,2] [1]Dr Heiko Pult – Optometry & Vision Research, DE; [2]Cardiff University, School of Optometry & Vision Sciences, UK; [3]Korb Associates and TearScience Inc., USA; [4]University of Waterloo, School of Optometry & Vision Sciences, CA
Purpose: The central portion of the upper (UL) and lower lid (LL) margins frequently do not touch during spontaneous blinking. The aim of this project was to investigate tear film (TF) spreading and central lid position of the closed eye in spontaneous blinks.
Method: A 0.15 l lissamine green drop (LGD) was placed on the anterior portion of the central LL margin of 15 subjects (9 female; median age= 45). UL and LL position were evaluated by high-speed video and slit-lamp during blinking. Images were analysed by Image-J Software. Lid margin thicknesses and corneal topography were measured with a Scheimpflug camera. A scaled model diagram was created to represent the central lid position of the closed eye in spontaneous blinking. TF spreading and lipid layer formation were simulated in a glass plate model.

Results: LGD was unaltered in consecutive blinks. Median LGD height was 0.4mm indicating a distance of >0.4mm between central anterior lid margins. UL margin remained perpendicular to the normal of the corneal surface during blinking. The LL margin tilted up from the fully open position by 22° (median) and it thinned by 0.3mm (median). The scaled diagram indicates an over-blink of the UL over the LL and a height off-set of the posterior lid margin of >0.7mm (y-axis). TF meniscus of the lower lid fused with the upper lid tear meniscus even without lid touch (in-vitro). Lipid spreading seemed to be related to TF volume.
Conclusions: Central lid margins frequently do not touch in complete, spontaneous blinks. They are also misaligned and tilted. The impact of lid postiion of tear film spreading remains to be quantified.
Financial disclosure: none

COMPARISON OF THE OCULAR TOLERANCE OF HYABAK® NEW FORMULA COMPARED TO STANDARD HYABAK® IN CONTACT LENS WEARERS – A PILOT STUDY.

Professor Christine Purslow[1,2] Mr Philip Gibson[1] Dr Katharine Evans[1]. Cardiff University[1], Plymouth University[2], United Kingdom
Purpose: This study was designed to evaluate the ocular tolerance of Hyabak® new formula , a preservative-free, hypotonic eye drop, in a group of volunteers who wear soft contact lenses, compared to the standard Hyabak.
Methods: Eleven healthy volunteers were recruited (mean age 28.1±7.6 years) from Cardiff University. All subjects were habitual soft contact lens wearers, with at least 12 months' experience. Ocular comfort, redness, corneal staining, tear meniscus height and pre-lens tear film stability were examined at baseline and after five days of product use. Subjects were randomised to use different drops in each eye for five days. They were instructed to apply one drop inside the lens prior to wear, and then 2-3 drops during the wearing period of at least 7 hours, for five consecutive days, after which all outcome measures were repeated.
Results: There were no significant changes in bulbar redness, limbal redness, corneal staining, tear meniscus height or pre-lens tear film stability following 5 days of drop application in these subjects (0.170 <1.000). 1.5="“ also="“ and="“ between="“ comfort="“ difference="“ formula="“ in="“ new="“ no="“ ocular="“ original="“ p="0.276).<br" reported="“ significant="“ the="“ there="“ versus="“ was="“>
Conclusions: Hyabak® new formula is tolerated well in this group of soft contact lens wearers, with no increase in redness or corneal staining, and comfort is miantained, compared to Hyabak® standard formulation.
[This research was supported by Laboratoires THEA, France]

MIGRATION AROUND THE EYELID MARGIN.

Christine Purslow[1,2], Clare Conaty[2], Alison Ng[2]. [1]Plymouth University, [2]Cardiff University, United Kingdom
Purpose: Cosmetic products are commonly applied to and close to the eyelid margins. The primary aim of this study was to demonstrate migration of products across the ocular muco-cutaneous junction and investigate the influence of position of application on such migration.
Methods: The study composed of two parts. In Part 1, each eye of ten male subjects (23.1±3.5yrs) was randomly assigned to receive a controlled application of petroleum jelly to either the inner (IEL) or the outer (OEL) eyelid margin, prior to measuring tear film stability and lipid layer patterns using the Keeler Tearscope™, at timed intervals up to 30 minutes, which were then compared to baseline data. In Part 2, six male subjects (age 22.8±3.1yrs) attended the laboratory for three sessions during which hydrogel contact lenses were fitted to both eyes and a fluorescein solution was applied in one of three ways to one eye only; directly onto the IEL, OEL, or via a liposomal spray to the closed lid. The lenses were harvested after 20 minutes and examined using UV spectrophotometry. Differences in UV absorbance over 400-600nm indicated fluorescein uptake compared to control lenses.
Results: Part 1: A significant change in the lipid layer pattern of the tear film was observed after five minutes for IEL (p=0.007), but not until 20 minutes for OEL (p=0.037). Tear film stability decreased significantly within five minutes for IEL application (p=0.010), but not until 20 min for OEL (p=0.045). Part 2: Contact lenses harvested after IEL application demonstrated significantly greater absorbance compared to control lenses (p=0.005). Where fluorescein was applied via spray or OEL, no significant difference in absorbance compared to controls was observed (p=0.098 and p=0.124, respectively). Comparing the application methods, absorbance following IEL was significantly increased compared to OEL (p=0.014) and spray (p=0.044).
Conclusions: Lipid-based, water-based and liposomal solutions migrate across the muco-cutaneous junction when applied in close proximity to the eye. All three applications studied showed some migration; however application to the IEL was found to be most effective in allowing for migration into the tear film. Application of a lipid based solution to the IEL exhibited migration that was 4 times faster than OEL application. This has implications for drug delivery and cosmetic use around the eyelid margins.

MARX LINE – ASSOCIATION WITH PARAMETERS OF THE OCULAR SURFACE SYSTEM.

Dieter Franz Rabensteiner[1], Ingrid Boldin[1], Gerold Schwantzer[2], Manuela Fischl[1], Christine Wachswender[1], Haleh Aminfar[1], Jutta Horwath-Winter.[1] Department of Ophthalmology,[1] Institute for Medical Informatics, Statistics and Documentation,[2] Medical University of Graz, Austria
Purpose: The Marx line (ML) can be recognised after the application of a vital dye (e.g. Fluorescein or Lissamine green). It is running along the inner eyelid, usually representing the mucocutaneous junction. Normally, the ML is located on the conjunctival side of the meibomian gland (MG) orifices. In ocular surface disease and due to ageing it may be completely or partially located on the cutaneous side. The purpose of this study was to reveal possible associations of the ML with parameters of ocular surface system.
Methods: Data of 223 consecutive patients with ocular discomfort from the dry eye unit of the Department of Ophthalmology, Medical University of Graz, Austria, were analysed retrospectively. The following tear film and ocular surface parameters were examined: presence of lid margin parallel conjunctival folds (LIPCOFs), Fluorescein break-up-time, Fluorescein as well as Lissamine green staining of the ocular surface and Schirmer test without local anaesthesia. Lid margins were evaluated according to ML (Yamaguchi score), vascularisation, expressibility and quality of MG secretion. Non-contact IR-Meibography was performed and gland dropout scored according to the meiboscore of Arita and colleagues.
Results: Changes of the ML were associated with age and the presence of MGD as defined by the MGD report. The following parameters showed a statistically significant association: LIPCOFs, vascularisation of the lid margin, MG expressibility, MG quality, MG dropout assessed by meibography. Furthermore demodex infestation and arterial hypertension were also linked with ML changes, which might be due to the correlation with age.
Conclusions: Changes of the ML are a good indicator for the presence of MGD. They are more likely to occur with increasing age and can also be present in blepharitis anterior with demodex infestation.

THE ANTI-EVAPORATIVE EFFECT OF THE TEAR FILM WAX ESTERS

Antti H. Rantamäki,[1] Susanne K. Wiedmer,[2] and Juha M. Holopainen.[1] Helsinki Eye Lab, Department of Ophthalmology,[1] Department of Chemistry,[2] University of Helsinki, Helsinki, Finland

Purpose: We studied the evaporation-retarding effect of wax esters (WEs) *in vitro*. The WEs selected for this study closely resembled the most abundant WE species in meibum.
Methods: A custom-built system was used to measure the evaporation rates through WE layers applied to the air-water interface at 35 °C and at reference temperatures of 30 °C and 41 °C. Additionally the melting points of the WEs were measured. Brewster angle microscopy (BAM) was used to assess the organization of the lipids at the interface and Langmuir film experiments were performed in order to investigate the stability of the WE layers under compression-relaxation cycles.
Results: Four of the total nineteen WEs retarded evaporation at 35 °C: behenyl palmitoleate (BP), behenyl oleate (BO), behenyl linoleate (BLN), and behenyl linolenate (BLNN) decreased evaporation by 20-40%. BP was the most efficient evaporation-retardant WE. At 30 °C the most effective evaporation-retardants were BLN and BLNN decreasing evaporation by ~50%, whereas BP and BO decreased evaporation by only 5-10%. At 41 °C each lipid decreased evaporation by only 2-4%. These four WEs melted within ~2 °C of the physiological temperature. Based on BAM the evaporation-retardant WE layers spread somewhat uniformly at the interface and the layers seemed to be partially in condensed phase. The isotherms suggested that the WE layers tolerated high surface pressures, but were unstable under compression-relaxation cycles.
Conclusions: The ability to retard evaporation is dependent on the state of the WE layer at given temperature. This evaporation-retarding condensed-like state exists in proximity to the liquid-to-solid phase transition ultimately defined by the length and the degree of saturation of the WE carbon chains. WEs as such are poor surfactants and they need to be accompanied by polar lipids to form stable lipid layers. [The Finnish Eye Foundation, the Academy of Finland, the Sigrid Juselius Foundation, the Nissi Foundation, and the Magnus Ehrnrooth foundation supported this study]

VITAMIN D ATTENUATES TLR3 INDUCED INFLAMMATION IN HUMAN CORNEAL EPITHELIAL CELLS

Rose Reins, Alison McDermott. University of Houston College of Optometry, Houston, TX, USA
University of Houston College of Optometry, Houston, TX
Purpose: Vitamin D has many diverse functions, including an important immunomodulatory role during infection and inflammation. Therefore, its influence on corneal inflammation needs to be thoroughly evaluated, with a view to the development of novel treatments for inflammatory conditions, such as dry eye syndrome, or infection. Our current studies evaluate the anti-inflammatory actions of vitamin D on human corneal epithelial cells in response to Toll-like receptor (TLR) 3 activation.
Methods: Telomerase-immortalized human corneal epithelial cells (hTCepi) were stimulated with 1,25D3 (10^{-7}M) or TLR3 agonist PolyI:C (1µg/ml) for 24 hours. Expression of IL-1ß, IL-6, IL-8, IL-23, IFNy, TNFa, MIP3a, MMP-9, LL-37, and TLR3 were analyzed by real time RT-PCR. Protein expression was examined using ELISA, Luminex assay, and flow cytometry. hTCepi were also stimulated with LL-37 (10µg/ml) and PolyI:C for 24 hours and IL-8 expression tested. All experiments were performed a minimum of three times.
Results: Stimulation with PolyI:C resulted in a significant increase in pro-inflammatory cytokine expression in hTCepi. Addition of 1,25D3 downregulated these cytokines following TLR3 activation. MMP-9 expression was also attenuated by 1,25D3 during PolyI:C stimulation. Additionally, 1,25D3 decreased TLR3 expression. Interestingly, the coordinated response of both 1,25D3 and PolyI:C resulted in increased production of the anti-microbial peptide, LL-37, above 1,25D3 stimulation alone. However, LL-37 was not responsible for the level of cytokine downregulation by 1,25D.
Conclusions: Vitamin D attenuated TLR3 activation by decreasing cytokine production and downregulating TLR3 expression following PolyI:C stimulation. At the same time, LL-37 expression was augmented. These results suggest an important role for vitamin D in protecting the ocular surface during inflammation and demonstrate its ability to dampen the potentially harmful effects of an inflammatory response. [This research was supported by NIH Grant EY13175, UHCO Core Grant EY07551]

EXTRACELLULAR VESICLES AND THE STRESS RESPONSE IN THE CORNEAL EPITHELIUM

Alexandra Robciuc[1,2], Antti H. Rantamäki[1], Matti Jauhiainen[2] and Juha M. Holopainen[1]
[1] Helsinki Eye Lab, Department of Ophthalmology, University of Helsinki, Helsinki, Finland;[2] Public Health Genomics Research Unit - National Institute for Health and Welfare, Helsinki, Finland
Purpose: The proper maintenance of homeostasis at the ocular surface is still under discussion, since the external environment contributes as an unpredictable component. We aimed to discover how the corneal epithelium contributes to the complexity of the tear fluid in stress and physiological conditions.
Methods: A cell culture of human corneal epithelial cells (HCEs) was exposed to stress stimuli - ultraviolet radiation (UVB), hyperosmolarity (HO) and lipopolysaccharide (LPS) - and secretion of specific lipid modifying enzymes was followed in the conditioned medium using basic proteomic techniques. Additionally, activation of the cellular stress response and IL-8 secretion were measured.
Results: We identified enzymes of the sphingolipid metabolism (acid and neutral sphingo-myelinases and ceramidases) in human tears and conditioned media and also showed that their secretion can be induced by stress. All stress stimuli investigated induced a pro-inflammatory response from the HCEs, but only UVB and HO induced a dose-dependent release/secretion of sphingomyelinases from the cells as well. In the attempt to identify the secretion route of the enzyme we discovered that the sphingomyelinases are released in association with extracellular vesicles. These vesicles exhibited a moderate yet significant anti-inflammatory effect on the non-stressed HCEs.
Conclusions: HCE cells release extracellular vesicles in response to stress and components of the sphingolipid metabolism are associated with these mobile membranes. Moreover, the released vesicles might have a significant impact on the inflammation at the ocular surface.
[This work was supported by grants from the Juselius Foundation, Finnish Academy, Helsinki University Central Hospital Research Foundation, Finnish Eye Foundation and Nissi Foundation.]

DRY EYE ANIMAL MODELS: TEAR OSMOLARITY AND OCULAR SURFACE DYSFUNCTION

Eduardo Melani Rocha[1], Davi L Marques[1], Monica Alves[1, 2] MD PhD, Carolina Maria Modulo[1] MSc, Adriana Andrade Batista Murashima[1], Lilian Eslaine Costa Mendes da Silva[1] MSc, MD PhD
[1] Faculty of Medicine of Ribeirão Preto, University of São Paulo
[2] Pontific Catholic University of Campinas Brazil
Purpose: to evaluate parameters of ocular surface in three animal models of dry eye Disease (DED): a) induced by topical benzalkonium chloride 0.2% (BAC) for 7 days; b) Diabetes Mellitus induced by streptozotocin (60 mg/kg iv) (DM) and c) Hypothyroidism induced by methimazole (500 mg/l of drinking water) for 4 weeks.
Methods: male rats were compared to controls (n=5-8/group). The tear film and blood osmolarity were measured. Phenol red thread tear test and fluorescein staining were performed. Cornea histology was evaluated and Western blotting was applied to compare the expression of Substance P and beta-III tubulin in corneas of those groups.
Results: BAC, DM and Hypothyroidism induced DED like condition. Tear flow were 7.6 ± 2.01 mm in controls, 3.40 ± 0.67 mm after BAC treatment and 3.40 ± 2.27 mm in DM (p=0.03). Fluorescein staining were mild in controls, DM and hypothyroid rats and 8.40 ± 1.36 after

BAC instillation. In controls, the tear omolarity was 284.0± 3.29 mOsm/l, in BAC it was 306 ±4.09 mOsm/l, in DM it was 337.8 ± 17.35 mOsm/l and in hypothyroidism it was 344.6 ± 4.32 mOsm/l (p=0.007). Blood osmolarity in DM group was 328.4 ± 1.33 mOsm/l, and in the control group it was 305.5 ± 2.1 mOsm/l (p=0.02). Cornea epithelium was significantly thinner at day 07 in the BAC group compared with control rats (p< 0.05). The expression of beta-III tubulin was similar between BAC and control corneas.

Conclusions: tear hyperosmolarity, lower tear secretion and ocular surface damage have been considered hallmarks of DED in humans. The ocular surface changes and hyperosmolarity in animal models resembled that of DED in humans. The present work helps to monitor the mechanisms of DED in those diseases.

Financial Support: CNPq, FAPESP, NAP-FTO USP.

COMPARISON OF TEAR FILM LIPID PROFILE AMONG BASAL, REFLEX AND FLUSH TEARS.

Athira Rohit[1, 2], Simon HJ Brown[3], Fiona Stapleton[1, 2], Mark Willcox[2]. Brien Holden Vision Institute, Sydney, Australia[1],The School of Optometry & Vision Science, the University of New South Wales, Sydney[2],The School of Health Sciences, University of Wollongong, Sydney[3].

Objective: To determine whether using tears collected by flushing the ocular surface with saline can be used as an alternative to basal tear collection for the identification and quantification of lipids in the tear film.

Methods: Tears were collected from ten participants with no history of ocular surface disease or contact lens wear. Up to 5 L of basal tears were collected from each eye using a microcapillary tube. Up to 15 L of reflex tears were collected by stimulating a sneeze reflex by gently inserting a sterile cotton bud in to the nasal passage or by self induced yawning. For "flush tears", 60 L of unit dose saline was installed into the inferior palpebral fornix using an eppendorf pipette, participants closed their eyes and rotated them twice, and fluid was then immediately collected by capillary tube. Each of the basal, reflex, and flush methods described above were used on three occasions with the order of methods randomized allowing at least 24 hr between each collection method. The lipid components of interest such as cholesterol esters, wax esters, triacylglycerides, total phospholipids including phosphatidylcholine and sphingomyelin and (O-acyl)- -hydroxy fatty acids (OAHFA) were analyzed from each tear sample using nano-electro-spray ionization tandem mass spectrometry.

Results: All targeted lipid classes including cholesterol esters (45.7+/-6.8%), wax esters (34.2+/-6.9%), triglycerides (3.3+/-0.6%), total phospholipids (9.4+/-1.5%) and OAHFA's (6.0+/-2.1%) were detected from basal tear samples. However, the hydrophobic components including wax esters and triacylglycerides and the hydrophilic components including total phospholipids and OAHFA's from the reflex and flush tear samples were below the limit of detection.

Conclusion: We were not able to confirm that flush or reflex tears show a similar lipid profile to basal tears, in contrast with a previous study examining tear protein profiles. We recommend that basal tears are used for tear lipid analysis, as the reflex or flush tears contain very low levels of most lipid components.

No commercial relationships.

The first author's PhD candidature is supported by the Tuition Fee Remmission Scholarship from the University of New South Wales, Sydney, Australia and a Faculty stipend from Vision Cooperative Research Center, Australia.

TOWARDS QUANTITATIVE IMAGING OF THE TEAR FILM DYNAMICS AND UNDERSTANDING ITS CLINICAL RELEVANCE

Jannick P. Rolland, University of Rochester, Rochester, NY, USA

Imaging has long been a valuable tool in science. Across a wide range of applications, a common denominator is the enormity of the datasets generated by modern imaging systems and the unique issues associated with their evaluation for their objective comparison and optimization. We hypothesize in this research that it will be highly advantageous to design a medical device to maximize its performance on the tasks for which the system is intended. The basic premise is that image quality must be evaluated by the usefulness of the image data for an observer performing some clinically or scientifically relevant task. Generic tasks include classification and estimation. Here, we seek to estimate the time-varying thickness of the tear film and associated volume across the cornea using a computational observer.

In this talk, we will report on the development of a tear-film nanoscale-class imager (hardware and algorithms) to capture the dynamics of the tear film in the context of Optical Coherence Tomography of the Ocular Surface (OSOCT). Specifically, we guide the design of the imager with a custom simulation tool that includes a computational observer to interpret the OSOCT data and estimate the tear film thickness from the imaging datasets. We will show that the developed tool enables us to accurately estimate simultaneously the thickness of the lipid and aqueous layers, which is critical in the investigation of tear film instability (TFI), a core mechanism of Dry Eye Disease. The current instrument built in the laboratory achieves an axial resolution of less than one micron by design, validated in experiments, a requirement to achieve thickness estimates in the nanometer class. Finally, given the measured dynamics of the tear-film thickness, we will show a path to evaluating the optical aberrations induced by the tear film and their impact on retinal image quality.

The computational framework presented is a paradigm shift in the development of an OSOCT instrument for applications that represent new frontiers in performance such as measuring the tear film dynamics.

[We acknowledge the NYSTAR Foundation, the NIH, and the Research to Prevent Blindness]

THE CORNEAL PAIN SYSTEM AND DRY EYE DISEASE

Perry Rosenthal

The tear deficiency/hyper-evaporation model of dry eye disease needs help in explaining the disparity between dry eye-like symptoms and signs of ocular surface desiccation, especially in the presence of generous tears. Moreover, its model needs to account for observations that tear metrics and Meibomian gland function presumed to cause dry eye-like symptoms in some people are asymptomatic in others. The neuropathic paradigm attributes them to pathologically lowered activation thresholds of certain corneal thermoreceptors, such as those of the TRPM8 family believed responsible for monitoring tear layer thickness through their sensitivity to drops in temperature. Recent studies suggest that as the tear layer becomes thinner, the rate of evaporative cooling at the corneal surface increases until it breaches the activation thresholds of underlying thermoreceptors and triggers the cascade of events that culminate in the restoration of a robust optical tear layer and silencing of their activity. It follows that as their activation sensitivity increases (and activation thresholds fall) so does their sensitivity to tear evaporation clinically manifested as corneal evaporative hyperalgesia. 'Sensitized' corneas would also be expected to require thicker tear layers to avoid triggering dry eye-like symptoms and be more sensitive to tear layer thinning accelerators such as moving air, Meibomian gland dysfunction, accelerated tear layer breakup, etc. Notably, the disparity between the intensity of symptoms and external signs is also characteristic of painful peripheral neuropathies. Might inadequately explained chronic corneal evaporative hyperalgesia be a form of neuropathic pain? Could the associated corneal inflammation that plausibly contributes to nociceptor sensitization be generated by ongoing barrages of nociceptor activity (neuroinflammation)? Can the paucity of tears, when present, be a downstream complication of corneal neuropathy and, while exacerbating symptoms, is not their primary cause?

The strength of a medical paradigm is measured by its ability to explain the otherwise unexplainable and predict the effectiveness of

novel treatments. The latter has yet to be demonstrated for this paradigm.

MANAGEMENT OF INTRACTABLE OCULAR SURFACE DISEASE WITH NEWLY DEVELOPED SCLERAL CONTACT LENS.

Soo Jeong Ryu,[1] Hyuk Jin Choi,[2,3] Joon Young Hyon,[1,2] Sang Mok Lee,[4] Joo Youn Oh,[2] Won Ryang Wee.[2] Department of Ophthalmology, Seoul National University Bundang Hospital, Gyeonggi, Korea,[1] Department of Ophthalmology, Seoul National University College of Medicine, Seoul, Korea,[2] Seoul National University Hospital Healthcare System Gangnam Center, Seoul, Korea,[3] Schepens Eye Research Institute, Massachusetts Eye & Ear Infirmary, Harvard Medical School, Boston, MA ,U.S.A.[4]

Purpose: To report cases of intractable ocular surface diseases managed with newly developed scleral contact lens.

Methods: Newly developed scleral contact lens were designed to be optimized for fitting in Asian eyes. This study included those patients with recurrent cornea epithelial defect, Stevens-Johnson syndrome, Graft-Versus-Host Disease, whose symptoms could not be controlled by conventional treatments. UCVA, BCVA, OSDI, ECC, and the status of ocular surface were evaluated before and after scleral contact lens fitting.

Results: Scleral contact lens was prescribed to 21 eyes of 13 patients. OSDI and visual acuity were significantly improved after scleral contact lens fitting during follow up periods of 12weeks. None of them was affected any contact lens related complication.

Conclusion: Scleral contact lens with optimized design for Asian eye can be useful treatment option for severe ocular surface diseases.

[No commercial relation ship]

LIPOPOLYSACCHARIDE INDUCTION OF PRO-INFLAMMATORY GENE EXPRESSION IN HUMAN CORNEAL, CONJUNCTIVAL AND MEIBOMIAN GLAND EPITHELIAL CELLS.

Afsun Sahin, Wendy R. Kam, Raheleh Rahimi Darabad, Yang Liu, David A. Sullivan. Schepens Eye Research Institute, Massachusetts Eye and Ear, Harvard Medical School, Boston, MA, USA

Purpose: We recently discovered that lipopolysaccharide (LPS), a bacterial glycolipid, induces the secretion of leuokotriene B4 by human corneal, conjunctival and meibomian gland epithelial cells (Arch Ophthalmol 2012;130:1013-1018). We hypothesize that this hormone action reflects an overall stimulation of inflammatory gene activity in these cells. Our goal was to test this hypothesis.

Methods: Immortalized human corneal (gift from Dr. James Jester, Irvine, CA), conjunctival (gift from Dr. Ilene Gipson, Boston, MA) and meibomian gland epithelial cells were cultured in the presence or absence of LPS (15 μg/ml) and ligand binding protein (150 μg/ml). Cells were then processed for RNA isolation and the analysis of gene expression by using Illumina BeadChips, background subtraction, cubic spline normalization and Geospiza software.

Results: Our results demonstrate that LPS stimulates a significant increase in pro-inflammatory gene expression in human corneal and conjunctival epithelial cells. This effect was associated with a marked upregulation of genes linked to cytokine and chemokine production, cytokine secretion, chemotaxis, Toll-like receptor signaling pathway, apoptosis and defense, and inflammatory and immune responses. In contrast, with the exception of the Toll-like pathways, none of these pro-inflammatory ontologies were induced by LPS in human meibomian gland epithelial cells.

Conclusions: Our findings support our hypothesis that LPS promotes inflammatory gene expression in human corneal and conjunctival epithelial cells. However, our results also demonstrate that LPS lacks the ability to induce such inflammatory activity in human meibomian gland epithelial cells. [Supported by NIH grant EY05612, the Margaret S. Sinon Scholar in Ocular Surface Research fund, ARVO/Pfizer and TUBITAK]

MEIBOMIAN GLAND DYSFUNCTION: ENDOCRINE ASPECTS.

Ozlem G Sahin[1], Elçin Kartal[2], Nusret Taheri[3] Dunya Goz Hospital, Department of Ophthalmology/Uveitis[1], Middle East Technical University Department of Statistics[2], Middle East Technical University Health Sciences Department of Biochemistry[3], Turkey

Purpose: To compare the hormone levels of patients with seborrheic meibomian gland dysfunction with controls.

Methods: This is a retrospective case-control study involving 50 patients and 50 controls. Blood work-up for hormones were studied in both groups by using macroELISA (Enzyme-linked immunosorbent assay). Statistical evaluation was done by using SPSS 15.0 independent samples t-test.

Results: There were statistically significant differences of serum testosterone and dehydroepiandrosterone sulphate levels between patients and controls.($p = 0.000$) Female gender showed statistically significant differences of serum thyroid-stimulating hormone and prolactin levels between patients and controls ($p = 0.014$ and $p = 0.043$), in addition to serum testosterone and dehydroepiandrosterone sulphate levels.($p = 0.000$ and $p = 0.001$) However, male gender showed statistically significant differences of only serum testosterone and dehydroepiandrosterone sulphate levels between patients and controls. ($p= 0,003$ and $p = 0,003$ respectively)

Conclusions: Increased serum levels of testosterone and dehydroepiandrosterone sulphate in both genders might be considered as diagnostic markers for seborrheic meibomian gland dysfunction.

[The authors have no proprietary or commercial interests in any concept or product discussed in this article. The authors have no grants to declare for this research]

OCULAR SIGNS AND SYMPTOMS IN CONTACT LENS WEARERS IN A CONTROLLED LOW HUMIDITY ENVIRONMENTAL EXPOSURE CHAMBER (LH-EEC) A NATURAL PROVOCATION RESEARCH MODEL.

Anne Marie Salapatek[1], Piyush Patel[1], Fiona Soong[1] , Jalaiah P. Varikooty[2] , Nancy J. Kier[2] , Lyndon Jones[2] [1]Inflamax Research, Mississauga, [2]CCLR, University of Waterloo, Waterloo, Canada

Purpose: To evaluate the effects of contact lens (CL) wear on the ocular surface under controlled conditions of low humidity and airflow, to assess the validity of a natural provocation research model for the study of dry eye and contact lenses.

Methods: The LH-EEC is validated to maintain uniform low humidity and comfortable room temps which mimic natural arid environs. Ten symptomatic CL wearers discontinued CL wear & used artificial tears tid for 48hrs prior to LH-EEC Visit. Upon return, subjects were randomly fit with etafilcon A: CL_A in one eye and narafilcon A: CL_B in the contralateral eye; then exposed to LH-EEC for 180mins with visual tasking. Total Ocular Symptom Scores (TOSS) were rated at entry and at set intervals within the EEC. Tear Break Up Time (TBUT), corneal staining and conjunctival staining were collected pre and post LH-EEC using Efron scale (0-4) on the NEI corneal grid and nasal & temporal conjunctival zones.

Results: After 180 mins in LH-EEC, mean increase from baseline was CL_A=+1.4±1.2 and CL_B=+1.0±0.7 for corneal staining and CL_A=+1.30±0.78 and CL_B=+1.20±0.72 for conjunctival staining. The mean reduction in TBUT was CL_A=-0.3±0.2s and CL_B=-0.8±0.7s. Symptoms escalated throughout the exposure period with TOSS increasing from baseline for both lenses (CL_A=+2.4±1.7 and CL_B of +3.30±1.16), with the mean change for CL_B being significant (p=0.02). Similarly, dryness symptom score showed a trend to increase for CL_A (+1.1±0.5) and a significant increase for CL_B (+1.40±0.31) (p=0.001).

Conclusions: The LH-EEC CL Dry Eye model exacerbates ocular symptoms and signs while controlling all environmental variables. After 180mins of exposure, signs indicative of ocular surface desiccation and an increase in dry eye symptoms were measurable. The LH-EEC model provides a valuable clinical research option for the study of CL and dry

eye, which provides rapid symptoms and signs typically found in longer periods of in-eye wear outside the LH-EEC.
Study support from CCLR-School of Optometry, University of Waterloo, Canada.

IN-VIVO WETTABILITY OF CONTACT LENSES WORN IN A LOW HUMIDITY ENVIRONMENTAL EXPOSURE CHAMBER (LH-EEC) SHOW COMPARABLE CHANGES TO TRADITIONAL FIELD TRIALS

Anne Marie Salapatek[1], Fiona Soong[1], Jalaiah P. Varikooty[2], Nancy J. Kier[2], Lyndon Jones[2], Piyush Patel[1].

Purpose: The purpose of this study was to use the LH-EEC, a natural provocation research model which tightly controls environmental variables (humidity, temp, airflow) to observe in-vivo CL wettability changes and to compare these results with those reported in typical CL field studies.

Methods: Ten symptomatic CL wearers were randomized and fit with etafilcon A: CL_A in one eye and narafilcon A: CL_B in the contralateral eye. They were exposed to LH-EEC for 180mins with visual tasking. Following CL insertion, measures of tear osmolarity were taken prior to LH-EEC entry and exit. Observations of blink rate and in vivo wettability grading (using a 0-4 scale with 0.25 steps) was performed at specified intervals throughout the visit. Dryness symptoms were rated from 0 (no discomfort) to 4 (constant discomfort) throughout the visit.

Results: After 180mins of LH-EEC, there was trend of increasing tear osmolarity for both CL_A (7.4±3.6mOsmol) and CL_B (4.80±3.23mOsmol), but this was not significant ($p>0.05$). Dryness symptom score showed non-significant increase from pre to post chamber for CL_A (+1.10±0.53) but a significant ($p=0.001$) increase for CL_B (+1.40±0.31). Blink rate significantly increased ($p<0.003$) from pre-EEC rates of 42.0±4.8blinks/min to avg. maximum of 61.2±4.3blinks/min. Lens wettability worsened significantly over time for both CL_A and CL_B 0.58±0.18 ($p=0.01$) and 0.65±0.25 ($p=0.03$) respectively. These values are comparable to changes in wettability seen after 8hrs of wear with both study materials (Luensmann et al; Keir et al.)

Conclusions: The LH-EEC exacerbates ocular symptoms and signs after 180min with CL wear. Significant changes in lens wettability were seen during the exposure and yielded values comparable to results shown in traditional trials with 8hrs CL wear. The LH-EEC provides a way to accelerate CL wear and provides noteworthy research options for a controlled provocation study of CL and dry eye signs and symptoms in a shorter time course.

Study Support by CCLR - School of Optometry, University of Waterloo, Canada

TEAR SECRETION CHANGE BY A DAILY INTRAPERITONEAL INJECTION WITH AICAR FOR 5 DAYS IN C57BL/6J AND DB/DB MICE.

Kokoro Sano, Motoko Kawashima, Takaaki Inaba, Akiko Ito, Tetsuya Kawakita, Shigeto Shimmura and Kazuo Tsubota. Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan

Purpose: AMP-activated protein kinase (AMPK) plays a key role in regulation of energy homeostasis in various organs including muscles and the liver. We investigated the effects of AMPK activator, 5-Aminoimidazole-4-carboxyamide ribonucleoside (AICAR) on tear secretion in mice.

Methods: Male C57BL/6JJc1 mice received a single intraperitoneal injection of 125 mg/kg AICAR (n=10), 250 mg/kg AICAR (n=12) or PBS as a control (n=10) per day for 5 days. Tear secretion was measured by cotton threads (Zone Quick) for 30 seconds at pre-administration and on day 5. For gene expression analysis, the same treatment was performed using different mice (n=5 or 6). Total RNA was extracted from the lacrimal glands of the mice, then real time PCR was performed for Nicotinamide phosphoribosyltransferase (Nampt). And we also used BKS.Cg-+$Lepr^{db}$/+$Lepr^{db}$/J (db/db) mice and measured tear secretion. Female db/db mice received a single intraperitoneal injection of 125mg/kg AICAR (n=5) or PBS as a control (n=5) per day for 5 days.

Results: AICAR treatments for 5 days increased tear secretion in C57BL/6JJc1 and db/db mice. In C57BL/6JJc1 mice, the tear secretion in both 125 mg/kg and 250 mg/kg AICAR-treated groups was significantly larger (5.25±2.75mm, 5.25±3.75mm) than in the control (2.75±1.75mm) ($p<0.01$, $p<0.01$). In the real time PCR, Nampt expression in the 250mg/kg AICAR group was significantly larger than in the control on day 5 ($p<0.01$).

Conclusion: A daily injection of AICAR for 5 days leads to an increase in tear secretion. The results suggest that pharmacological activation of AMPK may be a potential therapy for dry eye disease.

EPIDEMIOLOGY AND RISK FACTORS FOR MEIBOMIAN GLAND DYSFUNCTION (MGD)

Debra A. Schaumberg, ScD, OD, MPH

Meibomian gland dysfunction (MGD) can lead to an evaporative subtype of dry eye disease (DED) and is thought to play a role in the majority of cases of DED throughout the world. Epidemiological studies have indicated a substantial overlap between MGD and DED and that the evaporative and aqueous deficient subtypes of DED often occur together. MGD is often associated with symptoms of ocular surface irritation and visual fluctuation, as well as compromise of the normal ocular surface homeostasis; however, research has indicated age-related changes in the meibomian gland also occur among people who report no such symptoms. This presentation will review and update the epidemiologic investigation of MGD focusing on work done since the report of the International Workshop on MGD. Additional estimates of the prevalence of symptomatic and asymptomatic MGD from different populations have been published that reinforce the higher prevalence among Asian populations seen in prior work. Further work on identification of potential risk factors has suggested the possibility of a higher prevalence of MGD among men, and in association with pinguecula, higher diastolic blood pressure, diabetes, cardiovascular disease, rosacea, rheumatoid arthritis, hypercholesterolemia, and use of angiotensin II receptor blockers. There has been some progress in understanding the epidemiology of MGD but further work is needed.

"PLUNC", A NEW SURFACTANT PROTEIN OF THE TEAR FILM.

Martin Schicht, Fabian Garreis, Lars Bräuer and Friedrich Paulsen Institute of Anatomy, Friedrich-Alexander-University Erlangen-Nuernberg, Germany

Purpose: PLUNC (Palate Lung Nasal Clone) is a very hydrophobic protein that belongs to the family of surfactant proteins. PLUNC is a component of the innate immune system and could be identified in various human samples. The protein is produced by bronchial epithelial cells and immunohistochemical studies also showed the presence of PLUNC within the respiratory epithelium and submucosal glands. Recently, it was shown that PLUNC regulates the liquid volume of the airway surface by inhibition of the epithelial sodium channel (ENaC). This indicates that PLUNC could play a role in regulating the fluid balance of the lung. The PLUNC protein show evolutionary similarity to serum glycoproteins and to factors of the innate immune system, the BPI protein (bactericidal / permeability-increasing protein) and the lipopolysaccharide binding protein (LBP). Furthermore, it is able to directly act antimicrobial against Gram-negative organisms.

Methods: PLUNC expression was analyzed and quantified by means of real time-RT-PCR, Western-blot analysis, ELISA as well as immunohistochemistry. The activation and regulation of PLUNC transcription was studied in a human cornea cell line (HCE) as well as in a conjunctiva epithelial cell line (HCjE) after incubation with ocular pathogens by real-time RT-PCR. Furthermore, the PLUNC

concentration was quantified by ELISA in tears from patients with dry eye syndrome (DES) in comparison to healthy volunteers.
Results: Here, we present for the first time the detection of PLUNC in the ocular system and its regulation in the tear film with regard to rheological and antimicrobial properties. All investigated tissue samples cornea, eye lid, lacrimal gland and meibomian gland react show presence of PLUNC RNA and protein. In tears from DES patients PLUNC concentration is increased compared to controls. The stimulation experiments in cornea and conjunctiva cell lines with pathogens shows an increased expression of PLUNC.
Conclusion: PLUNC is a fluid- and immune regulatory protein and based on our findings we hypothesize that PLUNC may play a role during immune defense at the ocular surface and for the regulation of surface tension of the tear film.

PROTEOGLYCAN 4 (LUBRICIN) AS A NATURAL OCULAR SURFACE CONTACT LENS BOUNDARY LUBRICANT

Tannin A Schmidt.[1,] Michael Samsom,[1] Yuno Iwabuchi,[1] Amanda Chan,[1] Lakshman N Subbaraman,[2] Lyndon Jones,[2] Heather Sheardown,[3]
University of Calgary,[1] Calgary, Canada; University of Waterloo,[2] Waterloo, Canada; McMaster University,[3] Hamilton, Canada.
Purpose: Proteoglycan 4 (PRG4), also known as lubricin, is a mucin-like glycoprotein recently discovered at the ocular surface, where it functions as a boundary lubricant and appears to play a protective role. Friction may influence, and contribute to, dry eye disease and contact lens (CL) discomfort. As such, supplementation with PRG4 could combat heightened friction and potential CL discomfort. Therefore, the objective of this study was to determine whether PRG4 is an effective in vitro boundary lubricant at a human cornea-CL biointerface.
Methods: Human corneas, and model silicone hydrogel CL materials (pHEMA-TRIS, DMAA-TRIS) or commercial CL (Acuvue TrueEye® (TE), Acuvue Oasys® (OAS)), were mounted on a biomechanical testing machine with custom sample holders, forming a cornea-CL biointerface. Surfaces were articulated against each other at effective sliding velocities ranging from 0.3–30 mm/s under loads of 8-25 kPa. CL were first tested in saline, then in 300 ug/mL PRG4.
Results: PRG4 functioned as an effective friction-reducing boundary lubricant for model and commercial CL tested. Kinetic friction values were significantly lower in PRG4 for both commercial CL (TE 0.12±0.02, OAS 0.10±0.02, mean±sem), compared to their respective saline controls (TE 0.16±0.03, $p<0.05$; OAS 0.13±0.03, $p<0.01$). Similarly for model CL materials, (pHEMA-TRIS 0.17±0.030, DMAA-TRIS 0.13±0.03) compared to their saline controls (pHEMA-TRIS 0.20±0.03, $p<0.01$; DMAA-TRIS 0.16±0.03, $p<0.05$).
Conclusions: These data support the hypothesis that PRG4 is an effective ocular surface boundary lubricant for CL. Due to the possible association between ocular friction and discomfort, these data support the use of PRG4 as a therapeutic treatment for dry eye and symptomatic CL wearers. Future studies will examine PRG4 interaction with other CL and biomaterials, other lubricants (e.g. hyaluronan), and its lubricating ability on human eyelids.
This research was supported by NSERC & CIHR. Schmidt is a co-founder of Lubris, LLC.

LIFITEGRAST, AN INVESTIGATIONAL ICAM-1 DECOY, INHIBITS T-CELL ACTIVATION, ADHESION AND CYTOKINE RELEASE.

C.P. Semba[1]; [1] Shire Pharmaceuticals
Purpose: T-cells mediate, direct and promote the chronic inflammatory response in dry eye disease (DED). Lymphocyte function-associated antigen-1 (LFA-1) is the main integrin on T-cells. The binding of LFA-1 to ICAM-1 activates the T-cell mediated inflammatory cascade. Studies in DED patients have demonstrated increased expression of ICAM-1. Therefore, ICAM-1 is a logical target to halt or reduce chronic inflammation in DED.
Methods: A lipid bilayer system was created to mimic the binding of LFA-1 to ICAM-1, also called the immunological synapse (IS). The disassociation constant, and affinity and avidity to LFA-1 were measured in the presence of lifitegrast. A second study assessed the ability of lifitegrast to inhibit human Jurkat cell attachment to ICAM-1. In a third study, human peripheral blood mononuclear cells (PBMCs) were stimulated with staphylococcal enterotoxin B (SEB) to release cytokines whose presence in tears correlates with the clinical severity of DED. The concentration of lifitegrast needed for 50% inhibition of cytokine release (EC_{50}) was assessed.
Results: Lifitegrast prevented and rapidly reversed the formation of the IS by outcompeting ICAM-1 for its binding site on LFA-1 in a dose-dependent fashion, demonstrating high affinity and avidity for LFA-1. Lifitegrast demonstrated concentration-related inhibition of Jurkat cell attachment to ICAM-1 and potent concentration-dependent inhibition of cytokine release from human PBMCs, particularly Th1 and Th2 T-cell cytokines, IFN- and IL-4. The EC_{50}s of lifitegrast for IFN- , IL-1 , IL-1 , IL-10, and MIP-1 – cytokines that correlate with the severity DED – was below 1nM, much lower than levels of lifitegrast sustained in tears following a BID dose in humans.
Conclusions: Lifitegrast is a novel ICAM-1 decoy that demonstrated potent and dose-dependent effects on T-cell activation, adhesion, and cytokine release, events which contribute to chronic inflammation in DED. Lifitegrast ophthalmic solution 5.0% is currently being tested in a Phase 3 pivotal study for the signs and symptoms of DED.

Dr Semba is an employee of SARcode Bioscience Inc., a wholly owned subsidiary of Shire Pharmaceuticals.

CO-EXPRESISON OF COX-2 AND MMP9 IN DRY EYE INDUCED MOUSE LACRIMAL GLANDS.

Yuri Seo, MD[1], YongwooJi,MD[1], Jongwoo Shim, MD[1], Hyemi Noh, PhD[1],HyungKeun Lee, MD[1]. Institute of Vision Research, Department of Ophthalmology, Yonsei University College of Medicine, Seoul, Korea[1]
Purpose: Cyclooxygenase 2 (COX-2) and matrix metalloproteinase 9 (MMP9) are well known inflammatory markers in many pathologic conditions, including dry eye. The primary purpose of this study is to determine the expression levels and topographic localization of the COX-2 and MMP9 expression in lacrimal gland by using controlled environmental chamber dry eye mouse model. In addition, MMP levels were determined by treatment of COX-2 inhibitor, celecoxib.
Methods: Eight to 12 weeks-old C57BL6 mice were used to murine dry eye model. To induce dry eye, B6 mouse were exposed of controlled environmental chamber and injected 0.1% scopolamine three times a day for a week. Then, expressions of COX-2, MMP9 in dry eye induced lacrimal glands were examined using immunohistochemistry and quantitative real-time polymerase chain reaction(qRT-PCR). In addition, to determine the effect of celecoxib, one group of mice were topically treated with 0.02% celecoxibtidand the other group was treated by vehicle for a week after dry eye induction.
Results: By the qPCR analysis, COX-2 and MMP9 mRNA expression in dry eye induced lacrimal glands was significantly increased than control mouse. The expression of COX-2 and MMPs were localized on peripheral areas of acinar and boarder of lobules with immunohistochemical staining study. But, no ductal epithelium and infiltrating cells expressed neither COX-2 nor MMP9. Moreover, MMP9 expression was overlapped with the COX-2 expressed area in dry eye induced lacrimal gland. By topically applied celecoxib, the expression of COX-2 and MMP9 mRNA was significantly reduced
Conclusions: COX-2 and MMP9 expressions are enhanced in vivo murine dry eye model, which can be inhibited by topical celecoxib treatment. Considering the topographic pattern of expression of COX-

2 and MMP9, MMP9 expression may be regulated by the COX-2 activity in dry eye lacrimal gland.

A PROSPECTIVE RANDOMIZED COMPARATIVE STUDY ON THE EFFICACY AND SAFETY OF THREE DIFFERENT SURGICAL PROCEDURES FOR CONJUNCTIVOCHALASIS.

Seika Shimazaki-Den,[1] Daisuke Tomida,[1] Miki Iwasaki,[2] Murat Dogru,[1] Jun Shimazaki.[1] Tokyo Dental College Ichikawa General Hospital,[1] Chiba, Japan, Ryogoku Eye Clinic,[2] Tokyo, Japan,

Purpose: Conjunctivochalasis (CCh), which is a major cause of ocular discomfort or dry eye symptoms in the elderly people, can be resolved with surgical treatment. This study was conducted to elucidate the efficacy and safety of three different surgical methods for CCh.

Methods: Forty-three eyes of 25 patients (mean age 75.1±9.8 years, 18 females) observed more than 3 months after surgical treatment for CCh were assigned to three groups as follows; cautery group (C) : 15 eyes, tucking group (T) : 16 eyes, resection group (R) 12 eyes. The main outcome measures were the duration of procedure, complications, frequency of residual conjunctival folds (Tseng's grade 0-3), subjective tolerability of surgery, tear breakup time (TBUT) and subjective symptoms.

Results: The mean duration of procedures for cautery, tucking and resection was 4.1±2.0, 7.9±2.7 and 13.9±3.2 m, respectively, and the difference was significant (*P*<0.0001). Although conjunctival granulation due to suture was observed in all eyes which underwent tucking, it disappeared with topical steroids. The mean preoperative CCh grade in the surgical groups was 2.6±0.5 (C), 2.8±0.5 (T), 2.3±0.8 (R) which significantly decreased to 0.3±0.6 (C), 0.6±0.8 (T) and 0.4±0.9 (R). However, the percentages of residual CCh greater than grade 1 in the surgical subgroups were 26.7 (C), 43.8% (T) and 16.7 (R), respectively. There was no significant difference in subjective tolerability among the three groups. TBUT was significantly prolonged to 4.5±2.2 s from 3.3±1.8 s after surgery. Subjective symptom scores were significantly improved after surgery in the three groups.

Conclusions: Surgical treatment for CCh was effective and safe regardless of the method. Tucking method tended to be inadequate in terms of complications and residual CCh compared to cautery or resection.

A NEW EXAMINATION FOR ASSESSING THE OCULAR SURFACE SENSITIVITY: MAXIMUM OPENING TIME OF THE EYES

Jun Shimazaki[1], Murat Dogru[1], Seika Shimazaki-Den [1]Department of Ophthalmology, Tokyo Dental College Ichikawa General Hospital, Chiba, Japan

Purpose: It has been reported that symptoms and signs do not have a good correlation in dry eye (DE) diseases. Differences in ocular surface sensitivity may be attributable to these discrepancies. However, other than the corneal sensitivity testing by esthesiometry, no new clinical examinations have been developed. We hypothesized that the tolerability of the desiccation may be related to the ocular surface sensitivity, and conducted this clinical study to assess the efficacy of two new indices.

Methods: Fifty-eight subjects (19 males and 39 females) with a mean age of 57.4 + 16.0 years were included in this prospective study. The subjects were divided into the following 3 groups according to the Japanese DE criteria; DE patients (n=12), DE suspects (n=32), and non DE subjects (n=14). Following routine DE examinations including fluorescein staining (maximum score; 9 points), tear film break-up time (BUT) measurements, the maximum opening time (MOT) of the eyes was measured. In this measurement, subjects were instructed to keep their eyes open until they felt discomfort. The measurement was repeated three times, and the mean value was used for analysis. The difference between MOT and BUT (MOT-BUT difference: MBD), which might reflect sensitivity to desiccation, was also used for analysis. Following these measurements, the subjects completed a DE questionnaire and underwent Schirmer test.

Results: Both MOT and MBD were significantly shorter in DE and DE suspects compared with non-DE subjects (P=0.0002 and 0.0014, respectively). Females showed a significantly shorter MOT (P=0.0086) and MBD (P=0.0076) than the males. MOT and MBD both had a strong correlation with dryness symptoms (P=0.027 and 0.023, respectively). With regard to the correlations with other parameters, both MOT and MBD showed a significant correlation with BUT (P<0.001 and 0.041, respectively) but not with other parameters such as Schirmer values and fluorescein staining scores.

Conclusions: MOT and MBD are decreased in DE subjects, suggesting increased ocular surface sensitivity. Since MOT and MBD showed a good correlation with DE symptoms and signs, they appear to be promising parameters for the assessment of symptomatology in DE disease.

[There is no commercial relationship or grant support on the subjects]

DRY EYE RELATED QUALITY OF LIFE AND EYE MANIFESTATION IN OCULAR CHRONIC GRAFT VERSUS HOST DISEASE PATIENTS

Machiko Shimmura-Tomita, Hiroko Takano, Nozomi Kinoshita, Fumihiko Toyoda, Ayumi Ota, Katsuaki Tanaka, Akihiro Kakehashi. Department of Ophthalmology, Saitama Medical Center, Jichi Medical University, Saitama, Japan

Purpose: To evaluate Dry Eye related Quality of life Score (DEQS) and ocular manifestation in ocular chronic graft versus host disease (GVHD) patients.

Methods: We examined DEQS score, Schirmer test, tear break up time (BUT), fluorescein score, corneal sensation, conjunctival injection signs, the history of lacrimal punctum plugs, ocular complications, systemic GVHD and current systemic and local therapies of 9 cases of ocular chronic GVHD (5 males and 4 females; mean age 48.6 years old; mean follow up period 23.3 months) followed at our institution.

Results: The average DEQS score was 33.0 ± 16.8 (15-65). Schirmer score was over 10 mm in 4 eyes, 5-10 mm in 2 eyes, and less than 5 mm in 10 eyes. BUT in all eyes were less than 5 seconds, and the average fluorescein score was 4.7 out of 9. The average corneal sensation was 47 mm with Cochet-Bonnet test. Injection of bulbar and palpebral conjunctiva was observed in 6 eyes of 3 cases (their DEQS scores were 32, 38, and 65), and all of these eyes had surgery for secondary cataracts, as well as lacrimal punctum plugs. Of those 3 cases, 2 eyes in 1 case also had secondary glaucoma. All cases in this study suffered GVHD in other organs (dry mouth in 8 cases, liver dysfunction in 3 cases, lung dysfunction in 3 cases, skin impairment in 2 cases, and gastrointestinal dysfunction in 1 case). Current therapies were systemic steroid with cyclosporine in 2 cases, systemic immunosuppressant alone in 3 cases, local steroid in 3 cases, local sodium hyaluronate in 7 cases, local diquafosol sodium in 6 cases, and local rebamipide in 2 cases.

Conclusion: Dry eye related quality of life in ocular GVHD patients was low especially in inflamed eyes with secondary cataracts.

[Commercial relationship: none]

RE-ASSEMBLY OF THE STEM CELL NICHE IN LONG-TERM CULTURE OF LIMBAL EPITHELIAL CELL SHEETS

Shigeto Shimmura, Hideyuki Miyashita, Satoru Yoshida, Tetsuya Kawakita, Kazuo Tsubota. Department of Ophthalmology, Keio University School of Medicine.

Purpose: Corneal epithelial stem cells are located in the limbus, the junction between cornea and conjunctiva.Cultivated corneal epithelial cell sheets are used for ocular surface reconstruction of limbal stem cell deficiency patients.In this study, we devised a cultured medium that can be used to culture stratified limbal epithelial cell sheets for up to 5months in vitro.

Methods: Epithelial cells were isolated from human limbal tissue,

seeded in cell culture inserts andco-cultured with human mesenchymal stem cell-derived feeder cells. Epithelial sheets were engineered in mediumcontaining KGF and the Rho kinase inhibitor Y-27632 without air-lifting.
Results: Immunohistochemistry showed theexpressions of progenitor cell markers p63 and cytokeratin 15 in the basal layer, and corneal epithelium specificdifferentiation markers cytokeratin 3 and cytokeratin 12 in supra-basal layers in cultured sheets for up to 5 months.Melanocytes were also observed in the basal layer and provided pigments to basal epithelial cells. In addition, colonyforming efficiency did not change between 1 and 3 months (22.3%±16.0%, n=4 and 23.3% ± 7.3%, n=5, p = 0.90,Student's t test). These results suggest that our sheets maintain corneal progenitor cells for at least 3 months inculture.
Conclusions: Long term maintenance of cell sheets without impairment in progenitor cell population is possiblewith the use of KGF and Rho kinase inhibitors.

SLEEP DEPRIVATION AND TEAR FILM

Young Joo Shin
Purpose: To investigate whether sleep deprivation disturbs the tear film.
Method: Twenty healthy male subjects with no ocular disease were recruited. Sleep was deprivated in sleep deprivation (SD) group including 10 subjects. Ten subjects served as the control group. The tear film and ocular surface were evaluated at 2 pm, 10 pm, and 6 am and 2 pm) the next day. Tear osmolarity, Schirmer's test, tear film break-up time (TBUT), visual pain analogue scale (VAS), intraocular pressure (IOP), serum norepinephrine (NE) were measured.
Results: The mean tear osmolarity level increased in SD group at 6 am the next day compared with that in the control group ($p < 0.05$). The SD group showed a significantly shorter TBUT compared with the control group at 6 am the next day ($p < 0.05$). There were significantly lower tear secretion measured by Schirmer test in SD group compared with those in the control group at 6 am ($p < 0.05$). No significant change was shown in**.
Conclusion**:** Sleep deprivation induced tear hyperosmolarity, shortened TBUT, reduced tear secretion and triggered the development of ocular surface diseases. Sleep deprivation could exacerbate signs and symptoms in patients with ocular surface disease.

OPTIMIZING VISCOSITY OF OPHTHALMIC SOLUTIONS WITH THE COMBINATION OF TWO POLYMERS.

Peter A. Simmons[1], Bereth J. Beard[1], Joseph G. Vehige.[1]
[1]Allergan Inc., Irvine, California USA
Purpose: Artificial tear formulations typically contain a water-soluble polymer to provide enhanced residence time, retention of moisture, as well as binding to the mucin coat of the ocular surface or facilitation of wound healing. The purpose of this study was to investigate the potential advantages of combining two polymers, carboxymethylcellulose (CMC) and hyaluronic acid (HA) in a single formulation.
Methods. 0.5% CMC and 0.1% HA solutions were prepared in Phosphate Buffered Saline. These solutions were tested for bulk viscosity (Brookfield) in comparison to a solution that combined both CMC and HA. A rheometer (TA AR2000) also assessed viscoelastic properties simulating eye movements and blinking. All measurements were performed in triplicate
Results: Bulk viscosity of the individual CMC and HA solutions was 2.5 and 5.7 cps, respectively. The combination of polymers yielded a viscosity of 13.1 cps, 60% higher than predicted by the sum of the individual measurements. On the rheometer, shear rates were varied from 1/sec to 10,000/sec (representing the still eye to the rapid phase of blinking). At these shear rates, viscosity ranged from 6.4 cps to 3.2 for CMC and 9.5 cps to 3.0 cps for HA. For the combination, measurements at the same shear rates were 25.0 cps and 6.2 cps. Thus low-shear viscosity increased 57% while high-shear viscosity was unchanged relative to the addition of the individual viscosities.
Conclusions: These data suggest that combinations of these polymers may be utilized in order to formulate artificial tears with specific properties to optimize retention of fluid on the ocular surface (through higher low-shear viscosity) as well as minimizing blur and stickiness during blinking (through lower high-shear viscosity).
Commercial Relationship: The authors are employees of Allergan, Inc.

PROMPT VERSUS DELAYED APPLICATION OF AMNIOTIC MEMBRANE IN A PATIENT WITH STEVENS-JOHNSON SYNDROME

Kimberly C. Sippel, Jessica B. Ciralsky. Department of Ophthalmology, Weill Cornell Medical College, New York, NY, USA
Purpose: The recent literature has shown that amniotic membrane application to the entire ocular surface during the acute stage of Stevens-Johnson syndrome is associated with a significantly decreased risk of development of blinding cicatricial sequelae. However, the optimal timing of application has not been clearly defined.
Methods: Retrospective review of a single case.
Results: Both eyes of patient with acute Stevens-Johnson syndrome and severe ophthalmic involvement (i.e. extensive sloughing of the conjunctival and corneal epithelium) were treated with amniotic membrane application. However, due to the preferences expressed by the family, one eye was treated within 72 hours of diagnosis (i.e. the hyperacute stage) and the other at a later time point during the acute phase. The eye treated within 72 hours displayed significantly less cicatricial changes and improved vision compared to the eye treated at a later time point.
Conclusions: The window of opportunity in the acute phase during which it is possible to achieve a positive effect with amniotic membrane application appears to be small, and the procedure is optimally performed within 72 hours of diagnosis.
[This research was supported by a Research for Preventing Blindness grant.]

CONSTRUCT VALIDITY OF THE CURRENT SYMPTOMS QUESTIONNAIRE

Ping Situ,[1] Carolyn Begley,[1] Trefford Simpson,[2] Robin Chalmers,[1] Ziwei Wu.[1] School of Optometry, Indiana University, USA,[1] School of Optometry and Vision Science, University of Waterloo, CANADA[2]
Purpose: The Current Symptoms Questionnaire (CSQ) was adapted from the Dry Eye Questionnaire (DEQ) to quantify symptoms of ocular irritation at the time of testing. The aim of this study was to establish the construct validity of the CSQ through a meta-analytical approach.
Methods: Twelve studies that involved normal, dry eye subjects and contact lens wearers that took place between 2002 and 2011 were included in the meta-analysis. In each study, the CSQ was administered at baseline (no intervention) and after various experimental conditions. Using a prior clinical diagnosis and a self-assessment of dry eye as diagnostic criterion, baseline CSQ data were compared to habitual symptoms concurrently measured by the Dry Eye Questionnaire-5 (DEQ-5) using Receiver-Operating Characteristic (ROC) curve analysis. Factor analysis was used to determine the underlying factors of the CSQ. The changes in CSQ scores before and after various experimental conditions were also compared.
Results: Using the prior diagnosis of dry eye as diagnostic discriminator, both ROC curve and area under the ROC were similar between CSQ and the DEQ-5 (0.81 and 0.88 for CSQ and DEQ-5 respectively). Similar results were found using the criterion of self-assessment of dry eye (0.82 and 0.81 for CSQ and DEQ, respectively). Factor analysis indicated single underlying factor in the CSQ. The changes in CSQ scores before and after stimulation varied among different experimental conditions, with mean (±SD) ranging from 1.88 (±8.43) to 6.46 (±5.63).
Conclusion: The CSQ and standardized DEQ-5 scores performed

similarly to effectively segregate subjects with and without a prior diagnosis of dry eye. In addition, the uni-dimension of the CSQ suggests that it can provide a single score for the assessment of symptoms of ocular irritation under different experimental conditions.
[This research was supported by grants from NEI R01EY021794 (Begley) & NSERC Canada (Simpson)]

COMPARING SENSORY RESPONSES TO REPEATED OCULAR SURFACE STRESS WITH AND WITHOUT WEARING A CONTACT LENS

Ping Situ, Jun Zhang, Carolyn Begley. School of Optometry, Indiana University, USA

Purpose: In this study, we employed a repeated blink suppression paradigm (BSP), with and without contact lenses (CLs), to induce tear film break-up (TBU) to study its effect on the ocular sensory response and associated symptoms.

Methods: Subjects participated in 2 study visits, with CLs and without (NCL). In the NCL visit, subjects kept one eye open as long as possible (BSP) while fluorescein TBU was monitored, indicated discomfort using a "discomfort knob" (DK) on a 0-10 scale and rated discomfort and burning during and after each trial on 0-10 visual analogue scales (VAS). This procedure was repeated 10 times. The CL visit was identical, except subjects wore their CLs, TBU was viewed by retroillumination (RI), and the BSP was 30 sec. The Current Symptom Questionnaire (CSQ) was used to evaluate symptoms before and after 10 trials. TBU was quantified using custom MATLAB programs.

Results: TBU occurred in 95% NCL and 92% CL BSP trials. The discomfort intensity and slope measured by the DK was significantly lower while wearing CL compared to NCL (paired t-test, $p<0.01$). The VAS ratings of discomfort and burning were significantly lower while wearing a CL versus NCL, both during and after BSP trials (ANOVA $p=0.03$ and 0.02 for discomfort and burning, respectively). CSQ symptoms increased significantly after the 10 repeated BSP trials for both CL and NCL (AVG scores pre 10.85 vs. post 22.40, $p=0.01$), with no significant difference between CL and NCL trials (AVG score CL 17.10 vs. NCL 16.15, $p=0.64$)

Conclusion: TBU occurred with extended eye opening in most NCL and CL trials and was associated with increased discomfort and burning during and after each trial. After 10 repeated trials, symptoms of ocular irritation increased with and without CLs. This study demonstrates the feasibility of using a repeated BSP to induce and study subjective ocular sensory responses associated with TBU.

[This research was supported by a grant from NEI R01EY021794 (Begley)]

CORRELATION BETWEEN QUANTITATIVE MEASUREMENTS OF TEAR FILM LIPID LAYER THICKNESS AND MEIBOMIAN GLAND LOSS IN PATIENTS WITH OBSTRUCTIVE MEIBOMIAN GLAND DYSFUNCTION AND NORMAL CONTROLS

Jong Suk Song, Youngsub Eom, Jong Suk Lee, Su Yeon Kang, Hyo Myung Kim Department of Ophthalmology, Korea University College of Medicine, Seoul, South Korea

Purpose: To evaluate the correlation between tear film lipid layer thickness and other objective measurements such as tear film break-up time (TBUT), upper and lower meibomian gland losses, and Schirmer 1 test in patients with obstructive meibomian gland dysfunction (MGD) and normal controls.

Methods: Thirty eyes of 30 patients with obstructive MGD and 25 eyes of 25 normal controls were enrolled. Lipid layer thickness was measured using an interferometer. Tear film stability and tear production were evaluated by TBUT and Schirmer 1 test. Upper and lower meibomian gland losses were evaluated using noncontact meibography. The correlations among variables were evaluated in the obstructiveMGDgroup and the control group.

Results: TBUT was significantly shorter in the obstructive MGD group than in the control group ($P < .001$). Upper and lower meibomian gland losses were higher in the obstructive MGD group than in the control group ($P < .001$ and $P < .001$, respectively), and lipid layer thickness was significantly thicker in the control group than in the obstructive MGD group
($P = .028$). Lipid layer thickness was significantly negatively correlated with upper and lower meibomian gland losses in both groups.

Conclusions: Lipid layer thickness objectively measured with the interferometer was significantly thicker in the control group than in the obstructive MGD group. Lipid layer thickness was negatively correlated with upper and lower meibomian gland losses in the control group as well as in the obstructive MGD group.

[This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012R1A1A2042054), Seoul, South Korea]

LONG-TERM RESULTS OF THE TREATMENT BY 3% DIQUAFOSOL OPHTHALMIC SOLUTION IN DRY-EYE PATIENTS WITH SJÖGREN'S SYNDROME

Yukiko Sonomura,[1,2] Norihiko Yokoi,[2] Aoi Komuro,[2,3] Hiroaki Kato,[2] and Shigeru Kinoshita[2]

[1]Department of Ophthalmology, Kyoto Yamashiro General Medical Center, Kyoto, Japan; [2]Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan; [3]Komuro Eye Clinic, Kyoto, Japan.

Purpose: The 3% diquafosol sodium (DQS) ophthalmic solution containing the $P2Y_2$ receptor agonist as the active ingredient reportedly accelerates fluid transport from the conjunctival epithelium, facilitates the secretion of soluble mucin from conjunctival goblet cells, and stimulates the expression of membrane-associated mucin on the corneal epithelium. The purpose of this present study was to investigate the long-term results of 3% DQS treatment for dry-eye patients with Sjögren's syndrome.

Methods: This study involved 17 eyes of 17 female patients (mean age: 64.7 years) with Sjögren's syndrome. All 17 eyes were treated with 3% DQS ophthalmic solution 6 times daily for 6 months in substitution for sodium hyaluronate ophthalmic solution and artificial tears. Subjective symptoms and objective findings were then investigated each month for 6 months post-treatment. Subjective symptoms were assessed using the visual analog scale. Tear meniscus radius (TMR), fluorescein BUT (FBUT) and ocular-surface epithelial damage score were evaluated.

Results: Post-treatment, dryness was improved at 2 to 5 months ($p<0.04$), foreign body sensation was improved at 4 and 6 months ($p<0.02$), ocular pain was improved at 2 and 6 months ($p<0.03$), and ocular fatigue was improved at 1, 2, 3, and 6 months ($p<0.05$). However, the symptom of ocular discharge significantly worsened within 2 months ($p<0.03$). There was no significant increase of TMR, yet FBUT increased at 2, 3, 4, and 6 months ($p<0.05$). Conjunctival staining scores were significantly improved at 1 to 6 months ($p<0.005$), and corneal staining scores were improved at 2 to 6 months ($p<0.04$).

Conclusions: The results of this study show that 3% DQS ophthalmic solution is effective for the improvement of subjective symptoms and objective findings in patients with Sjögren's syndrome.

Commercial relationships and grant support: None to report for all authors.

DECELLULARIZATION OF PORCINE LACRIMAL GLAND TISSUE FOR DEVELOPMENT OF A LACRIMAL GLAND SCAFFOLD.

K. Spaniol[1], A. Kunze[1], M. Metzger[2], G. Geerling[1], S. Schrader[1]. 1: Department of Ophthalmology, University Hospital Düsseldorf, 2: Institute for Tissue Engineering and Regenerative Medicine, University Hospital Würzburg

Purpose: In cases of severe dry eye due to lacrimal gland insufficiency engineering of a lacrimal gland tissue construct in vitro might be a promising future treatment approach. Aim of this study was to evaluate structure and main basement membrane components of native porcine lacrimal gland tissue before and after a decellularization process in order to develop an acellular scaffold for lacrimal gland regeneration.
Methods: Lacrimal glands were extracted from six domestic pigs after euthanasia. Glands were cut into four pieces, two were left native and two were decellularized using sodium desoxychelate in ultra-pure water over night. Tissue pieces were embedded in paraffin and OCT. Morphology was examined histologically by hematoxylin&eosin (H&E) staining. Feulgen staining was used to evaluate absence of DNA in decellularized tissue. Expression of basement membrane markers (laminin, collagen IV, fibronectin) was evaluated by immunostaining.
Results: Histology showed an intact connective tissue matrix after the decellularization process. Immunohistochemistry revealed the expression of major basement membrane components such as collagen IV, laminin and fibronectin in native lacrimal gland tissue and these components were still detectable after the decellularization of the lacrimal gland tissue. Efficacy of the decellularization process was demonstrated by complete absence of nuclei in the lacrimal gland tissue, as assessed by DAPI-staining.
Conclusions: Decellularization of lacrimal gland tissue generates an intact acellular scaffold with preserved acinar structures, containing major basement membrane components such as collagen IV, laminin and fibronectin. An intact extracellular matrix with preserved basement membrane structures is a prerequisite for essential cellular processes like adhesion, migration, proliferation and stem cell maintenance. Therefore decellularized lacrimal gland tissue might be a promising scaffold for lacrimal gland regeneration in vitro.
[This work was supported by the Research Commission of the Medical Faculty of the Heinrich Heine University Duesseldorf]

HIGHER PLASMA LEVELS OF OESTRADIOL ARE ASSOCIATED WITH INCREASED OCULAR DISCOMFORT IN WOMEN.

Fiona Stapleton, Ulrike Hampel, Noor Badarudin, Moneisha Gokhale, Michele Madigan, Isabelle Jalbert, Blanka Golebiowski.
School of Optometry and Vision Science, University of New South Wales, Sydney, Australia.
Purpose: Alterations in sex hormone levels have been implicated in dry eye. This study aimed to examine the relationship between symptoms of ocular discomfort and circulating oestrogen and androgen levels.
Methods: A cross-sectional, single visit study was conducted. The study involved 74 subjects without ocular surface disease, including 52 females (age 35±13yrs, range 19-70) and 22 males (age 34±14yrs, range 20-75). Subjects completed the Dry Eye Questionnaire (DEQ5), the Ocular Comfort Index (OCI) and numerical ratings (1-100) of comfort, dryness, foreign body sensation (FB), burning and watering. Tear osmolarity (TearLab) and tear volume (Phenol Red Thread test) were assessed; conjunctival goblet cell density (GCD) was examined using impression cytology. Plasma concentrations of oestradiol (E2), free testosterone and Dehydroepiandrosterone sulfate (DHEA-S) were determined using specific ELISA; 3 -Androstanediol-Glucuronide (3 -diol-G) concentration was assessed with radioimmunoassay. Associations were examined using Pearson's and Spearman's correlations; differences between groups were assessed using Independent samples t-test or Mann-Whitney U test.
Results: Reduced levels of DHEA-S and 3 -diol-G in females were associated with higher tear osmolarity (r=-0.35, *P*=0.01) and reduced tear volume (=0.30, *P*=0.03) respectively. In females, higher levels of E2 were associated with increased ocular symptoms (DEQ5 =0.36, *P*=0.01; dryness =0.36, *P*=0.01; FB =0.37, *P*=0.01; burning =0.28, *P*=0.048). Tear volume was lower in females (*P*=0.02) but there was no difference in tear osmolarity or GCD. No associations were detected between hormone levels and ocular symptoms in the male group. Concentrations of E2 and DHEA-S decreased with age for all subjects (E2 =-0.30, *P*=0.01; DHEA-S r=-0.41, *P*<0.001), but there was no association between age and symptoms.
Conclusions: Taken together, our observations indicate that higher plasma levels of oestradiol are implicated in increased ocular discomfort in females without ocular surface disease. Furthermore, circulating androgen precursors and metabolites may also have a role in tear function.
Funding support: Faculty of Science, University of New South Wales and Blackmores Australia

INSIGHT INTO HOW TO REDUCE THE FORMATION OF RECURRENT EROSIONS GAINED FROM CORNEAL DEBRIDEMENT STUDIES IN THE MOUSE.

Mary Ann Stepp[1], Sonali Pal-Ghosh[1], Ahdeah Pajoohesh-Ganji[1], Christophe Cataisson[2], Daniel Saban[3]. GWUMC, Department of Anatomy and Regenerative Biology and Department of Ophthalmology Washington DC 20037[1], NCI/NIH, Bethesda, MD, 20892[2], Duke University Eye Center, Durham, NC 27710[3],
Traumatic corneal abrasions are the leading ophthalmic cause of emergency room visits and trauma to the cornea increases the risk of recurrent erosions. An *in vivo* mouse model has been developed that reproducibly induces recurrent epithelial erosions in wild-type mice spontaneously within two weeks after a single 1.5 mm corneal debridement wound using a dull blade. Removing the corneal epithelial basement membrane by manual keratectomy or with a rotating burr allows corneas to heal without developing erosions. To determine the causes of the differences in healing outcomes after dull blade compared to rotating burr wounds, we conducted a series of experiments involving flow cytometry, 3D confocal imaging, cytokine arrays, and QPCR to look at the events that occur at the time of injury that promote development of recurrent erosions several weeks later. Data show that 1) there are more monocytes (CD45+/ Ly6C hi/ Ly6G+/ CD11b hi/ F480 low/ CD11c+) and T cells (CD45+/GL3 hi) recruited 6 hr after dulled blade wounds, 2) at the time of wounding, chemokine levels are higher in stromal wound beds after dulled blade compared to rotating burr wounds, and 3) resident corneal epithelial and stromal cells initiate a stronger cytokine transcriptional response to rotating burr wounds. Despite the fact that rotating burr wounds remove the BMZ, damage more of the subbasal nerves, and induce transcription of higher levels of several cytokine mRNAs within resident corneal cells, corneas wounded with this method heal without developing erosions. While we have more to learn about why recurrent erosions develop, these data give us important insight into the distinct roles played by local deposition of cytokines compared to the transcriptional responses of cells within damaged tissues to injury.

SYSTEMIC ISOTRETINOIN SIDE EFFECTS ON HUMEN MEIBOMIAN GLAND.

MN. Suarez, Cátedra de Oftalmología
Facultad de Medicina de Montevideo.Uruguay
Purpose: Evaluate meibomian gland side effects of oral isotretinoin therapy for acne vulgaris patients
Methods: Ten young patients treated with sytemic isotretinoin(dosage 0.5mg/kg/day)for 6-12 months were studied.Complete ophthalmological examination,Schirmer tests,Break up time,conjunctival impression cytology,and in vivo confocal microscopy of meibomian gland were performed in this group of patients.
Results: Chronic eye redness,meibomian gland pattern distortion in palpebra transillumination,severe meibomian gland changes were found in all patients with confocal microscopy.Conjunctival impression cytology revealed epithelial lytic changes in this group of patients.

Conclusions: Oral isotretinoin therapy appears to have a potential toxic side effect on human meibomian gland ,particularly in young patients
No commercial relationship

QUANTIFYING TEAR FILM INFLAMMATORY MARKERS USING A NOVEL, MULTIPLEX ELECTROCHEMILUMINESCENT TECHNIQUE

Lakshman Subbaraman, Mirunalni Thangavelu, David McCanna, Lyndon Jones
Centre for Contact Lens Research, School of Optometry and Vision Science, Waterloo, ON, Canada

Purpose: To optimize a novel, multiplex electrochemiluminescent array technique (Meso Scale Discovery, Rockville, MD) to quantify tear film inflammatory markers and to validate the signal linearity and recovery of tear samples in cytokine and chemokine assay kits.

Methods: Tear samples were collected from 10 participants over two visits and 4uL from each participant was pooled to be used for linearity and recovery analyses. 1ul of individual tears were tested using both the human pro-inflammatory 9-plex and human chemokine 9-plex assays. Manufacturer's protocol was followed for these assays. Linearity of the signal intensity was tested using 1uL, 2uL and 3uL of pooled tear samples. Two standards (Std 3 and Std 4) from both the kits were spiked with 1uL and 2uL of pooled tears to assess recovery.

Results: Interleukin-8 (448.8±50.8 pg/mL) and Interleukin-6 (24.4±3.1 pg/mL) were linear in the cytokine array for all volumes tested. Average concentration determined for IL6 and IL8 in tear samples were 7.9±4.3 pg/mL and 415.7±188.6 pg/mL respectively. Interleukin-10 was not detected in any of the samples and Interleukin-1B was below the lower limit of detection (LLOD). In the chemokine array, IL-8 (500.9±31.7 pg/mL), MCP -1 (400.7±79.1 pg/mL) and IP-10 (591720.1±5699.9 pg/mL) were linear at all volumes, MCP-4 and MDC were linear only at 2ul and 3ul volumes. Recovery was good for all chemokines except for IP 10; the endogenous signal masked the signal from the spike. Average concentration of IL8 and MCP-1 were 513.5±411.6 pg/mL and 574.3±496.8 pg/mL respectively.

Conclusions: A novel, multiplex electrochemiluminescent assay with superior sensitivity and recovery that provides a rapid and convenient method for quantifying tear film cytokines has been optimized. This novel technique can be employed to determine cytokine and chemokine expression in low volumes of tears collected from patients with dry eye and contact lens related dry eye.

Conflict of interest: None

IMPACT OF ENVIRONMENTAL CHANGES ON IN VITRO CORNEAL EPITHELIAL WOUND HEALING

Lakshman Subbaraman, Emma V. Dare, Lyndon Jones
Centre for Contact Lens Research, School of Optometry and Vision Science, University of Waterloo, Waterloo, Canada

Purpose: Damage to the corneal epithelium can result in severe vision loss. Therefore, rapid wound healing is essential. To study wound healing in vitro a scratch model is often used. The purpose of this study was to compare speed of wound closure using three differing commercially available cell culture media under both normoxic and hypoxic culture conditions.

Methods: Immortalized human corneal epithelial cells were cultured in normoxic and hypoxic conditions (2% oxygen) in either Dulbecco's Modified Eagle Medium: Nutrient mixture F-12 (DMEM/F-12) with 10% fetal bovine serum and 10 ng/ml epidermal growth factor, keratinocyte serum-free media (KSFM), or EpiLife®. A scratch wound was made on the cell monolayers and cell recovery and wound closure were followed for up to 48 hours by measuring the area of the wound using ImageJ software. Cultures were also stained for Connexin 43 (Cx43), zona occludens-1 (ZO-1), and Ki-67.

Results: Wound healing with supplemented DMEM/F-12 was significantly faster than both KSFM (p=0.046) and EpiLife (p=0.001) with the majority of wounds being closed 10 hours after wounding. In addition, hypoxic culture significantly delayed wound healing by an average of 32.4% across all time points. Six hours after wounding, only cultures in DMEM/F-12 stained for abundant ZO-1 and Cx43, indicating epithelial barrier formation. In the DMEM/F-12 and KSFM cultures, Ki-67 staining revealed that cells were entering the cell cycle at a distance from the wound site, followed likely by migration to the wound. Very few cells in the EpiLife cultures showed evidence of proliferation.

Conclusions: During the 48 hours post scratch, an in vitro model found that culture in supplemented DMEM/F-12 led to superior wound healing and tight junction formation, when compared with two other culture media. This result indicates the importance of the culture medium choice when designing a wound healing experiment.

EFFICACY OF CONTACT LENS SOLUTIONS AGAINST *ACHROMOBACTER XYLOSOXIDANS* BIOFILMS USING CONFOCAL MICROSCOPY

Lakshman Subbaraman, David J. McCanna, Jaclyn Chang, Lyndon Jones. Centre for Contact Lens Research, School of Optometry & Vision Science, University of Waterloo

Purpose: Biofilms of *Achromobacter xylosoxidans* (Ax) can develop in contact lens (CL) cases. These microorganisms can attach to the CL and cause microbial keratitis. This study evaluated the antimicrobial efficacy of CL solutions against Ax biofilms by measuring the damage to cell membranes of Ax using confocal microscopy.

Methods: Ax biofilms were formed by incubating the bacteria overnight on glass coverslips and were then exposed to CL solutions for four hours. Commercial CL solutions evaluated contained the antimicrobials polyhexamethylene biguanide (PHMB*), polyquaternium*-1(PQ1) and alexidine (ALX), and PQ1 and Aldox (AD). After exposure, the bacteria were stained with SYTO 9 and propidium iodide (PI). Using a confocal microscope, the number of cells with damaged cell membranes was determined. In addition to evaluating CL solutions, four concentrations of benzalkonium chloride (BAK) 0.05%, 0.01%, 0.005% and 0.001% in phosphate buffer saline were also evaluated to demonstrate dose related effects at exposure times as short as 5 minutes.

Results: PQ1-ALX-based solution caused the greatest damage to the Ax cell membranes. The other formulations tested based on PHMB and PQ1 with AD caused some of the bacteria to lose membrane integrity but did not cause as much damage to the bacteria cell membranes ($p<0.05$) as the PQ-ALX formulation. Dose effects of the preservative BAK could be seen at 5 minutes of exposure time. BAK at 0.005% and 0.01% caused an increase in the number of cells that were permeable to PI compared to the phosphate buffered control (27.5% and 51% respectively, $p<0.05$). All the Ax bacteria were permeable to PI after exposure to 0.05% BAK.

Conclusions: One of the five lens care systems tested caused a substantial number of Ax bacteria to lose membrane integrity. This method was also able to detect the effect different concentrations of BAK have on the membrane integrity of the Ax biofilm bacteria. Understanding the ability of antimicrobials to damage bacteria cell membranes could help in the development of lens care solutions that can reduce and/or eliminate Ax biofilms from lens cases.

Funding: NSERC Canada

QUANTIFICATION OF LIPOCALIN-1 IN TEARS AND CONTACT LENS DEPOSITS USING A SANDWICH ELISA TECHNIQUE

Lakshman Subbaraman, Rajan Mistry, Mirunalni Thangavelu, Lyndon Jones
Centre for Contact Lens Research, School of Optometry and Vision

Science, University of Waterloo
Purpose: Lipocalin-1 has been identified as a tear biomarker in dry eye disease, Sjogren's syndrome and Diabetic Retinopathy. The purpose of this study was to optimize a sandwich enzyme linked immuno-sorbent assay (ELISA) using an antibody pair for the detection and quantitation of lipocalin-1 in tear samples and worn contact lens (CL) extracts, using minimum sample volume.
Methods: Tear samples were collected using a microcapillary method from 6 volunteers; 1uL from each subject was combined to form a pooled sample. Worn CLs (senofilcon A) were collected after a week of wear from 5 CL wearers. Immediately upon collection, CLs were incubated individually overnight in 1.5mL of TFA:ACN solution. Three aliquots (650uL, 600uL, 100uL) of the extract were dried and the samples were stored at -80°C until further analyses. Human Lipocalin-1 ELISA pair kit (Sino Biological Inc., catalogue number SEK11583) was used and several modifications were made to the manufacturer's recommended protocol.
Results: The modifications made to the protocol improved linearity (R^2=1) and yielded optimal signal at 450nm for tear samples at 2,000,000X dilution from a 1uL sample. CL extracts at 500X dilution yielded signal that did not fall within the range of the standard curve. The 1000X and 2000X dilutions were determined to be optimal. Furthermore, sample preparation was optimized to detect lipocalin-1 from a 0.75uL aliquot to detect 5 abundant tear proteins (lysozyme, lipocalin-1, albumin, lactoferrin and IgA) from one single tear aliquot using this technique (this compares with Western blotting, which requires 1uL per sample). The average amount of lipocalin-1 detected was 2.3±0.89 ug/uL in tears and 0.43±0.36 ug in CL deposits.
Conclusions: A high throughput, sandwich ELISA technique has been optimized to quantify lipocalin-1 in tears and contact lens deposits. This technique will be extremely useful when quantifying several proteins from a single tear aliquot.
Funding: NSERC Canada

A STATE OF THE ART ANALYSIS OF OSMOLARITY AS A DIAGNOSTIC MEASURE FOR DRY EYE DISEASE

Benjamin D. Sullivan. TearLab Corp., San Diego, CA, USA
Purpose: To evaluate the current state of the art of the basic science and clinical utility of tear osmolarity.
Results: A review of the current literature reveals that tear osmolarity "demonstrated the highest correlation to disease severity and was found to be the single best metric to diagnose and classify dry eye disease [1,2]." Consistent with earlier studies, that found osmolarity to be "superior in overall accuracy to any other single test for dry eye diagnosis, even when the other test measures were applied to a diagnosis within the sample groups from which they were derived, [3]" recent data suggest that "the results showed a significantly higher tear film osmolarity in patients with severe keratoconjunctivitis sicca compared with the healthy controls. Testing tear film osmolarity can be a very effective objective diagnostic tool in the diagnosis of dry eye disease. [4]" Further, "tear osmolarity > 305 mOsm/L was selected as cut-off value for dry eye: the LR+ was 10.99, the highest value as compared to those found for the other tests; the PPV+ value was also very high (98.4)…[Osmolarity] showed a good performance in dry eye diagnosis, higher than the other tests considered, mainly in severe dry eye. Tear osmolarity values should be interpreted as an indicator of DED evolutionary process to severity. [5]" Despite more recent data that demonstrates that osmolarity is the least variable of all the common signs for dry eye disease [6], the two most commonly cited historical misconceptions are that the clinical value of osmolarity is lessened because of its variability [7] and because it doesn't correlate with the other signs [8]. We now know that the marker is heteroscedastic, and that osmolarity provides a linear measure of tear instability; meaning that eye-to-eye or temporal differences are what clinicians should be looking for to help confirm diagnosis of the disease, as normal subjects are exceptionally stable, while DED subjects become unstable as they lose homeostasis [2,9]. Moreover, a review of the literature demonstrates that none of the signs of DED are correlated with each other [10], not just osmolarity, and the implications of this lack of correlation on selection criteria for clinical trials is discussed. [1] Sullivan BD et al., IOVS 2010;51(12):6125, [2] Lemp MA et al., AJO 2011;151(5):792, [3] Tomlinson A et al., IOVS 2006;47(10):4309, [4] Jacobi C et al., Cornea 2011;30(12):1289, [5] Versura et al., Curr Eye Res. 2010;35(7):553. [6] Sullivan BD, et al. Cornea. 2012 Sep;31(9):1000-8. [7] Khanal S, et al. Br J Ophthalmol. 2012 Mar;96(3):341-4. [8] Messmer EM, et al. Dev Ophthalmol. 2010;45:129-38. [9] Keech A, et al. Curr Eye Res. 2013 Apr;38(4):428-36. [10] Sullivan BD, et al., Acta Ophthalmol. 2012 Dec 28. Disclosures: Commercial Relationship, TearLab, Corporation: Benjamin Sullivan (I, E, P).

REGULATION OF THE HUMAN MEIBOMIAN GLAND IN HEALTH AND DISEASE

Sullivan DA, Ding J, Kam WR, Liu Y, Rahimi Darabad R, Sahin A, Liu S. Schepens Eye Research Institute, Massachusetts Eye and Ear and Harvard Medical School, Boston, MA, USA
Meibomian gland function is critically important in maintaining the health and integrity of the ocular surface. This gland, through its production and secretion of lipids, promotes the stability and prevents the evaporation of the tear film. Conversely, meibomian gland dysfunction (MGD) leads to a decreased stability and an increased evaporation of the tear film. Indeed, MGD is the major cause of dry eye disease. Given the importance of this tissue, it is extraordinary that, until recently, very little was known about the physiological regulation of the human meibomian gland. Significant breakthroughs in our understanding have come about through several recent discoveries in our laboratory. First, we discovered that we could immortalize human meibomian gland epithelial cells that function like primary cells. These immortalized cells possess a normal karyotype and respond to numerous agents (e.g. sex steroids, glucocorticoids, mineralocorticoids, pituitary hormones, growth factors, neurotransmitters, bacterial toxins and antibiotics) with alterations in proliferation, differentiation, cAMP accumulation, signaling pathways, gene expression and lipogenesis. We believe that these cells represent an ideal preclinical human model for drug discovery. Our second discovery was that we could create a model of human MGD *in vitro*. Exposure of the immortalized cells to isotretinoin, a well-known risk factor for MGD *in vivo*, reduces the activity of survival mediators, inhibits proliferation and leads to cell death. We have discovered topical treatments that may counteract these adverse MGD-inducing effects. Our third discovery was the identification of human meibomian gland genes that may promote the development of MGD. These studies have also identified genes that may be responsible for the typical absence of intraglandular inflammation and bacterial infection in human MGD, and have revealed novel targets for potential therapeutic intervention. Overall, these recent results have significantly advanced our understanding of the regulation of the human meibomian gland in health and disease. [Supported by NIH grant R01EY05612, the Margaret S. Sinon Scholar in Ocular Surface Research Fund, and the Guoxing Yao & Yang Liu Research Fund]

ANALYSIS OF THE FATTY ACID COMPOSITION OF HUMAN MEIBUM

Tomo Suzuki,[1,2] Sayaka Kamada,[1,2] Satoshi Fujiwara,[3] Tetsuya Tajika,[4] and Shigeru Kinoshita.[1] Department of Ophthalmology, Kyoto Prefectural University of Medicine,[1] Kyoto, Japan; Kyoto City Hospital, Kyoto, Japan; [2] Shimadzu Techno-Research, Inc., Kyoto, Japan;[3] Senju Pharmaceutical Co., Ltd., Kobe, Japan.[4]

Purpose: Many previous studies have focused on the analysis of lipids contained in human meibomian gland secretion (meibum) in an attempt to elucidate the pathogenesis of dry eye and meibomian gland dysfunction. However, the findings of those reports have varied due to difficulties in collecting pure meibum and the performance of precise lipid analysis. The purpose of this present study was to re-evaluate the methods of meibum collection and analysis of fatty acid composition of human meibum.
Methods: This study involved 6 healthy volunteers (3 males and 3 females) ranging 30-39 years of age. In each subject, after warm compression of the eye lids by use of an electronic device (EH-SW50, Panasonic Corp. Osaka, Japan) (40° C, 10 minutes), a meibum sample was obtained under a surgical microscope. Immediately after gently squeezing the eyelid margin by use of a Yoshitomi Meibomian Gland Compressor® (T.M.I. Co. Ltd., Saitama, Japan), a Daviel cataract spoon that had been thoroughly washed in high-grade organic acetone and hexane and then air-dried to avoid possible lipid contamination was used to collect each sample. The meibum was transmethylated and then analyzed using gas chromatography-mass spectrometry (GC-MS).
Results: The composition of fatty acids of meibum was found to be similar between the male and female normal subjects, and to be composed of 40% saturated and 60% unsaturated fatty acids. Monounsaturated fatty acids (52.0-56.5%) and branched-saturated fatty acids (34.7-37.6%) were the major components, while straight-saturated fatty acids (2.3-2.9%) were minor. These results were reproducibly achieved. Oleic acid (C18:1n9C, 32%) was the most dominant of all fatty acids.
Conclusions: The findings of this study show that the meibum in normal human subjects contains both unsaturated and branched-saturated fatty acids.
Funding: Supported by grant 23890181 from the Japanese Ministry of Education.
Financial disclosure: Senju Pharmaceutical Co. Ltd.

THE 2% REBAMIPIDE EYE DROP TREATMENT FOR DRY EYE

Hiroko Takano, Nozomi Kinoshita, Machiko Shimmura-Tomita, Fumihiko Toyoda, Ayumi Ota, Katsuaki Tanaka, Akihiro Kakehashi. Department of Ophthalmology, Saitama Medical Center, Jichi Medical University, Saitama, Japan
Purpose: Rebamipide is a mucosal protective and ulcer-healing agent developed in Japan and widely prescribed in Asia. The rebamipide, a gastro protective drug, increase the gastric mucus production and its form of eye drop was approved in Japan. The 2% rebamipide eye drop (Mucosta ophthalmic suspension UD2%; Otsuka Pharmaceutical Co, Ltd, Tokyo, Japan) is newly developed for the treatment of dry eye. The rebamipide upregulates mucin secretion and production and suppress superficial punctate keratopathy on the ocular surface of patients. We evaluate 2% rebamipide eye drop for the treatment of dry eye patients.
Methods: We treated 48 dry eye patients (47 females and 1 male 27 to 89 years old, mean age was 66.8) using the 2% rebamipide eye drops 1 to 4 times a day with other treatments. We examined tear break up time (BUT), fluorescein score and asked the questions about their conditions using Dry eye related quality of life score (DEQS).
Results: The follow up periods were 2 to 15 month. They applied the 2% rebamipide eye drops 4 times daily (12 cases), 3 times (5 cases), twice (27 cases) or once (3 cases). The fluorescein scores varied 3 to 9 and BUT were less than 5 seconds in those cases. The changes of those were within the seasonal change. The DEQS scores were 0 to 77 (mean 23+-21). Sixteen cases (33.3%) asserted significant improvement of their complaint, though their DEQS scores were 0 to 75 (mean 21). Three cases failed to use because of the bitter taste (two cases) and the bottle size is too small for her finger bent because of RA (one case). There was no change for worse on the cornea and conjunctiva.
Conclusion: The 2% rebamipide eye drop improved the complaint in dry eye patients and thought to be clinically useful for the treatment of dry eye.
[Commercial relationship: none]

REPROGRAMMING OF ADIPOSE DERIVED STEM CELLS INTO EPITHELIAL CELLS BY DEFINED CHEMICALS FOR OCULAR SURFACE RECONSTRUCTION

Xiao Wei Tan[1], Melina Sentiwan[1], Gwendoline Goh[1], Gary Hin Fai Yam[1] , Donald Tan [2,3,4,] Jodhbir S. Mehta[1,2,3,4] [1]Tissue engineering and stem cell research group, Singapore Eye Research Institute [2]Yong Loo Lin School of Medicine, National University of Singapore [3]Singapore National Eye Centre [4]Department of Clinical Sciences, Duke-NUS Graduate Medical School, Singapore
Purposes: Severe ocular surface disease, such as Stevens-Johnson syndrome, ocular cicatricial pemphigoid and chemical injury, are devastating conditions that may result in limbal stem cell deficiency and visual loss. In patients with bilateral disease where almost the entire corneal and conjunctival surface is severely damaged and scarred, no satisfactory long-term treatment method is available. Therefore we aim to evaluate the potential usage of adipose derived stem cells (ADSC) as corneal epithelial replacement for these blinding conditions.
Methods: ADSCs were initially characterized by defined medium induction and cell surface glycoprotein analyzing. Mesenchymal-epithelial transition (MET) progress was then induced with a combination of chemicals including retinoic acid and histone deacetylase (HDAC) inhibitor. The MET progression and transformation rate were evaluated by immunostaining, flow cytometry and real-time PCR with a panel of specific epithelial and mesenchymal cell markers, such as E-cadherin, N-cadherin, ZO-1, cytokeratin etc. cell migration rate and adhesion were compared.
Results: Human ADSCs were identified to be CD73, CD29, CD105, CD90 and CD166 positive and could differentiate into osteoblasts, chondrocytes, and adipocytes hence proved to be mesenchymal stem cells. After incubation with retinoic acid and HDAC inhibitor, mesenchymal cell markers were down-regulated and epithelial cell markers were up-regulated. After treatment with those factors, cell migration speed slowed down and there were less cell-matrix attachment.
Conclusions: Retinoic acid and HDAC inhibitor successfully induced the MET of ADSCs hence generated cells with epithelial cell phenotype. Future in vivo study is necessary to prove the efficacy of ADSC as a substitute of limbal stem cell for ocular surface reconstruction. This new treatment modality may offer new hope to patients afflicted with these devastating diseases, where no satisfactory long-term treatment regime is currently available.

QUERCETIN INTAKE IMPROVES LACRIMAL FUNCTION REMEDIAL EFFECT OF QUERCETIN INTAKE ON LACRIMAL FUNCTION.

Yasuhisa Tanaka,[1] Takaaki Inaba,[1] Jun Shimazaki,[2] Kazuo Tsubota,[1] [1]Department of Ophthalmology, Keio Universiy School of Medicine, [2] Department of Ophthalmology, Tokyo Dental College, Ichikawa General Hospital
Background. Prevention of decline in mental and physical functions in the older population has become an important public policy issue in Japan, a country experiencing a super-aging society. Consequently, studies that elucidate mechanisms of aging systematically are imperative for preventing various aging-related diseases. Sensory organs are particularly vulnerable to aging, so that many of eye diseases are closely related to aging. In the course of various studies, anti-oxidizing properties of polyphenol have been gaining medical attention as a preventive factor against aging and/or lifestyle diseases. Quercetin is

one of polyphenolic compounds abundantly contained in onions and apples. Much evidence is available on quercetin as a functional food factor particularly for its prominent antioxidant activity among the various polyphenolic compounds.
Purpose: To clarify efficacy of antioxidant activity of quercetin in attenuating pathology of dry eye disease.
Results: As a result of feeding the experimental diet with 0.5% of quercetin to the decreased tear production model mice, no changes in the body weight and food intake were observed; however, the decrease in tear volume was significantly suppressed. Further, changes in the lacrimal gland morphology and the blood chemical values indicated improvements in the pathological features.
Conclusion: Elucidating the association between the antioxidant effects of quercetin and tear secretion is crucial for preventing and/or treating dry eye disease, as it is assumed to be increasingly prevalent as well as lifestyle diseases.

NERVE GROWTH FRACTOR IN HUMAN CULTURED CONJUNCTIVAL CELLS UNDER DRY EYE MIMICED HYPEROSMOAR STRESS

Hungwon Tchah[1], Soon-Suk Kang[2], Eun-Soon Kim[2], Jae Yong Kim[1], Jin-A Sin[2], In-Suk Song[1]
[1]Department of Ophthalmology, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea.[2]Asan Institute for Life Sciences, Asan Medical Center, Seoul, Korea.
Purpose: To evaluate the effect of nerve growth factor (NGF) on cultured human primary conjunctival epithelial cells (pHCEC) under hyperosmolar stress.
Methods:, RT-PCR for NGF RNA production and ELISA for NGF protein production were assessed with pHCECs in normal osmolar medium (307 mOsm/L) or higher-osmolarity media, 350, 400, and 450 mOsm/L. Apoptosis analysis by FACSCalibur flow cytometer was done with pHCEC cultured in normal osmolar or 400 mOsm/L medium with/without NGF neutralizing antibody and/or recombinant human NGF. Bcl-xL, Bax, phospho-JNK, and cleaved caspase-3 expression were detected by Western blotting of pHCEC cultured in above mentioned conditions.
Results: NGF levels in cultured pHCECs were up-regulated during hyperosmolar conditions. Number of apotic cells significantly were increased in hyperosmolar condition and even more increased in NGF neutralizing antibody-added hyperosmolar condition. When recombinant human NGF is added in hyper osmolar medium, number of apoptotic cells were decreased. Phospho-JNK, Bax, and cleaved caspase-3 expression were up-regulated and Bcl-xL expression was down-regulated in hyperosmolar condition, but these phenomena were reversed with addition of recombinant NGF..
Conclusions: Our results suggest that hyperosmolarity induces apoptosis of pHCECs by JNK signaling pathway. Up-regulated NGF under hyperosmolar condition may contribute, at least in part, to reduce apoptosis of pHCEC and may be beneficial in recovering conjunctival damage due to chronic hyperosmolar stress, like dry eye condition.
(Authors have no financial interest in any materials used in this study)

INCREASED TEAR FILM BREAK-UP TIME AS A SENSITIVE INDICATOR IN THE DIAGNOSIS OF EARLY DRY EYE IN TYPE 2 DIABETICS.

Rajarathna Thangavel[1], Manjula Jain[2], Rajesh Jain[1], Pamela D'Souza[1].
Department of Ophthalmology[1], Department of Pathology[2], Lady Hardinge Medical College, New Delhi, India.
Purpose: To report the high sensitivity of tear film break-up time(TBUT) in the diagnosis of dry eye disease in its early stages in patients with Type 2 Diabetes.
Methods: Tear film break-up time(TBUT) was assessed in 100 patients with Type 2 Diabetes and was compared with cytological changes in the conjunctiva.
Results: Our study showed that TBUT has high sensitivity in diagnosing early dry eye disease. Increased tear film breakup time was seen in patients who were asymptomatic or minimal symptoms but had conjunctival cytological changes of dry eye.
Conclusions: Our study demonstrates i) the high sensitivity of TBUT and ii) the ease of procedure that could permit its use as a routine office screening procedure in Type 2 diabetic patients. Tear film stability is essential for the maintenance of healthy ocular surface, especially in diabetics, who are prone to ocular surface disorders. Early diagnosis of tear film instability can help initiate preventive measures and early treatment of dry eye.
The authors have no financial disclosures.

TEAR SUPPLEMENT EFFECTS ON TEAR PHYSIOLOGY AND WETTABILITY IN CONTACT LENS WEAR

A. Tomlinson, R. Fagehi, K. Oliver
Vision Sciences, Glasgow Caledonian University
Purpose: To investigate the effect of sustained use of eye drops on tear evaporation, osmolarity, and lens wetting in contact lens (CL) wear.
Methods: 12 habituated subjects (4 male & 8 female; age 37.54± 15.93 yrs) wore Acuvue Oasys (Johnson & Johnson) lenses for the 4 weeks of the study. The subjects attended for 5 visits; visit 1, baseline, without lens wear; visit 2, one week of lens wear without applying drop; visit 3, after a week of lens wear and using the first drop 4 times per day (allocated randomly, either Refresh Contacts or Optive Plus (Allergan, Irvine, CA); visit 4 after a week with lenses but no drops; visit 5 a week of lenses wear, using the second eyedrop.
Tear evaporation and osmolarity were evaluated at each visit at a temperature of 21 °C and two relative humidities (RH), 40% and 10%. Contact lens wettability (de-wetting) was evaluated (at 21 °C and 40% RH) by thin film interferometry measuring onset latency, drying duration, peak latency and maximum speed of drying. [1]
Results: CL wear increased evaporation at both 40/10% RHs (p= 0.07, 0.02 respectively). Generally a week's use of eye drops did not significantly reduce evaporation at either RH (p= 0.411 at 40%, 0.787 at 10% RH). The evaporation rates after use of Refresh and Optive Plus were the same at both RHs. No significant effects were found for the eye drops on the tear osmolarity.
All the wettability parameters were significantly improved after 1 week's use of both eye drops (Refresh & Optive). The beneficial effect with Refresh for drying duration and peak latency was greater than for Optive Plus (p=0.006, 0.028 respectively).
Conclusions: In the present study the integrity of the lipid layer (LL) (and tear evaporation) was not significantly affected by the use of eye drops in CL wear. This is probably because the LL of 'young' CL wearers was adequate and could not be enhanced, sufficiently to overcome the disruptive effect of the presence of the lens. CL wettability did improve with both drops with a slight superiority for Refresh Contacts.
[1]Fagehi R et al. Application of Thin Film Interferometry to Measurement of Contact Lens Wettability In-vivo; ARVO 2011 ***E-abstract: 6524****.*

S100A8 AND S100A9 ACTIVATE A RANGE OF DISEASE MEDIATORS VIA TRANSCRIPTION IN THE CONJUNCTIVA OF PTERYGIUM PATIENTS

Louis Tong,[1,2,3,4] Wanwen Lan,[4] YoonPin Lim,[3] AiHua Hou,[4]
Singapore National Eye Center,[1] Duke-NUS graduate medical school,[2] National University of Singapore,[3] Singapore Eye Research Institute,[4] Singapore
Purpose: Pterygium is a fibrovascular growth in the ocular surface with corneal tissue destruction, matrix dysregulation and varying

extent of chronic inflammation. The calcium binding S100A8/9 proteins, known to activate the innate defence, have been found to be dysregulated in wide range of ocular surface diseases in our previous work. We investigated the ability of S100A8/9 to regulate the expresssion of the panel of disease-inducing genes involved in pterygium pathology.
Methods: Four paired samples of pterygium and uninvolved conjunctiva tissues from the same eyes were evaluated for differences in global transcript levels using a genechip microarray. Five pairs of samples from another 5 patients were evaluated for protein levels using nano-ESI LCMS/MS and compared with the iTRAQ method. Primary conjunctival fibroblasts were cultured from tissues. After serum starvation for 24 hours recombinant S100A8, S100A9 or the heterodimer form of these were added to the media. Transcript levels were evaluated by reverse transcription qPCR.
Results: The molecules upregulated at both transcript and protein levels include S100A8/A9, the inflammatory chemokine (CXCL1), matrix proteins (vimentin, biglycan, and gelsolin), annexin-A2, thymosin 4 and RAB10. The protease related molecules chymase1 and SERPINA1 were found to be downregulated. On adding S100A8, S100A9 or the heterodimer, transcripts of all the dysregulated proteins, but not the S100A8 or A9, increased at 6 or 24 hours.
Conclusions: Since raised S100A8/9 also occurs in dry eye, meibomitis and corneal neovascularisation, we conclude that they are *bona fide* alarmins in the ocular surface. These are likely early upstream molecules in pterygium that are responsible for a large repertoire of key observed abnormalies, with the exception of depressed chymase and SERPINA1, which may be driven by non-S100A8/A9 triggers.
Grants: Singapore National Research Foundation NMRC/CSA/045/2012 and Ministry of Health's National Medical Research Council NMRC/1206/2009

IS INFLAMMATION THE CORE MECHANISM IN DRY EYE DISEASE? DISAGREE.

Kazuo Tsubota. Keio University School of Medicine
Dry eye is caused by multifactorial risks, including aging, inflammation, video display terminal (VDT) use, smoking, etc. Inflammation is definitely one of the important risk factors for the development of dry eye; however, we believe that the core mechanism of dry eye disease is unstable tear film. According to a recent survey on dry eye, most of the dry eye patients have short tear film break-up time with preserved lacrimal gland function. Those patients have not only ocular symptoms but also decreased visual function. An unstable tear film causes decreased visual function, and patients feel relief with closed eyes, a condition where unstable tear film does not affect the eye. However, inflammation is not a major risk factor, such as for VDT work. Inflammation is important but not the core mechanism.

WORK PRODUCTIVITY LOSS AMONG VISUAL DISPLAY WORKERS WITH DRY EYE DISEASE: OSAKA STUDY

Miki Uchino[1], Yuichi Uchino[1], Murat Dogru[1], Motoko Kawashima[1], Norihiko Yokoi[2], Aoi Komuro[2], Yukiko Sonomura[2], Hiroaki Kato[2], Shigeru Kinoshita[2], Debra A. Schaumberg[3,4], Kazuo Tsubota[1]. Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan[1], Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan[2] , Department of Ophthalmology & Visual Sciences, University of Utah, Salt Lake City, UT, USA[3], Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA[4]
Purpose: To estimate the impact of dry eye disease (DED) on work performance and productivity in office workers.
Methods: Six hundred seventy-two Japanese young and middle-aged office workers were recruited in this cross-sectional study. At-work performance deficits and productivity loss were measured by the Japanese version of Work Limitations Questionnaire (WLQ-J), completed by e-mail. Dry eye tests including the Schirmer test, tear film break up time, fluorescein and lissamine staining were performed. Respondents were classified into three groups including definite DED, probable DED, and non-DED using the Japanese dry eye diagnostic criteria.
Results: Of the 672 office workers, 553 subjects (82.3%) including 366 men and 187 women completed the questionnaire and underwent clinical evaluation. Forty-six subjects (8.3%) were diagnosed as definite DED, 198 subjects (35.8%) were diagnosed as probable DED, and 309 subjects did not have DED. For the total workplace productivity loss, the non-DED group lost 3.56%, those with probable DED lost 4.06%, and those with definite DED lost 4.82%, indicating significantly worse performance and productivity (trend test p=0.01). For the four subscales, DED was associated with significantly lower on-the-job time management (trend test p=0.009) and mental performance/interpersonal functioning (trend test p=0.01). Annual DED productivity losses were estimated to be $6,160 per employee by total production and $ 1,178 per employee calculated by wage.
Conclusions: This study indicates that there is a significant impact of DED on total productivity of Japanese workers.
The authors make the following disclosures: Shigeru Kinoshita: consultant for Santen Pharmaceutical Co., Ltd.
Debra A. Schaumberg: Board membership for SARcode Bioscience, Mimetogen, Inc., consultant for Eleven Biotherapeutics, SARcode Bioscience, Mimetogen, Pfizer, Inc., Alcon, Inc., Allergan, Inc., Inspire Pharmaceuticals, and Resolvyx, expert testimony for Attorneys for Private Plaintiffs, Payment for development of educational presentations for Allergan, stock options for Tear Lab, Mimetogen.
Kazuo Tsubota: consultant for Santen Pharmaceutical Co., ltd.
This study was supported by a grant-in-aid from the Ministry of Health Labour and Welfare, the Ministry of Education, Science, Sports and Culture, Grant-in-Aid for Young Scientists (B), 2279192, 2010.

GALECTIN-3 CONTRIBUTES TO THE OCULAR SURFACE INFLAMMATORY RESPONSE.

Yuichi Uchino[1], Jerome Mauris[1], Julia Dieckow[1], Ashley M. Woodward[1], Francisco Amparo[1], Reza Dana[1], Flavio Mantelli[2], Pablo Argüeso.[1] Schepens Eye Research Institute, Harvard Med School, Boston, MA, USA,[1] Dept of Ophthalmology, University of Rome, Italy[2]
Purpose: Galectin-3, a carbohydrate-binding protein expressed by the ocular surface epithelia, provides barrier function under physiological conditions though interaction with transmembrane mucins on the apical glycocalyx. Here, we investigated whether galectin-3 is altered in dry eye patients and/or modulates cytokine receptor activation in corneal epithelial cells.
Methods: Galectin-3 in tear fluid collected from the inferior fornix of normal subjects (n=11) and patients with dry eye (n=20) was analyzed by quantitative Western blot with recombinant protein as calibration standards. siRNA targeting galectin-3 was introduced into stratified cultures of human corneal-limbal epithelial (HCLE) cells by lipofectamine-mediated transfection. Stratified HCLE cell cultures were treated for 6 hr with serum-free growth-media alone or with recombinant human IL-1 (10 ng/ml). Secreted IL-8, IL-1 receptor 1 (IL1R1) and GAPDH were quantified by Western blot.
Results: The levels of galectin-3 detected in normal tear samples were 0.12±0.14 ng/µg total protein (range, 0.00-0.41). In dry eye, there was a significant increase in galectin-3 content in tears (0.38±0.37 ng/µg total protein; range, 0.04-1.36). Treatment of stratified HCLE cells with galectin-3 siRNA decreased galectin-3 protein biosynthesis by 90%. IL-8 protein levels were increased in the culture media of control HCLE cells treated with IL-1 , but not in cells treated with galectin-3 siRNA, indicating that galectin-3 mediates IL1R1 activation. The amount of IL1R1 in total cell protein extracts was not affected by either IL-1 stimulation or galectin-3 knockdown.

Conclusions: Increased levels of galectin-3 in the tear fluid may reflect alterations of the ocular surface glycocalyx barrier in dry eye patients. Galectin-3 regulates IL-1-mediated inflammatory responses on the ocular surface epithelia, however the molecular mechanisms of this regulation remain to be elucidated.
Supports: NIH EY014847, Japan Eye Bank Association, Uehara Memorial Foundation.

ANTI-INFLAMMATORY EFFECTS OF REBAMIPIDE EYE DROPS ON ALLERGIC CONJUNCTIVITIS

Mayumi Ueta[1,2], Chie Sotozono[1], Ayaka Koga[1,2], Norihiko Yokoi[1], and Shigeru Kinoshita[1]
[1]Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan
[2]Faculty of Life and Medical Sciences, Doshisha University, Kyoto, Japan
Purpose: Rebamipide, a gastroprotective drug, has been reported to suppress gastric mucosal inflammation. In Japan, rebamipide eye drops have recently been approved for the treatment of dry eye disease. The purpose of this present study was to evaluate the anti-inflammatory effects of rebamipide eye drops on allergic conjunctivitis.
Methods: Five patients with allergic conjunctivitis with giant papillae (vernal conjunctivitis or atopic keratoconjunctivitis) instilled rebamipide eye drops 3-4 times daily for varying periods of time. All 5 patients had dry eye with decreased tear-film break-up time. Changes in the size of the giant papillae in each patient was evaluated using Image J software. Moreover, IL-8 levels in the tears of another 6 eyes of allergic conjunctivitis without giant papillae, who were treated with rebamipide eye drops for dry eye disease, were examined using cytokine bead assay.
Results: Attenuation of the giant papillae was observed in all 5 patients. In 2 patients with severe disease, the addition of rebamipide contributed to the attenuation of giant papillae that had enlarged despite the administration of tacrolimus and steroids. In 3 patients with mild disease, who were or were not treated with anti-allergy drugs, the addition of rebamipide eye drops also resulted in the attenuation of their giant papillae.
IL-8 levels in the tears of allergic conjunctivitis patients without giant papillae decreased after treatment with rebamipide eye drops.
Conclusions: Our findings suggest that rebamipide eye drops might attenuate giant papillae in allergic conjunctivitis patients and also attenuate IL-8 levels in the tears of those patients. In addition to the treatment of dry eye, rebamipide eye drops may also be useful for the treatment of allergic conjunctivitis.

OPTICAL ABERRATIONS OF THE CORNEAL SURFACE: 3 MONTHS FOLLOW UP INVESTIGATION.

Balamurali Vasudevan. College of Optometry, Midwestern University, Glendale, AZ, USA
Purpose: To assess the natural history of progression of corneal higher-order aberrations in young-adult population for 3 months, and to study the effect of age on the changes in corneal aberrations.
Methods: Twenty two young adult subjects (ages: 23-39 years) participated in this study. Three measurements of corneal aberrations were obtained using an iTrace corneal aberrometer at each session. All the measurements were obtained with 4mm and 7mm pupil diameters at 8AM following a 24 hour washout from contact lenses. Measurements were repeated by the same investigator after 3 months. The parameters investigated were root mean square (RMS) of corneal higher-order aberrations (HOA), corneal spherical aberration (SA) and coma.
Results: Paired t-tests and intraclass correlation (ICC) performed between baseline and follow up visits for RMS of corneal HOA (t=1.059, p=0.30; ICC=0.98), SA (t=0.794, p=0.43; ICC=0.90) and coma (t=1.06, p=0.30; ICC=0.63) demonstrated that there were no significant difference and were highly correlated. Bland and Altman analysis revealed that measurements performed at both the visits were highly repeatable. Total RMS HOA, coma and spherical aberration did not vary as a function of age in these subjects. In addition, there was no significant effect of pupil diameter.
Conclusions: The corneal aberrations were not significantly different between the visits separated by 3 months. Any dynamic changes in optical aberrations observed could primarily be attributed to changes induced by tear film.

LIPID RELEASE FROM LIPID-BASED EYE DROPS CAN BE ASSISTED BY TEAR FILM SODIUM CHLORIDE

Joseph G. Vehige[1], Peter A. Simmons[1], Bereth J. Beard[1], Prem Mohanty[1]
[1]Allergan Inc., Irvine, California USA
Purpose: Lipid-containing eye drops and sprays are intended to provide unique benefits to dry eye patients that are deficient in meibomian gland secretion. For such products to provide this benefit, lipid must be delivered efficiently to the eye allowing an enhancement of lipid layer structure and function. This study modeled the role of tear film sodium chloride on the mechanism of lipid release from a stable lipid-based eye drop designed for efficient lipid delivery to the ocular surface.
Methods: Real–time oil droplet size (Z average: d.nm) and uniformity (polydispersion index, PDI) were measured using a dynamic light scattering instrument (Malvern, model ZEN3690) for a lipid-based eye drop (Refresh Optive Advanced, Allergan), undiluted, diluted 20:1 with distilled water and diluted 20:1 with 9.0% NaCl (0.45% final NaCl concentration). All samples were run in triplicate using multiple scans (one every 2 minutes) to measure Z-average and PDI changes over time.
Results: Undiluted, or diluted with water, the Z-average and PDI were stable at all time points. Dilution with water caused only a small decrease in droplet size, from 433 to 420 nm, with no significant change to PDI. Adding NaCl caused a marked initial (at 2 min) increase in Z-average to 1081.5 +/- 136.3 nm, with a continued increase over time to 2037.7 +/- 69.87 nm at 8 min. PDI for the samples with added NaCl increased beyond the limit of measurement, indicating a rapid loss of uniformity.
Conclusions: This lipid-based formulation maintained a stable droplet size with or without addition of distilled water. When mixed with saline, simulating mixture with native tear fluid on eye, the formulation was rapidly destabilized showing an increase in lipid droplet size and loss of uniform structure, demonstrating lipid phase separation. This separation is consistent with rapid delivery of lipid from the instilled drop into the lipid phase of the tear film.
Commercial Relationship: The authors are employees of Allergan, Inc.

QUANTITATIVE COMPARATIVE PROTEOMICS OF HUMAN TEAR FLUID AND LABIAL SALIVA PROVIDES PHYSIOLOGICAL/ BIOCHEMICAL EVIDENCE SUPPORTING SALIVARY GLAND TRANSPLANTATION FOR DRY EYE TREATMENT.

Peter Verhaert[1,4], Remco Crefcoeur[1], Peter Raus[1,2,3], Maithili Krishnan[1], Martijn Pinkse[1], Leen Slaets[1,4], Gordon Laurie[5], Niels Hellings[1,4]. [1] Department of Biotechnology, Delft University of Technology, Delft, Netherlands; [2] Miró Center for Ophtalmology, Mol, Belgium; [3] Faculty of Medicine and Pharmacy, Free University of Brussels; [4] Biomedical Research Center, Hasselt University, Hasselt, Belgium; [5] Laboratory for Cell Biology, University of Virginia, VA, USA.
Purpose: At Miró transplantation of labial salivary glands into a 'dry eye' is sucessfully employed to treat a variety of patients suffering from drye eye syndrom. We are performing extensive comparative quantitative proteomics and peptidomics analysis of both tear fluid as well as labial saliva, to collect biochemical evidence for the validity of this treatment.

Methods: GeLC MS/MS on QTOF (Waters QTof Premier) tandem mass spectrometry equipment. Immunohistochemistry using protein antibodies to orthogonally validate the Results:
Results: In labial saliva 191 different proteins could be unequivocally detected, whereas in tear fluid this number was 132. Between both body fluids, 81 proteins overlapped, one of them being lacritin. Immunocytochemistry revealed that lacritin immunoreactivity is indeed being produced in transplanted labial salivary glands.
Conclusions: Our data indicate that labial salivary gland transplantation into a drye eye effectively represents the translocation of an active lacritin secretin tissue at the site of the diseased tear gland. As lacritin is known to stimulate lacrimal gland secretion of both the aquaeous, mucous as well as lipid components of the tear fluid, this may well represent (part of) the cure observed after this treatment.
Future Perspectives: More in depth analysis of both the proteome and the peptidome of the human tear will undoubtedly yield highly valuable biochemical information on the natural composition of the healthy (as well as the diseased) tear, which may ultimately lead to even more elegant (and personalized) treatment of patients suffering from dry eyes, which may include innovative peptide and/or protein additives to artificial tears.
[We acknowledge financial support from the Netherlands Proteomics Centre (NPC) to the Delft NPC Analytical Hotel]

RECOVERY OF CONJUNCTIVAL GOBLET CELL DENSITY IN MILD – MODERATE DRY EYE PATIENTS AFTER TREATMENT WITH HYALURONIC ACID – VITAMIN B12 BASED EYEDROPS.

Piera Versura, Vincenzo Profazio, Giuseppe Giannaccare, Emilio C. Campos. Ophthalmology Unit, DIMES, Alma Mater Studiorum University of Bologna, S. Orsola-Malpighi Teaching Hospital, Bologna – Italy. piera.versura@unibo.it
Purpose: To evaluate the recovery of conjunctival goblet cells (CGC) density after topical treatment with Artelac Rebalance® eyedrops in dry eye (DE) patients.
Methods: Twenty mild-moderate DE patients were evaluated at enrollment (V0), after 2 days of washout (baseline, V1), and after 1 (V2) and 2 months (V3) of treatment (one drop/eye/3 times daily). Parameters for ocular discomfort (OSDI score), tear film quality (Schirmer test I, TFBUT, tear osmolarity), ocular surface damage (Laser Scanning Confocal Microscopy LSCM observations, Oxford grading score, conjunctival imprint cytology, PAS-positive GC number/mm^2) and inflammation (scraping cytology and exuded serum albumin) were measured. Data were statistically analyzed and expressed as mean ± SD, significance $p<0.05$. Pearson (r) or Spearman ( ) correlation coefficients among different variables were also calculated.
Results: Variables showed a statistically significant improvement (always $p<0.01$) at V3 versus V1, as follows: OSDI score (21.4±9.4 vs 36.2±10.6), TFBUT (7.5±1.9 vs 5.4±2.3 s), Oxford grading of surface damage (0.46±0.50 vs 1.23±0.41 score), tear osmolarity (293±6.5 vs 301.5±7 mOsm/L), CGC density (144.2±42.6 vs 121.3±31.1 cells/mm^2), scraping cytology score (2.1±1.0 vs 4.4±1.2), imprint cytology (1.08±0.4 vs 1.37±0.46) and percentage of serum albumin in tears (9.1±4.2 vs 20.9±10.9 %). CGC recovery was confirmed with LSCM counts and was correlated to tear osmolarity and scraping score reduction (r -0.55, -0.45 respectively, $p<0.0001$).
Conclusions: Recovery of CGC density and ocular surface damage with a parallel improvement in tear film quality were shown after a two month treatment with Artelac Rebalance® eyedrops in mild to moderate DE patients.
Commercial disclosure . Bausch + Lomb supported the study by supplying the necessary amount of product, which is not reimbursed by the public health system in Italy.

PREDICTIVITY OF TEAR PARAMETERS IN THE ONSET OF OCULAR GRAFT VERSUS HOST DISEASE (GVHD) DRY EYE AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

Piera Versura[1], Giuseppe Giannaccare[1], Francesca Bonifazi[2], Giuseppe Bandini[2], Lorenza Ridolfi[3]
[1]Ophthalmology Unit, [2]Haematology section, DIMES, Alma Mater Studiorum University of Bologna, [3]Emilia-Romagna Transplant Reference Center, S. Orsola-Malpighi Teaching Hospital, Bologna– Italy, piera.versura@unibo.it
Purpose: to evaluate the predictive value of tear parameters identifiable before Hemopoietic Stem Cell Transplantation (HSCT) in the onset of ocular Graft Versus Host Disease (GVHD)-related dry eye (DE).
Methods: 65 haematological patients were enrolled and analyzed before (T0) and after (T1) receiving HSCT, medium period 9±6 months postoperatively. Diagnostic performance of tear lysozyme, lactoferrin, exudated serum albumin (tSA), lipocalin and total tear protein (TP) at T0 was evaluated and compared to performance of OSDI, Schirmer test, BUT and Oxford grade vital staining. Patients were divided at T1 as Group 1, 17 patients [ocular GVHD following NIH severity score, Filipovich et al, 2005) and Group 2, 48 patients (DE non GVHD-related). Results were related to ocular surface inflammation (conjunctival scraping cytology score) and metaplasia (conjunctival imprint cytology score), recipient age, donor type, HSC source, T-cell depletion methods (chemiotherapy CT or radiotherapy RT).
Results: A statistically significant difference was shown at T0 in BUT, imprint cytology score, % tSA between groups. A significant decrease in Schirmer test and BUT while an increase in tSA was shown at T1. Epithelial metaplasia and inflammatory cell infiltration were detected at T1 in group 1. BUT and tSA exhibited the higher diagnostic performance (cut-off values at T0, respectively, $\leq$ 6 seconds and >15 %, LR+ 7.6 and 8.2) and high predictive values either alone (PPV+: 75 and 81) and taken together in a combination (LR+: 11.2, PPV+: 85). Pre- post variations were statistically correlated to recipient age, previous CT, peripheral blood stem cells (PBSC) as source ($p<0.01$).
Conclusions: Ocular surface involvement in GVHD is a frequent complication following HSCT. Ocular surface changes were evidenced already before HSCT. BUT and tSA were found to be predictive for the onset of ocular GVHD.

THE EFFECT OF NONINVASIVE TEAR FILM BREAK-UP TIME MEASUREMENT ON TEAR MENISCUS HEIGHT

Shinya Watanabe,[1] Shizuka Koh,[1] Hitoshi Watanabe,[1,2] Chikako Ikeda,[1] Yoshihiro Takai,[1] Motokazu Tsujikawa,[1] Naoyuki Maeda,[1] Kohji Nishida[1]. [1] Department of Ophthalmology, Osaka University Graduate School of Medicine, Osaka, Japan, [2] Kansai Rosai Hospital, Amagasaki, Japan
Purpose: To investigate the effect of noninvasive tear film break-up time (NIBUT) measurement on lower tear meniscus height (TMH).
Methods: Twenty-three eyes of 23 patients with aqueous-deficient dry eye and 23 eyes of 23 normal subjects were enrolled. All the subjects underwent imaging with a Keratograph 5M (Oculus, Wetzlar, Germany) equipped with a modified tear film scanning function. TMH images were captured and measured with an integrated ruler before and immediately after NIBUT measurements in each subject. During NIBUT measurements, subjects were instructed to keep their eyes open as long as possible and the recording was discontinued at the next blink.
Results: At baseline, the TMH values of normal and dry eye groups were 0.20±0.05mm and 0.14±0.03mm, respectively. TMH values of dry eye were significantly smaller than those of normal eye ($p<0.001$). TMH values after NIBUT measurement in normal and dry eye were 0.30±0.13mm and 0.18±0.08mm. In both groups, TMH significantly increased after NIBUT measurements ($P<0.001$, $p=0.03$). NIBUT values of dry eye (4.9±3.6 seconds) were significantly less than those of

normal eye (8.6±5.5 seconds) (p<0.001). There were no significant correlation between NIBUT and the amount of TMH change.
Conclusions: Forced eye opening required for NIBUT measurement increased TMH possibly due to reflex tear secretion. Since reflex tearing can be an obstacle to the interpretation of aqueous tear-sourced data, TMH should be measured before NIBUT measurement for accuracy. [The authors have no commercial relationship.]

THE EFFECT OF TEMPERATURE ON THE SURFACTANT PROPERTIES OF HUMAN AND RABBIT TEARS.

Xiaojia Eric Wei,[1,2] Zhenjun Zhao,[1,2] Thomas J. Millar,[3] Mark DP. Willcox.[2] Brien Holden Vision Institute,[1] School of Optometry and Vision Science, University of New South Wales,[2] School of Natural Science, University of Western Sydney,[3] Sydney, Australia

Purpose: Previous studies suggest that high surface pressure contributes to maintaining tear film stability. Our results showed rabbit tears have higher maximum surface pressure (MSP) and a higher concentration of divalent cations compared to humans. Divalent cations appear to play an important role in maintaining high surface pressure. As surfactant properties are sensitive to temperature, we sought to examine the effect of temperature on surfactant properties of human and rabbit tears.
Methods: Tears were collected from normal adult humans (n=10) and rabbits (n=6) and pooled for each group. The influence of temperature on tear surfactant property was measured on a Langmuir trough with various concentrations of divalent cations (either at human or rabbit levels) in the artificial tear subphase buffer at either 20 or 35°C.
Results: The -A profiles of both human and rabbit tears were affected by temperature, which shifted to the right at 35°C indicating an increase in the area being occupied by each molecule at the surface. However, the MSPs were unaffected by temperature change, remaining at 26 and 37mN/m respectively. At 20°C, the rabbit tears exhibited a stronger hysteresis between the expansion and compression phases compared to the cycle at 35°C. The hysteresis of human tears did not appear to be temperature dependent. When the concentration of divalent cations was reduced to the human level, rabbit tears demonstrated a decrease in the MSP from 37 to 32mN/m at 35°C, but showed no change at 20°C. In contrast, the surfactant properties of human tears were unaffected by increasing divalent cation concentrations to rabbit levels at either temperature.
Conclusions: The surfactant properties of both human and rabbit tears are sensitive to temperature. As the effect of temperature and divalent cations was much more pronounced with rabbit tears, this may indicate that they contain unique components which work together with the divalent cations to contribute to tear film stability.

PRODUCTION OF CONTACT LENS INDUCED MICROBIAL KERATITIS IN AN ANIMAL MODEL WITHOUT PRIORDAMAGE TO THE OCULAR SURFACE

Mark DP Willcox, Ajay K Vijay. School of Optometry and Vision Science, University of New South Wales, Sydney, NSW, Australia
¹School of Optometry and Vision Science², University of New South Wales², Sydney², Australia²

Purpose: Contact lens wear is associated with the production of microbial keratitis (MK), especially byPseudomonas aeruginosa strains. Previous studies that have used mice, rats, and Guinea Pigs have shownthat in the absence of damage to the ocular surface (i.e. scratching the cornea with a needle) inflammationbut not infection is produced during wear of P. aeruginosa adherent to contact lenses. The aim of this studywas to determine whether Rabbits wearing lenses colonised by different strains of P. aeruginosa succumbed tomicrobial keratitis without prior damage to their corneas.
Methods: 6 month old New Zealand White rabbits were used. Silicone hydrogel and hydrogel contact lenseswere washed and soaked in 1x108 cfu/ml of strains of P. aeruginosa (Paer1 [isolated from a non-infectiouskeratitis] and 6294 [isolated from MK]). Bacteria cells were allowed to adhere to lenses for 24h, lenses thenwashed three times in PBS, and applied to one eye of each rabbit. Rabbits eyes were examined by slit lampexamination at baseline, and after 6h, 24h and 48h of lens wear. Levels of redness, chemosis, ulceration andinfiltration were recorded. Numbers of bacteria on lenses were verified by plate counting.
Results: Strain Paer1 adhered to lenses to levels of 5.7 to 7.2 log10 cfu/lens depending on lens type. Strain6294 isolated from MK adhered at similar levels to all contact lenses. When lenses colonised with Paer1 wereworn there was increased redness at 6, 24 and 48h but no chemosis, infiltration or ulceration at any timepoint. However, when lenses were colonised with 6294 and worn, 50% of rabbits developed frank ulceration ofthe cornea with increases in conjunctival redness, chemosis and infiltration of the cornea after 24h of lenswear, and rabbits were euthanized at that time point. Baseline slit lamp examination of the corneas of allrabbits showed no breaks in the epithelium.
Conclusions: This study has shown that a strain of P. aeruginosa isolated from MK can cause frank infectionof the cornea during lens wear in the absence of any damage to the eye. This model may be useful to studythe virulence factors of the bacteria and the pathogenesis of MK.

THE EFFECT OF INCREASED PERIOCULAR HUMIDITY ON LIPID LAYER THICKNESS AND OCULAR COMFORT OF SYMPTOMATIC CONTACT LENS WEARERS.

Timothy Willis[1] Caroline Blackie[1,2] and Donald Korb[1,2]. TearScience Inc, Morrisville,[1] NC, Korb Associates, Boston, MA.[2]

Purpose: To determine whether increasing periocular humidity would 1) increase the thickness of the tear film lipid layer over the front surface of a soft hydrogel contact lens (CL), 2) increase comfort for symptomatic CL wearers.
Methods: All symptomatic CL wearers (n = 25; 5 males, 17 females) had worn their CLs for a minimum of 2 hours and a maximum of 5 hours prior to examination. Inclusion criteria: minimum CL wear of twice a week for 5 or more hours at a time; minimum symptom score when wearing the lenses = 3 on a scale of 1-5 (1 = asymptomatic, 5 = intolerable discomfort). Exclusion criteria: all toric lenses; all lenses of over 7.75D, age over 50 years, keratoconus. At three time points (immediately prior to wearing goggles, after 20 mins of continuous goggle wear and 15 minutes after the goggles were removed) patients were required to fill out a symptom questionnaire to assess their ocular comfort. The lipid layer thickness (LLT) was semi-quanitatively graded using an interferometer immediately following each symptom assessment.
Results: The mean age of the patients was 37.9+/-10.4 years. After the goggles had been worn for 20 mins, 88% reported an increase in their CL wearing comfort of at least one grade and 72 % evidenced an increase in LLT of at least 30nm (1 grade). After the goggles had been removed for 15 mins, only 12% maintained a comfort improvement of at least one grade; 8% reported feeling 1 grade worse than baseline, and only 8% maintained the increase in LLT of at least one grade (30nm); 12% evidenced a decrease in LLT of 30nm from baseline.
Conclusion: Increasing the periocular humidity results in an increase in the LLT of the tear film over the front surface of the contact lens and simultaneously improves lens-wearing comfort. Presumably, the increased LLT is due to decreased evaporation, which results in a thicker overall tear layer, improved protection of the ocular surface, improved hydrodynamic lubrication for the lid wiper and increased CL wearing comfort.

A MOLECULAR-LEVEL VIEW ON THE ORGANIZATION OF LIPIDS IN THE TEAR FILM.

Alicja Wizert[1], D. Robert Iskander[1], Lukasz Cwiklik.[2] Wroclaw

University of Technology, Wroclaw, Poland,[1] Academy of Sciences of the Czech Republic, Prague, Czech Republic.[2]
Purpose: To study biophysical properties of a realistic tear film model at the molecular level and under conditions that physiologically occur during blinking.
Methods: An innovative tear film model studied by molecular dynamics (MD) has been proposed. Coarse-grain MD simulations were performed for the water/lipid/air systems under thermodynamic equilibrium conditions at different lateral compressions to mimic varying surface pressure conditions expected during a blink. Additionally, non-equilibrium MD simulations were performed to study the dynamics of the system. The polar components (POPC, POPE, SM, Cer) of the lipid system were chosen directly to reflect the experimentally assessed lipidom of the tear film, whereas the lipids of the Meibomian origin (TG, CE) were used to compose a thick non-polar layer.
Results: Simulations demonstrate that polar phospholipids separate non-polar lipids from the water phase, constituting a monomolecular platform whereas the non-polar lipids form a relatively thick layer located at the top of the polar film. Under high compression not only does the large phospholipid surface undulate irregularly but also tends to extrude (into the non-polar medium) phospholipid aggregates organized into elongated three-dimensional structures or droplets attached to the monolayer.
Conclusions: The proposed model provides a stable layer, even when treated with high pressure. The lateral compression affects phospholipid monolayer forcing it to undulate, but no fracture occurs in the timescale of nanoseconds. Because the thick non-polar lipid layer eliminates disorders present in the polar phase, the outermost surface stays smooth even in high lateral pressure. Also, the polar mid-layer may have more complex structure than initially thought. The action of the eye lids during blinks introduces substantial variations of the lateral pressure. Under such dynamic conditions a monolayer covered with inversed micelles or other irregular three-dimensional structures, due to its elasticity, seems more suitable to model the real human tear film.
[Authors thank Wroclaw Center for Networking and Supercomputing for supplying computational resources.]

SALIVARY BIOMARKERS FOR DISEASE DETECTION

David Wong
Saliva has long been considered a “mirror of the body” that reflects the state of overall health. A wide range of systemic diseases, such as diabetes and Sjögren’s syndrome, have oral manifestations that clinicians (physicians and dentists) can encounter in patients at various stages of disease development.
In recent years, sparked by initiatives from the National Institute of Dental & Craniofacial Research (NIDCR), saliva has attracted widespread interest as a diagnostic medium for rapid, point-of-care testing. The advantages of using saliva for disease diagnostics include ease of access, noninvasive sample collection, increased acceptance by patients, and reduced risks of infectious disease transmission. Oral samples are readily accessible as whole saliva or by sampling secretions from specific glands or gingival crevicular fluid.
Advances in the science of salivary diagnostics have led to identification of disease signatures of candidate biomarkers and/or confirmation of genetic susceptibility for systemic conditions, particularly in molecular oncology. With the development of the salivary proteome, transcriptome, micro-RNA, metabolome and microbiome as diagnostics alphabets (salivaomics) fully enable saliva to be translated for personalized individual medicine applications. Salivary biomarkers panels have been developed for oral cancer, lung cancer, pancreatic cancer, breast and ovarian cancers. Coupled with the development of point-of-care technologies and the emerging trend of chairside screening for medical conditions, the clinical impact of scientifically credentialed salivary biomarkers for disease detection will include the improvement of access to care, reducing health disparities and impacting global health.

A PRELIMINARY POPULATION-BASED ASSOCIATION STUDY: DOES EDAR GENE AFFECT MEIBOMIAN GLAND DEVELOPMENT?

Jianjiang Xu, Jingyu Yang, Jiaxu Hong.
Department of Ophthalmology and Visual Science, Eye and ENT Hospital, Fudan University, Shanghai, China.
Purpose: EDAR gene plays an important role in the development of ectodermal derivatives. Asian-specific mutation in derived EDAR allele was found associated with enlargement of meibomian glands(MGs) in mouse. This study was conducted to test the hypothesis that EDAR contributes to population differentiation regarding MG morphology.
Methods: The derived EDAR allele attained high frequency in East Asian and moderate in Uygur, absent in European. We recruited 37 unrelated individuals and divided them into 3 groups according to ethnicity (Chinese(Ch):17; Uygur(Uy):13; Europeans(Eu):7). DNA was extracted from blood samples for genotyping, we designed primers to genotype EDAR V370A polymorphism (rs3827760). Observation of MGs was under laser scanning confocal microscopy(LSCM) in vivo, we measured glandular acinar unit density(MGAUD) and acinar longest/shortest diameter(MGALD/MGASD) as main parameters for comparative analysis to investigate the association of genotype and phenotype.
Results: Genotyping revealed that derived EDAR allele frequency was consistent with expectations under Hardy–Weinberg equilibrium,as reported previously. LSCM demonstrated that MGASD was significantly smaller in Ch(31.6±7.5um) compared with Uy(43.2±21.6um;p=0.0368) and Eu(48.0±19.2um;p=0.0252). However, values of MGAUD (Ch: 114.6±28.0/mm^2; Uy: 111.0±39.5/mm^2; Eu:117.8±35.6/mm^2) and MGALD (Ch: 89.5±13.0um; Uy: 82.7±19.5um; Eu: 82.5±14.5um) had no significant differences(p>0.05) among populations. Linear regression analysis indicated that EDAR V370A was not correlated with MG density and diameter(p>0.05,Spearman).
Conslucions: The difference of derived EDAR allele frequency did exist among populations. Based on limited phenotype data provided by LSCM, we didn’t demonstrate the impact of population difference on MG morphological pattern, no association was found between genotype and phenotype. Due to small sample size and other reasons, we didn’t get outcomes consistent with those in previous animal experiments. Further population-based association studies need to be carried out to unequivocally test our hypothesis and reach a sound conclusion.
[This work was supported by grants from the Key Clinic Medicine Research Program, the Ministry of Health, China (2012–2014); National Science and Technology Research Program, the Ministry of Science and Technology, China (2012BAI08B01); National Natural Science Foundation of China (81170817, 81200658); Scientific Research Program, Science and Technology Commission of Shanghai Municipality, Shanghai (11231200602, 11DZ2260900). The sponsor or funding organization had no role in the design or conduct of this research. The authors declare no financial relationship with the organization that sponsored the research exists.]

IN VITRO EXPANSION OF ACTIVATED KERATOCYTES FROM HUMAN CORNEAL STROMA FOR TISSUE ENGINEERING APPLICATIONS.

Gary Hin-Fai Yam, Nur Zahirah Binte M Yusoff, Jodhbir S Mehta.
Tissue Engineering and Stem Cell Group, Singapore Eye Research Institute, Singapore.
Purpose: Human corneal stromal keratocytes (hCSK) are quiescent. They become proliferative fibroblasts under serum and cytokine culture with a loss of keratocyte markers. Although these cells are crucial in corneal wound repair, there exists a development of corneal haze and opacity. Hence, *in vitro* propagation and maintenance of genuine CSK is critical for corneal tissue engineering and ocular clarity.

Methods: Collagenase-isolated hCSK were grown in serum-free or serum-added medium supplemented with soluble human amniotic stromal extract (ASE, our preparation), Rho-associated coiled coil forming protein serine/threonine kinase (ROCK) inhibitor Y-27632 and insulin-like growth factor (IGF1) (collectively named as ERI). Cell growth and CSK marker expression were analyzed by mitotic index, collagen matrix contraction assay, quantitative PCR and immunofluorescence. The ability to reverse activated keratocytes to genuine CSK was studied.
Results: With ERI supplement, hCSK cultured in 0.5% serum showed typical dendritic morphology, unlike the short slender fibroblastic cells under serum culture (0.5% or 2%) without ERI. The former were moderately proliferative (~2% mitotic index) and had reduced expression of CSK markers (ALDH1A1, ALDH3A1, Col8A2, keratocan and lumican), but there was a lack of stress F-actin fibers and global collagen matrix contraction. The activated keratoctyes were passaged 10 times and re-expressed CSK markers when returned to serum-free condition plus ERI. Our cocktail also generated proliferative activated CSK of non-human primate, porcine, bovine, rabbit and mouse origins with dendritic cell morphology.
Conclusion: ERI supplement helped *in vitro* transient propagation of activated keratoctyes, which regained CSK gene expression profile and characteristics when returned to serum-free condition. This could provide sufficient number of genuine CSK for corneal tissue engineering without the risk fibroblastic change.
[This work was supported by Biomedical Research Council Translational Clinical Research Partnership Grant (TCR0101673), Singapore]

MEIBOMIAN GLAND DYSFUNCTION (MGD) PREVALENCE: SENSITIVITY TO DIAGNOSTIC CRITERIA.

Nisha Yeotikar,[1] Hua Zhu,[1,2] Negar Babaei,[1] Daniel Tilia,[1] Eric Papas.[1,2,3] Brien Holden Vision Institute,[1] School of Optometry and Vision Science, University of New South Wales,[2] Vision CRC,[3] Sydney, Australia
Purpose: To investigate the sensitivity of MGD prevalence to various diagnostic criteria.
Methods: 179 subjects (105 females) aged between 25-65 years, with no pre-existing ocular and systemic abnormalities were recruited. At a single visit, clinical measures of meibomian gland (MG) structure and function were collected including meibum quality (MQ) and MG expressibility (MGE) of the lower lid, and infra-red meibography of the upper lid (UL) and lower lid (LL). MG loss factor (MGLF) was calculated by taking a ratio of the area of MG drop-out to the total area of MG measured. The prevalence of MGD was calculated using 8 diagnostic criteria that were defined using various combinations of MQ, MGE and MGLF.
Results: The prevalence values resulting from applying various diagnostic criteria are shown in the table below:

MGD Criterion	Description	Prevalence (%)	95% CI
Criterion 1	OD/OS (MQ & MGE = 1) or (MQ/MGE > 1)	63.7	56.1-70.6
Criterion 2	OD & OS (MQ & MGE = 1) or (MQ/MGE > 1)	35.2	28.3-42.7
Criterion 3	MGLF: OD/OS (UL/LL > 1)	39.1	32.0-46.7
Criterion 4	MGLF: OD/OS (UL & LL > 1)	15.6	10.8-22.0
Criterion 5	MGLF: OD & OS (UL & LL > 1)	10.1	6.2-15.7
Criterion 6	Criteria 1 + 3	31.8	25.2-39.3
Criterion 7	Criteria 1 + 4	14.5	9.9-20.7
Criterion 8	Criteria 2 + 5	7.8	4.5-13.0

Based on the overlap of the prevalence estimate confidence intervals (CI), there was redundancy among the original 8 criteria allowing them to be collapsed into 3 groups of increasing stringency as: I (Criterion 1), II (Criteria 2, 3 or 6) and III (Criteria 4, 5, 7 or 8). There was an overall increase in the MGD prevalence with age ($p < 0.01$) irrespective of the criteria applied.
Conclusions: The prevalence of MGD depends on the diagnostic criteria chosen. Diagnostic stringency can be systematically increased by applying the following hierarchical scheme:
I - MQ & MGE = 1 or MQ/MGE > 1 in either eye;
II - same as I in both eyes simultaneously;
III - same as II plus MGLF > 1 in both upper and lower lids.
The prevalence rates associated with each of these levels are: I - 64%, II - 35% and III - 8%.

THE EFFECT OF TOPICAL ANTI-GLAUCOMA EYE DROPS ON HUMAN TENON'S CAPSULE FIBROBLASTS SURVIVAL

Nilgun Yildirim[1], Gokhan Kus[2], Selda Kabadere[2], Ruhi Uyar[2], Afsun Sahin[1]. Eskisehir Osmangazi University Medical School, [1]Department of Ophthalmology and [2]Physiology
Purpose: Glaucoma is one of the leading causes for blindness. There are several topical antiglaucoma eye drops. However, there is limited data about their effects on human Tenon's capsule fibroblast (HTF), which is a key player in wound healing in the eye. The purpose of this study is to investigate the cell viability in HTFs exposed to topical antiglaucoma medications.
Subjects and methods: Small biopsy samples containing HTF were obtained during strabismus surgery with informed consent from patients. The human tenon explants were cultured in a medium consisting of DMEM supplemented with 10% FBS. The explants were incubated at 37 0C in a humidified incubator with 5% CO2. Primary HTFs that migrated out from the tissue were propagated in the same medium. When confluence achieved, cells were detached with 0.25 % trypsin, centrifuged at 1200 rpm, 4 0C for 5-10 min and counted with a CEDEX (Roche) cell counter. HTFs of less than passage 5 were used. Experimental groups were designed as follows: Control: Complete medium only; travatan, lumigan, xalatan; oftagen, glokoprost; timolol, timosol; Tomec, Cosopt, Azarga; Duotrav, Xalacom, Ganfort; Alphagan, Combigan, Azopt. Primary cells were treated treated for 5 or 10 min. After then, cells were washed in PBS and maintained in DMEM for 48 hrs before analyses. Cellular survival in the presence or absence of the drugs was determined by 3-3-(4,5-D-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide thyazolyl blue (MTT) assay. Data were expressed as the mean percent fraction of control ± standard error of mean and statistical significance ascertained by one way analysis of variance followed by Tukey's multiple comparison test. All results are the mean of at least three independent assays and the p value less than 0.05 was considered to be significant.
Results: Cell viabilities after treatment with topical antiglaucoma drops at 5 and 10 minutes were significantly reduced in all experimental groups except Travatan, Duotrav and Alphagan treated cells. This effect might be due to having less preservative in these agents.
Conclusions: Almost all topical antiglaucoma medications have deleterious effects on ocular surface. Newer topical antiglaucoma agents with less preservative or preservative free agents are needed to improve ocular surface side effects. Further studies are needed to evaluate the effect of these agents on ocular surface.

IS SHORT BUT A DIFFERENT TYPE OF DRY EYE DISEASES, OR MERELY AN EARLY STAGE OF AQUEOUS DEFICIENCY? DIFFERENT TYPE

Norihiko Yokoi. Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan
In this debate, I will argue that short breakup time dry eye (SBUTDE) is indeed a unique type of dry eye disease. I will present reasons connected to tear volume, reflex tear secretion, severity of symptoms, degree of ocular surface epithelial damage, and pattern/area of tear film breakup. In cases of SBUTDE, unlike those of aqueous tear deficient dry eye (ATDDE), the tear volume is generally within the normal range. However, ATDDE is characterized by decreased tear volume. Similarly, reflex tear secretion is also close to normal in SBUTDE, whereas ATDDE is again characterized by a decrease in reflex tear secretion. Even though SBUTDE is not

associated with severe corneal damage, it is accompanied by relatively severe symptoms of discomfort and visual disturbance. In ATDDE cases, when such severe symptoms occur, they are associated with severe damage to the ocular surface epithelium. In SBUTDE cases, the short BUT results in minimal ocular surface damage and conjunctival surface damage is rarely if ever seen. This is in contrast to ATDDE, in which a greater degree of conjunctival surface damage than corneal epithelial damage is often observed. In addition, SBUTDE is differentiated from ATDDE in terms of its pattern and area of tear-film breakup. In SBUTDE, clinicians observe undetermined patterns of breakup before the establishment of the precorneal tear film or circular patterns of breakup (spot break; which occurs instantaneously at eye opening) often appearing in the relatively central part of the cornea. In general, the area of breakup is not associated with corneal damage. In contrast, ATDDE is characterized by a linear pattern of breakup (line break), generally appearing in the lower part of the cornea and generally associated with corneal epithelial damage within the breakup region. In closing, I would like to introduce SBUTDE, a unique form of dry eye which is widely recognized in Japan, and present the detailed characteristics of this unique disease.
The author has no financial interests to disclose.

EFFECT OF TEAR FILM INSTABILITY ON HIGH ORDER ABERRATIONS OF THE CORNEAL SURFACE AFTER LASER SUBEPITHELIAL KERATOMILEUSIS.

Kyung Chul Yoon, Hyun Ho Jung, Han Jin Oh. Department of Ophthalmology, Chonnam National University Medical School and Hospital, Gwangju, Korea

Purpose: To evaluate the influence of tear film instability on higher order aberrations (HOAs) of the corneal surface after laser subepithelial keratomileusis (LASEK).

Methods: Thirty-one patients who underwent LASEK were divided into the dry eye (16 patients, 32 eyes) and the non-dry eye groups (15 patients, 30 eyes). Best-corrected visual acuity (BCVA), spherical equivalent (SE) refraction, ablation depth, tear film parameters and Ocular Surface Disease Index (OSDI) questionnaire were evaluated in both groups. Total HOA root mean square (RMS), third-order coma, third-order trefoil and fourth-order spherical aberration (SA) of the corneal surface immediately and at 10 seconds after blinking were measured using Pentacam (Oculus, Inc.).

Results: In the dry eye group, total HOA RMS, coma and trefoil significantly increased except for SA at 10 seconds after blinking compared with those measured immediately after blinking. In the dry eye group, the changes of total HOA RMS, coma and trefoil were correlated negatively with TBUT (R = -0.420, P = .026; R = -0.473, P = .011; R = -0.439, P = .020, respectively) but were correlated positively with OSDI score (R = 0.433, P = .022; R = 0.499, P = .017; R = 0.532, P = .010, respectively).

Conclusions: In patients after LASEK, the tear film instability can be associated with a significant increase of total HOA RMS, coma and trefoil. Furthermore, it may cause a reduction in optical quality.

[Commercial interests or grant support: none]

THE ROLE OF SECRETED FRIZZLED-RELATED PROTEIN 1 (SFRP1) IN CORNEAL EPITHELIUM

Jingjing You[1], Li Wen[1], Michele C Madigan[2], Gerard Sutton.[1,3] 1. Save Sight Institute, University of Sydney, Australia, 2. School of Optometry and Vision science, University of New South Wales, Australia, 3. Vision Group, Australia

Purpose: SFRP1 is associated with cell proliferation, migration and apoptosis in various cell types [1]. We previously detected higher extracellular (in tears) and lower intracellular (in cornea) SFRP1 in control compared to keratoconus samples [2, 3]. The expression and function of SFRP1 in human corneal epithelium is however not fully understood. In this study, we investigated the effects of exogenous SFRP1 on human corneal epithelial cell proliferation and its anti-oxidative potential.

Method: Primary limbal corneal epithelial cells (HCE-P) were cultured from human corneas (Lions NSW Eye Bank). Immunolabeling for corneal epithelial cell markers (CK-3, CK-19, vimentin and p63) was used to characterise the primary cells. Proliferation of cells treated with and without SFRP1 (0.5, 1 and 5 µg/mL) was assessed using Alamar blue assays (16 hrs post-treatment). SFRP1 anti-oxidative potential was evaluated by measuring proliferation on HCE-P cells treated with H_2O_2 only (500 µM), and with H_2O_2 (500 µM) + SFRP1 (1 µg/mL).

Result: HCE-P cells showed increased proliferation compared to untreated cells, in a dose-dependent fashion. HCE-P cell proliferation increased ~1-, 1.2- and 1.3-fold for cells treated with 0.5 µg/mL, 1 µg/mL and 5 µg/mL SFRP1 respectively, compared to untreated cells. Furthermore, we noted significantly increased cell viability ($p = 0.04$) in cells treated with H_2O_2 + SFRP1 (~88% cell viability), compared to cells exposed to H_2O_2 alone (~74% cell viability).

Conclusion: We found that SFRP1 can promote HCE-P cell proliferation and partially protect cells from H2O2 oxidative stress. As a naturally expressed protein in human cornea, SFRP1 could be important for maintaining corneal homeostasis and in corneal epithelial wound healing.

1. Esteve P, Bovolenta P: *Tohoku J Exp Med* 2010, 221(1):11-17.
2. You J *et al*: *Mol Vis* 2013, 19:509-515.
3. Sutton G *et al*: *Clin Experiment Ophthalmol* 2010, 38(1):43-48.

[This research is supported by the Sydney Eye Hospital Foundation, Sydney Foundation for Medical Research, Sydney Medical Foundation and Sydnovate, University of Sydney, Australia]

EXPERIENCE FROM RUNNING THE FIRST DRY EYE CLINIC IN POLAND.

Zbigniew Zagórski, Katarzyna Molenda, Agnieszka Kudasiewicz-Kardaszewska, Marta Piecyk-Sidor. Zagórski Eye Surgery Centers in Cracow and Lublin.

Purpose: to present the results of the first 100 patients examined at the dry eye clinic opened earlier this year in Cracow. The purpose of the clinic is to introduce recommendations of the DEWS and MGD Workshops into clinical practice, which was not practiced in outpatient clinics in Poland. The main reasons were lack of experienced ophthalmologists and necessary equipment as well as time shortage in busy general clinics.

Methods: At the beginning we dedicated one and later two afternoons to deal with dry eye and MGD patients only. Our equipment includes Oculus Keratograph M5, MG evaluator acc. to Korb, HS BQ slit lamp with photo and video attachment, HRT III confocal attachment for Meibomian gland evaluation and instruments for in-office gland expression, including Maskin MG probes. Specially trained ophthalmologists and technicians are involved in diagnostic procedures carried out in the following order: OSDI questionnaire and index, Interblink interval, Lower tear meniscus height, Non-invasive tear break up time and ocular surface protection index, Eye redness degree (Optional), Corneal and conjunctival staining score (Oxford scale), Schirmer Test (Optional), Standardized MG expression (Korb), Meibography, Confocal microscopy of MG (Optional).

Results: Separation of dry eye patients allows performing planned procedures without disturbing regular schedule of general ophthalmology clinic. Appropriate treatment in concordance with the guidelines of DEWS and MGD workshops is recommended according to the results of the diagnostic tests. Correlations between test results and symptoms are analyzed before and during the treatment.

Conclusion: Separate clinic for dry eye patients requiring specialized and time consuming diagnostic and therapeutic procedures can significantly improve the management level.

The authors have no commercial relationship to disclose.

TEAR CYTOKINE PROFILES AND THEIR CLINICAL CORRELATIONS WITH OCULAR SURFACE PARAMETERS OF DIFFERENT AGES

Hua Zhu,[1,2,3] Nisha Yeotikar,[1] Negar Babaei,[1] Amali Ariyavidana,[1] Maria Markoulli,[2] Daniel Tilia,[1] Brien Holden,[1,2,3] Eric Papas[1,2,3] [1]Brien Holden Vision Institute, [2]School of Optometry and Vision Science, University of New South Wales, [3]Vision CRC, Sydney, Australia

Purpose: Inflammatory mediators have been associated with the pathogenesis of ocular surface diseases including dry eye disease. The aim of the study was to assess tear cytokine levels and their clinical correlations with ocular surface parameters in normal/asymptomatic subjects of different ages.

Methods: One hundred and sixteen healthy subjects (67 females and 49 males), 64 aged 25-44 yr and 52 aged 45-65 yr, were recruited. Tear samples were collected from the right eye using a microcapillary tube after flushing with 60 µL of sterile saline. Profiles of 27 cytokines in each sample were assessed using a Bio-Plex assay kit (Bio-Rad) then normalized by the total protein concentration, measured with a BCA assay kit, to levels of cytokines per unit of protein. Clinical parameters, including meibomian gland functions, phenol red thread test, tear film break-up time, and corneal and conjunctival fluorescein staining, were evaluated. Correlations between cytokine levels and clinical parameters were determined by Spearman ranked correlation coefficient. Levels of cytokines between two independent sample groups were compared using nonparametric Mann Withney U test.

Results: There was a mild correlation between age and levels of IL-1 ($r = 0.211$, $p = 0.023$), IL-7 ($r = 0.248$, $p = 0.007$), IL-8 ($r = 0.230$, $p = 0.013$), IL-12p ($r = 0.32$, $p = 0.0001$), and IL-13 ($r = 0.205$, $p = 0.027$). The levels of cytokines IL-7, IL-12p and IL-13 were significantly higher in the older age group than the younger group ($p < 0.05$). The tear volumes tested with phenol red thread correlated inversely with the levels of IL-1 ($r = -0.20$, $p = 0.035$), IL-1b ($r = -0.21$, $p = 0.026$), IL-7 ($r = -0.20$, $p = 0.031$), and IL-8 ($r = -0.31$, $p = 0.001$). TBUT showed a mild positive correlation with levels of cytokines Eotaxin ($r = 0.264$, $p = 0.004$), IL-15 ($r = 0.245$, $p = 0.008$), and RANTES ($r = 0.239$, $p = 0.01$).

Conclusion: The levels of tear cytokines change with age and some clinical ocular surface parameters. Certain cytokines may be associated with clinical signs of dry eye.

TREATMENT OF OCULAR ROSACEA WITH LOW DOSE DOXYCYCLINE.

Manfred Zierhut[1], Deshka Doycheva[1], Christoph Deuter[1], Ines Pfeffer[2], Martin Schaller[2], Bianca Sobolewska[1], [1]Center for Ophthalmology, [2]Department of Dermatology, University Tuebingen, Germany

Purpose: To determine the efficacy of once-daily systemic treatment with doxycycline 40 mg in a slow-release form.

Material and Methods: Fifteen patients with ocular rosacea were enrolled in a retrospective observational case series. Patient complaints and clinical findings were evaluated. The mean duration of treatment was eight months (range 5-12 months).

Results: At the baseline visit, 73.3% of patients had severe complaints and 80% severe blepharitis. After 12 weeks of systemic therapy, severe complaints and blepharitis were seen in only 13.3% and 20% of the patients ($P = 0.01$). Follow-up investigations 6-17 months after discontinuation of the treatment showed further significant improvement of complaints.

Conclusions: Anti-inflammatory dose of slow release doxycycline 40mg daily is an effective and safe therapy of ocular rosacea.

NOTE

NOTE

7th International Conference on the Tear Film & Ocular Surface:
Basic Science and Clinical Relevance, Taormina 2013

Sessions

Translating tear film and ocular surface protein discoveries into treatments

Cutting-edge surgery: induction and treatment of ocular surface disease

New and emerging clinical endpoints for ocular surface disease

Macro to micro: imaging approaches for understanding the ocular surface

Meibomian gland dysfunction: update

Ocular surface senescence: programmed or preventable?

Innate and adaptive immunity: for whom the bell tolls

Microbiome: role in inflammation and infectious disease

New paradigms in understanding corneal sensation and pain

Corneal homestasis, repair and regeneration

Prime time: TFOS debates

TFOS workshop on contact lens discomfort: reports

Register at www.tfos2013.org

tfos

Sponsored by Alcon. Also sponsored by Allergan, Bausch & Lomb, Santen, SIFI, TearLab, Vistakon, Horus, Novaliq, TearScience, NicOx, SOOFT, Otsuka, AVR, Iris Pharma, Senju, Menicon, Eleven Biotherapeutics and EyeGate.