6th International Conference on the Tear Film & Ocular Surface: Basic Science and Clinical Relevance

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6th International Conference on the Tear Film and Ocular Surface: Basic Science and Clinical Relevance

Conference Program & Abstract Book

Florence, Italy

September 22-25, 2010

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Preface

During the past several decades, a significant, international research effort has been directed towards understanding the composition, function and regulation of the preocular tear film. This effort has been 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 has been stimulated by the knowledge that alteration or deficiency of the tear film, which occurs in innumerable individuals throughout the world, may lead to desiccation of the ocular surface, ulceration and perforation of the cornea, an increased incidence of infectious disease, and potentially, pronounced visual disability and blindness.

To promote further progress in this field of vision research, the 6th International Conference on the Tear Film & Ocular Surface: Basic Science and Clinical Relevance will be held at the Firenze Fiera, Florence, Italy, from September 22 to 25, 2010. This Conference, which is sponsored by the Tear Film & Ocular Surface Society (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 companies with an interest in the treatment of tear film or ocular surface disorders.

To help achieve this objective, numerous scientists, clinicians and industry representatives from many countries, including Argentina, Australia, Austria, Belgium, Brazil, Bulgaria, Canada, China, Croatia, Czech Republic, Finland, France, Germany, Greece, Hong Kong, Hungary, India, Italy, Japan, Netherlands, New Zealand, Singapore, Slovenia, South Korea, Spain, Sweden, Switzerland, Thailand, Turkey, United Arab Emirates, United Kingdom and the United States have registered as active 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

Acknowledgments

The Tear Film & Ocular Surface Society expresses its appreciation to Sabrina Zappia and CITYNet (www.citynetonline.it), Julie Karimi and Jaka Congressi (www.jaka.it), Haydée Marangoni and h.design, and the Managers of Firenze Fiera, Lungarno Hotels, Il Convivium Firenze and Context Travel for their assistance with, and/or contributions to, this Conference.

Recognition

The Tear Film & Ocular Surface Society congratulates the following individuals, who were the recipients of the Conference Travel Awards: Philipp Ackermann, Danielle Augustin, Colin Cerretani, Laura Contreras-Ruiz, Thomas Fuchsluger, Fabian Garreis, Anna Guzman-Aranguez, Maria Markoulli, Maryam Mokhtarzadeh, James Mun, Kyung-Sun Na, Jose Ricardo, Stefan Schrader, Yuichi Uchino and Eric Xiaojia Wei.

THURSDAY, SEPTEMBER 23, 2010

Opening Remarks

8:00 *Stefano Bonini (Italy)*

Claes H. Dohlman Conference Address

Chairperson – Stefano Bonini (Italy)

8:05 HEALTH AND DISEASE OF THE OCULAR SURFACE. Shigeru Kinoshita. Kyoto Prefectural University of Medicine, Kyoto, Japan

Session I

Sugar Can Be Good for You: Glycobiology & Mucins

Chairpersons - Pablo Argueso (USA), Monica S Berry (UK) & Anna Guzman-Aranguez (Spain)

8:30 **Keynote Address:** REPROGRAMMING OF CELLULAR TRANSCRIPTION BY SIGNALING THROUGH MUC1. Michael A. Hollingsworth, Michelle E. Behrens, Samuel J. Erb. Eppley Institute for Research in Cancer, Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE, USA

8:55 **Keynote Address:** ROLE OF SPDEF IN PULMONARY GOBLET CELL DIFFERENTIATION. Tom Korfhagen, Perinatal Institute, Section of Neonatology, Perinatal and Pulmonary Biology, Cincinnati Children's Hospital, Cincinnati, Ohio

9:20 TEAR FILM: EPITOPES FACING THE OUTSIDE WORLD. Sarah Baos[1,2], Terence McMaster, David Phillips, Monica Berry[1] [1]HH Wills Physics Laboratory and [2]Academic Unit of Ophthalmology, University of Bristol, Bristol, UK

9:35 CELL SURFACE MUCIN O-GLYCANS IMPAIR NANOPARTICLE DELIVERY TO CORNEAL EPITHELIAL CELLS. A. Guzman-Aranguez,[1] J. Pintor,[1] P. Argüeso.[2] [1]Department of Biochemistry, School of Optics, Complutense University, Madrid, Spain; [2]Schepens Eye Research Institute and Department of Ophthalmology, Harvard Medical School, Boston, MA, USA

9:50 THE MECHANISM OF MUCIN SECRETION FROM ISOLATED RABBIT CONJUNCTIVAL TISSUE BY DIQUAFOSOL. Yuko-Takaoka Shichijo, Tadahiro Murakami, Atsuyoshi Dota, Katsuhiko Shinomiya, Osamu Katsuta, Masatsugu Nakamura. Research and Development Division, Santen Pharmaceutical Co., Ltd., Nara-Osaka, Japan

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

International Dry Eye WorkShop: Updates

Chairpersons - Donald R. Korb (USA), Kyung-Sun Na (South Korea) & John E. Sutphin (USA)

10:40 **Keynote Address:** RECENT ADVANCES IN DEFINING AND CLASSIFYING DRY EYE DISEASE. A. J Bron. Nuffield Laboratory of Ophthalmology, University of Oxford, UK

10:55 **Keynote Address:** INTERNATIONAL DRY EYE WORKSHOP: UPDATE ON THE EPIDEMIOLOGY OF DRY EYE. Kelly K. Nichols, OD, MPH, PhD Ohio State University College of Optometry, Columbus, OH, USA

11:10 **Keynote Address:** METHODOLOGIES TO DIAGNOSE AND MONITOR DRY EYE DISEASE. Murat Dogru, M.D, Ph.D. Johnson and Johnson Ocular Surface and Visual Optics Department, Keio University School of Medicine, Tokyo, Japan

11:25 **Keynote Address:** UPDATE ON THE DEWS REPORT: THERAPY AND MANAGEMENT. Michael A. Lemp, Georgetown University

11:40 **Keynote Address:** ADVANCES IN THE DESIGN AND CONDUCT OF CLINICAL TRIALS IN DRY EYE DISEASE. Gary N. Foulks, MD, FACS. Kentucky Lions's Eye Center, Lexington, KY, USA

11:55 **Keynote Address:** DEWS WORKSHOP UPDATE: CLINICAL AND BASIC RESEARCH IN DRY EYE. Ilene K. Gipson. Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School, Boston MA, USA

12:10 **Poster Viewing & Lunch**

Poster Discussion I

Chairpersons - Philipp Ackermann (Germany), Thomas J. Millar (Australia) & Friedrich P. Paulsen (Germany)

13:40 REFRACTIVE SURGERY ALTERS CONJUNCTIVAL GOBLET CELLS IN PATIENTS WHO DEVELOP DRY EYE. M Shatos[1], D Ryan[2], K Bower[2], C Coe[2], L Peppers[2] E Guilbert[1], J Doherty[1], R Hodges[1], D Dartt[1] [1]Opthal/Harvard Med Sch, Schepens Eye Research Institute, Boston, MA;[2] Walter Reed Army Medical Center, Washington, DC

13:45 LUBRICIN AS AN OCULAR SURFACE-CONTACT LENS BOUNDARY LUBRICANT: DOSE-DEPENDENT & SYNERGISTIC EFFECTS. S. Morrison[1], B. Snider[1], B.D. Sullivan[2], E. Truitt III[3], D.A. Sullivan[4], T. Schmidt[1] [1] University of Calgary, Calgary, Canada; [2] TearLab Corp., San Diego, CA; [3] Singularis, Inc., San Diego, CA; [4] Schepens Eye Research Institute and Harvard Medical School, Boston, MA

13:50 PHOSPHOLIPIDS IN TEARS, CONTACT LENSES AND MEIBUM? Jennifer T. Saville[1], Zhenjun Zhao[2], Mark D.P. Willcox[2,3], Todd W. Mitchell[4] and Stephen J. Blanksby[1]. [1]School of Chemistry and [4]School of Health Sciences, University of Wollongong, NSW 2052, [2]Brien Holden Vision Institute and [3]School of Optometry and Vision Science, University of New South Wales, NSW 2052, Australia

13:55 A NEW MOUSE MODEL OF DRY EYE DISEASE (*Tet-mev-1* Mice) : OXIDATIVE STRESS AFFECT FUNCTIONAL DECLINE IN LACRIMAL GLAND. Yuichi Uchino,[1,2,3] Tetsuya Kawakita,[2] Masaki. Miyazawa,[3] Takamasa Ishii,[3] Hiromi Onouchi,[3] Kayo Yasuda,[3] Shigeto Shimmura,[2] Naoaki Ishii[3,] Kazuo Tsubota[2]. Ophthalmology, Tokyo Electric Power Company Hospital[1], Ophthalmology, Keio University School of Medicine,[2] Tokyo, Japan, Molecular Life Science, Tokai University School of Medicine,[3] Kanagawa, Japan

International Meibomian Gland Dysfunction Workshop: Reports

Chairpersons - Colin Cerretani (USA), James P. McCulley (USA) & Eric B. Papas (Australia)

14:00 **Introduction:** TEAR FILM & OCULAR SURFACE SOCIETY: A REPORT FROM THE INTERNATIONAL WORKSHOP ON MEIBOMIAN GLAND DYSFUNCTION. Kelly K. Nichols. Ohio State University, College of Optometry, Columbus, OH, USA

14:05 **Keynote Address:** DEFINITION & CLASSIFICATION OF MEIBOMIAN GLAND DYSFUNCTION. J. Daniel Nelson. Health Partners Medical Group, Minneapolis, MN, USA

14:20 **Keynote Address:** ANATOMY, PHYSIOLOGY & PATHOPHYSIOLOGY OF THE MEIBOMIAN GLAND. Erich Knop. Eye Clinic Research Laboratory, Charite-Univ Med, Berlin, Germany

14:35 **Keynote Address:** TEAR FILM LIPIDS, AND LIPID-PROTEIN INTERACTIONS IN HEALTH AND DISEASE. Ben J. Glasgow. Jules Stein Eye Institute UCLA, Ophthalmology, Los Angeles, CA, USA

14:50 **Keynote Address:** EPIDEMIOLOGY OF, AND RISK FACTORS FOR, MEIBOMIAN GLAND DYSFUNCTION. Debra A. Schaumberg. Harvard Medical School, Brigham Womens Hospital, Boston, MA, USA

15:05 **Keynote Address:** EVALUATION, DIAGNOSIS & GRADING OF SEVERITY OF MEIBOMIAN GLAND DYSFUNCTION. Alan Tomlinson. Glasgow Caledonian University Vis Sci, Glasgow, Scotland, UK

15:20 **Keynote Address:** MANAGEMENT & THERAPY OF MEIBOMIAN GLAND DYSFUNCTION. Gerd Geerling. Department of Ophthalmogy, University of Wuerzburg, Wuerzburg, Bavaria, Germany

15:35 **Keynote Address:** DESIGN & CONDUCT OF CLINICAL TRIALS. Penny Asbell. Mount Sinai Medical Center, Ophthalmology, New York City, NY, USA

15:50 **Poster Session I (with Coffee & Tea)**

The Tearome: Not Just Hype

Chairpersons – Darlene A. Dartt (USA), Gordon Laurie (USA) & James Mun (USA)

16:25 **Keynote Address:** EICOSANOIDS IN THE OCULAR SURFACE AND TEAR FILM. Michal L. Schwartzman. Departments of Pharmacology & Ophthalmology, New York Medical College, Valhalla, New York, USA

16:50 **Keynote Address:** RAB GTPASES IN REGULATED SECRETION AND DISEASE. Miguel C. Seabra. Molecular Medicine, Imperial College London, London, UK; Faculdade de Ciencias Medicas, Universidade Nova de Lisboa, Portugal; and Instituto Gulbenkian de Ciência, Portugal

17:15 **Keynote Address:** ANTI-AGING APPROACH FOR THE TREATMENT OF DRY EYE. Kazuo Tsubota[1], Motoko Kawashima[1], Takaaki Inaba[1], Murat Dogru[1], Yoko Ogawa[1], Shigeru Nakamura[1], Ken Shinmura[2], Akihiro Higuchi[1], Tetsuya Kawakita.[1] Department of Ophthalmology[1], Department of Internal Medicine[2], Keio University School of Medicine, Tokyo, Japan

17:40 COLD-SENSITIVE CORNEAL AFFERENTS IMPLICATED IN BASAL TEAR PRODUCTION, TRPM8 NERVE MEMBRANE RECEPTORS, AND DRY EYE DISEASE. Harumitsu Hirata and Michael L. Oshinsky. Department of Neurology, Thomas Jefferson University, Philadelphia, PA, USA

17:55 MESENCHYME/EPITHELIUM INTERACTION IN EYELID AND MEIBOMIAN GLAND MOPHORGENESIS. Winston W-Y Kao, Yujin Zhang, Chia-Yang Liu and Mindy K. Call. Edith J. Crawley Vision Research Center, Department of Ophthalmology, University of Cincinnati

18:10 – 19:10

Poster Session I (with Wine & Hors d'oeuvres)

Chairpersons - Philipp Ackermann (Germany), Thomas J. Millar (Australia) & Friedrich P. Paulsen (Germany)

1 CHARACTERIZATION OF MUCIN-TYPE GLYCOPROTEINS IN MARINE MAMMAL TEARS. Robin Kelleher Davis,[1,2] Pablo Argueso.[1,2] Schepens Eye Research Institute,[1] Harvard Medical School,[1,2] Boston, MA, USA

2 DETECTION OF TEAR GLYCOPROTEINS AND GLYCOSYLATION MOITIE.S P. Ramamoorthy, J.J. Nichols College of Optometry, The Ohio State University

3 OCULAR SURFACE MUCINS IN ADULTS WITH CYSTIC FIBROSIS. Katharine Evans[1], Rachel North[1], Christine Purlsow[1], Monica Berry[2]. School of Optometry & Vision Sciences, Cardiff University[1]; Academic Unit of Ophthalmology, University of Bristol, Bristol Eye Hospital[2], UK

4 IMPACT OF DIFFERENT CONTACT LENS MATERIALS ON MUCIN FRAGMENTATION: RELATION TO SYMPTOMS. M. Berry[1], Paul Murphy[2], Christine Pursolow[2], H. Pult[2,3]; [1]Academic Unit of Ophthalmology, University of Bristol, Bristol Eye Hospital, Bristol, United Kingdom; [2]Contact Lens and Anterior Eye Research (CLAER) Unit, School of Optometry and Vision Sciences, Cardiff University, Cardiff, United Kingdom. [3]Optometry and Vision Research, Weinheim, Germany

5 REGULATION OF GOBLET CELL DIFFERENTIATION IN THE CONJUNCTIVA: THE ROLE OF THE TRANSCRIPTION FACTOR, SPDEF. Ilene K Gipson[1], Albert Alhatem[1] Gang Chen[2]. Jeffrey Whitsett[2], and Hans Clevers[3] [1]Schepens. Eye Research Institute, Harvard Medical School, Boston MA, [2]Cincinnati Children's Hospital Medical Center, University of Cincinnati School of Medicine, Cincinnati, OH and [3] Hubrecht Institute, Utrecht, The Netherlands

6 UNCHANGED GOBLET CELL COUNTS AND EPITHELIAL METAPLASIA IN SEASONAL ALLERGIC CONJUNCTIVITIS OUTSIDE THE POLLEN SEASON. Amarilla Veres, Krisztina Kosina-Hagyó, János Németh INSTITUTIONS:Semmelweis University, Dept. of Ophthalmology

7 **Discussion:** REFRACTIVE SURGERY ALTERS CONJUNCTIVAL GOBLET CELLS IN PATIENTS WHO DEVELOP DRY EYE. M Shatos[1], D Ryan[2], K Bower[2], C Coe[2], L Peppers[2] E Guilbert[1], J Doherty[1], R Hodges[1], D Dartt[1] [1]Opthal/Harvard Med Sch, Schepens Eye Research Institute, Boston, MA;[2] Walter Reed Army Medical Center, Washington, DC

8 RESOLVINS RVD1 AND THE ASPIRIN-TRIGGERED RESOLVIN 17 (R)-RVD1 BLOCK HISTAMINE-STIMULATED INCREASE IN CA^{2+} AND ACTIVATION OF EXTRACELLULAR REGULATED KINASE (ERK)1/2 TO PREVENT CONJUNCTIVAL GOBLET CELL SECRETION Dartt DA[1, 3], Li D[1, 3], Hodges RR[1, 3], Shatos M[1, 3], and Serhan CN[2,] INSTITUTIONS:[1]Schepens Eye Research Institute, [2]Brigham and Womens Hospital, [3]Harvard Medical School, Boston, MA

9 NONCLINICAL PHARMACOLOGY, OCULAR DISTRIBUTION, AND SAFETY OF MIM-D3, A NOVEL NGF MIMETIC FOR THE TREATMENT OF DRY EYE. Karen Meerovitch, Teresa Lama and Garth Cumberlidge. Mimetogen Pharmaceuticals Inc. Montreal, Quebec, Canada

10 ISOLATION, CULTURE OF MOUSE LACRIMAL GLAND EPITHELIAL CELLS. Tetsuya Kawakita,[1] Shinya Kobayashi,[1] Motoko Kawashima,[1] Naoko Okada,[1] Kenji Mishima,[2] Masataka Ito,[3] Ichiro Saito,[2] Shigeto Shimmura,[1] Kazuo Tsubota[2]. [1] Department of Ophthalmology, Keio University School of Medicine,[2] Department of Pathology, Tsurumi University,[3] Department of Anatomy, National Defense University,[3] Japan

11 **Discussion:** A NEW MOUSE MODEL OF DRY EYE DISEASE (*Tet-mev-1* Mice): OXIDATIVE STRESS AFFECT FUNCTIONAL DECLINE IN LACRIMAL GLAND. Yuichi Uchino,[1,2,3] Tetsuya Kawakita,[2] Masaki. Miyazawa,[3] Takamasa Ishii,[3] Hiromi Onouchi,[3] Kayo Yasuda,[3] Shigeto Shimmura,[2] Naoaki Ishii[3,] Kazuo Tsubota[2]. Ophthalmology, Tokyo Electric Power Company Hospital[1], Ophthalmology, Keio University School of Medicine,[2] Tokyo, Japan, Molecular Life Science, Tokai University School of Medicine,[3] Kanagawa, Japan

12 EVALUATION OF LIPID OXIDATIVE STRESS STATUS IN DRY EYE DISEASE. Tais H. Wakamatsu[1AB],Murat Dogru[1A,2],Yukihiro Matsumoto[1AB],Takashi Kojima[1AB],Minako Kaido[1AB],Osama M.A. Ibrahim[1AB],Ayako Igarashi[2],Enrique A. Sato[1AB],Yoshiyuki Ichihashi[1B],Jun Shimazaki[2]and Kazuo Tsubota[1B] [A]J&J Ocular Surface and Visual Optics, [B]Ophthalmology, [1]Keio University School of Medicine, Tokyo, Japan;[2]Ophthalmology, Tokyo Dental College, Chiba, Japan

13 MAINTENANCE EFFECT OF EXPERIMENTAL DRY EYE AFTER DEPRIVATION OF DESICCATING STRESS IN C57BL/6 MICE. Kyung-Chul Yoon Department of Ophthalmology, Chonnam National University Medical School and Hospital

14 ADENOSINE A2A RECEPTOR UP-REGULATION IN THE MALE NOD MOUSE DRY EYE MODEL. Stina K. Carlsson[1], Daniel Diez[2], Sarah F. Hamm-Alvarez[2], Kai-Jin Wu[2] and J. Peter Gierow.[1] School of Natural Sciences, Linnaeus University, Kalmar, Sweden[1] Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, School of Pharmacy, Los Angeles, USA[2]

15 CHANGES OF ION TRANSPORTERS AND AQUAPORINS IN RABBIT LACRIMAL ACINI AND DUCTS DURING PREGNANCY. Chuanqing Ding[1], Michael Lu[1], Yanru Wang[2] Cell & Neurobiology[1], Physiology & Biophysics[2], University of Southern California, Los Angeles, CA 90089, USA

16 $P2X_7$ RECEPTORS INTERACT WITH a_{1D}-ADRENERGIC AND MUSCARINIC RECEPTORS IN RAT LACRIMAL GLAND ACINI. Robin R. Hodges and Darlene A. Dartt, Schepens Eye Research Institute; Department of Ophthalmology, Harvard Medical School, Boston, MA.

17 CROSSING OF PATHS: A DEFECT IN A MAJOR REGULATORY PROTEIN OF THE SECRETORY PATHWAY INCREASES DEGRADATIVE PATHWAY ACTIVITY. Lilian Chiang[1], Tanya Tolmachova[2], Alistair N. Hume[2], Joel Schechter[3], Miguel C. Seabra[2], Sarah Hamm-Alvarez[1,3] University of Southern California [1]School of Pharmacy, [3]Keck School of Medicine , Los Angeles CA, USA, [2]Cell and Molecular Biology, Division of Biomedical Sciences, Faculty of Medicine, Imperial College, London

18 EVALUATING BIOCHEMICAL PATHWAYS IN THE MEIBOMIAN GLAND Thomas J. Millar[1]and Frank Schirra[2] School of Natural Sciences, University of Western Sydney, Australia,[1] Klinik für Augenheilkunde, Universitätsklinikum des Saarlandes, Homburg/Saar, Deutschland[2]

19 MORPHOGENESIS OF THE MOUSE MEIBOMIAN GLAND. Mindy K. Call[1], Chyong Jy Nien[2], James V. Jester[2], Winston W-Y Kao.[1] [1]Edith J. Crawley Vision Research Center, University of Cincinnati, Cincinnati, OH, USA. [2]Gavin Herbert Eye Institute, University of California Irvine, CA, USA

20 DO MARINE MAMMALS HAVE A UNIQUE TYPE OF MEIBOMIAN GLAND? Nadja Knop,[1] Erich Knop, [1] Robin Kelleher Davis.[2,3] Research Laboratory, Dept of Ophthalmology CVK, Charité - Universitätsmedizin Berlin, Germany; [1] Schepens Eye Research Inst, [2] Harvard Medical School, [2,3] Boston, MA, USA

21 VASOACTIVE INTESTINAL PEPTIDE ACTIVATES THE ADENYLYL CYCLASE PATHWAY IN HUMAN MEIBOMIAN GLAND EPITHELIAL CELLS. Wendy Kam and David A. Sullivan, Schepens Eye Research Institute and Harvard Medical School, Boston, MA, USA

22 REGULATION OF THE PROLIFERATION AND DIFFERENTIATION OF HUMAN MEIBOMIAN GLAND EPITHELIAL CELLS. Shaohui Liu and David A Sullivan, Schepens Eye Research Institute and Harvard Medical School, Boston, MA, USA

23 DIFFERENCES IN MEIBOMIAN GLAND PHYSIOLOGY BETWEEN PRE- AND POST-MENOPAUSAL WOMEN. Tomo Suzuki[1,2], NorihikoYokoi[2], Aoi Komuro[2], and Shigeru Kinoshita[21]Department of Ophthalmology, Kyoto City Hospital, Kyoto, Japan; [2]Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan

24 A SOLUTE GRADIENT IN THE TEAR MENISCUS TO EXPLAIN MARX'S LINE, ITS FORWARD MIGRATION AND MEIBOMIAN GLAND DYSFUNCTION – A NEW HYPOTHESIS. AJ Bron[1], N Yokoi [2], E.A.Gaffney [3], JM Tiffany [1]..[1]. Nuffield Laboratory of Ophthalmology, University of Oxford, UK; [2]. Department of Ophthalmology, Kyoto Prefectural University of Medicine, Japan; [3]. Mathematical Institutes, University of Oxford, UK.

25 HYPERLIPIDEMIA - A PREDISPOSING FACTOR TO MEIBOMIAN GLAND DYSFUNCTION. Souhad Lawand. MD, Ph.D Zulekha hospital, Ophthalmology department. UAE – Sharjah

26 PREVALENCE OF NON-OBVIOUS MEIBOMIAN GLAND DYSFUNCTION (NOMGD) IN A DRY EYE STUDY. C.A. Blackie[1,2], D.R. Korb[1,2] [1]Korb Associates, Boston, MA; [2]TearScience, Morrisville, NC.

27 COMPARISON OF THREE CONTEMPORARY THERAPIES FOR THE MANAGEMENT OF MEIBOMIAN GLAND DYSFUNCTION. Jennifer P. Craig[1], Stuti Misra[1], Elizabeth Robinson[2] [1]New Zealand National Eye Centre, Department of Ophthalmology and [2]Department of Epidemiology and Biostatistics, University of Auckland, New Zealand

28 CLINICAL SAFETY STUDY OF A NOVEL EYELID WARMING DEVICE USING MOIST HEAT TECHNOLOGY. Felicity Gill, Paul Murphy, Christine Purslow School of Optometry & Vision Sciences, Cardiff University, UK

29 MANAGEMENT OF LID MARGIN DISEASES WITH BLEPHACLEAN. Michel Guillon, Cecile Maissa, Stéphanie Wong OTG Research and Consultancy, London UK.

30 BLEPHASTEAM®: A NOVEL EQUIPMENT TO TREAT MEIBOMIAN GLAND DYSFUNCTION (MGD). A CLINICAL AND LABORATORY STUDY. V Profazio, P. Versura, MG Tedeschi, C. Coslovi, M. Cellini, E C Campos Ophthalmology Unit, Alma Mater Studiorum University of Bologna

31 HUMAN TEARS AND MEIBUM LIPIDOMES: A ROADMAP FOR SUCCESSFUL ANALYSIS. Igor Butovich, Department of Ophthalmology, UT Southwestern Medical Center, Dallas, TX

32 DRY EYE AND HUMAN TEAR LIPID COMPOSITIONAL, CONFORMATIONAL AND FUNCTIONAL RELATIONSHIPS USING SPECTROSCOPY. Douglas Borchman, Gary N Foulks, Marta C Yappert. University of Louisville

33 CHARACTERISATION OF MEIBUM LIPIDS IN ASIANS WITH AND WITHOUT DRY EYE. Louis Tong,[1,2,3] Sin-Man Lam,[4] Shyam S Chaurasia,[1] Siew-Sian Yong,[1] Guanghou Shui,[4] Markus R Wenk[4] [1]Singapore Eye Research Institute, [2]Singapore National Eye Center, [3]Duke-NUS Graduate Medical School, [4]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore

34 **Discussion:** PHOSPHOLIPIDS IN TEARS, CONTACT LENSES AND MEIBUM? Jennifer T. Saville[1], Zhenjun Zhao[2], Mark D.P. Willcox[2,3], Todd W. Mitchell[4] and Stephen J. Blanksby[1]. [1]School of Chemistry and [4]School of Health Sciences, University of Wollongong, NSW 2052, [2]Brien Holden Vision Institute and [3]School of Optometry and Vision Science, University of New South Wales, NSW 2052, Australia

35 COMPARISON OF MASS SPECTROMETRY LIPID PROFILES USING VISUAL AND COMPUTER-BASED TECHNIQUES. Kelly K. Nichols, OD, MPH, PhD;[1] Jianzhong Chen, PhD,[2] Kari B. Green-Church, PhD[2] College of Optometry;[1] Mass Spectrometry and Proteomics Facility;[2] The Ohio State University, Columbus, OH, USA

36 MODELLING MEIBOMIAN LIPID FILM STRUCTURE USING X-RAY REFLECTIVITY. Chendur K. Palaniappan[1], Shiwani R. Raju[1], Michael James[2] and Thomas J. Millar[1] School of Natural Sciences, University of Western Sydney[1], Bragg Institute, Australian Nuclear Science and Technology Organisation, Sydney[2]

37 VISCOELASTICITY OF HUMAN MEIBOMIAN LIPID FILMS AT THE AIR-LIQUID INTERFACE. Shiwani R. Raju, Chendur K. Palaniappan and Thomas J. Millar. Schoolof Natural Sciences, University of Western Sydney, Australia

38 VISCOELASTIC AND STRUCTURAL CHANGES OF MEIBOMIAN LIPIDS WITH TEMPERATURE. Danielle L. Leiske,[1] Michelle Senchyna,[2] Howard A. Ketelson,[2] Gerald G. Fuller.[1] Stanford University, Stanford, CA,[1] Alcon Research, Ltd. Fort Worth, TX,[2] USA.

39 THE INFLUENCE OF HUMAN MEIBOMIAN LIPIDS ON THE WETTING PROPERTIES OF A DROPLET. Danielle L. Leiske,[1] Cécile Monteux,[2] Michelle Senchyna,[3] Howard A. Ketelson,[3] David Meadows,[3] Gerald G. Fuller.[1,]. Stanford University, Stanford, CA USA,[1] ESPCI, Paris, France,[2] Alcon Research, Ltd. Fort Worth, TX USA.[3]

40 HUMAN TEAR LIPID BREAKS UP BY DEWETTING C. Cerretani[1], C. J. Radke[1,2] [1]Chemical Engineering Department and [2]Vision Science Group University of California, Berkeley

41 TEAR EVAPORATION REDUCTION BY MODEL THIN OILY FILMS C. Cerretani[1], C.J. Radke1,[2] [1] Dept. of Chemical Engineering, Univ. of California, Berkeley, CA [2] Vision Science Group, Univ. of California, Berkeley, CA

42 THE EFFECT OF MEIBOMIAN LIPID FILMS ON EVAPORATION OF WHOLE TEARS *IN VITRO*. George H. Herok,[1,2] Shiwani R. Raju,[1] Thomas J. Millar[1,] School of Natural Sciences, University of Western Sydney[1], Department of Medical and Molecular Biosciences, University of Technology, Sydney[2]

43 IF TEAR EVAPORATION IS SO HIGH, WHY IS TEAR OSMOLARITY SO LOW? P. Ewen King-Smith[1], P. Ramamoorthy[1], K.K. Nichols[1], R.J. Braun[2], J.J. Nichols[1]. College of Optometry, The Ohio State University[1], Department of Mathematical Sciences, University of Delaware[2]

44 THE ROLE OF AQUEOUS TEAR EVAPORATION IN NORMALS AND PATIENTS WITH DRY EYE DISEASE. James P. McCulley, M.D., F.A.C.S, F.R.C. Ophth (U.K.) UT Southwestern Medical School

45 COMPUTATIONAL MODELING OF TEAR FILM DYNAMICS ON AN EYE-SHAPED DOMAIN. K.L. Maki,[1] R.J. Braun,[1] P. Ucciferro,[1] W. D. Henshaw[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

46 ON COMPUTATIONAL MODELS OF TEAR FILM AND OSMOLARITY DYNAMICS. R.J. Braun,[1] P.E. King-Smith,[2] J.J. Nichols[2] and P. Ramamoorthy.[2] [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 47 MEASURING OSMOLARITY WITH THE TEARLAB™. Santosh Khanal, Thomas J Millar. School of Natural Sciences, University of Western Sydney, Australia 48 Longitudinal Variation in Signs & Symptoms of Dry Eye Disease as Compared to a Composite Severity Index. Benjamin D. Sullivan[1], Baris Sonmez[2], Ebru Comert[2], Michael S. Berg[1], Michael A. Lemp[3]. [1]TearLab Corp. [2]Ondokuz Mayis Universitesi [3]Georgetown University

49 TEAR FILM OSMOLARITY IN DRY EYE DISEASE. Christina Jacobi, Friedrich E Kruse, Claus Cursiefen. Department of Ophthalmology, University of Erlangen-Nuremberg, Erlangen, Germany

50 EVALUATION OF TEAR OSMOLARITY IN PATIENTS UNDERGOING PHACOEMULSIFICATION CATARACT SURGERY.Arturo E. Grau (MD), Maria C. Morales (PhD), Juan A. Durán (MD, PhD). Instituto Clínico-Quirúrgico de Oftalmología, Bilbao, Vizcaya, Spain

51 EFFICACY OF TOPICAL PLASMA RICH IN GROWTH FACTOR IN THE TREATMENT OF DRY EYE. Arturo E. Grau MD, Silvia López-Plandolit MD, María C. Morales PhD,Vanesa Freire PhD-Student and Juan A. Durán MD, PhD. Instituto Clínico-Quirúrgico de Oftalmología, Bilbao, Vizcaya, Spain

52 METHODS FOR ITRAQ ANALYSES FOR QUANTITIAVE ANALYSIS OF PROTEIN EXPRESSION LEVELS IN TEAR FILM. Kari B. Green-Church,[1] Liwen Zhang,[1] Sruthi Srinivasan,[2] Mirunalni Thangavelu,[2] Christopher Paulette,[2] Kelly K. Nichols.[2] Mass Spectrometry and Proteomics Facility[1] College of Optometry,[2] The Ohio State University, Columbus, OH, USA.

53 COMPARATIVE TEAR PROTEIN PROFILING OF DRY EYE, BLEPHARITIS AND CONTROL PATIENTS BY MALDI-TOF MASS SPECTROMETRY AS A NEW DIAGNOSIS TOOL Nerea González[1], Ibón Iloro[2], Felix Elortza[2] and Tatiana Suárez[1]. [1]Bioftalmik Applied Research S.L. Viacaya Technology Park, Building 800, 48160, Derio, Spain. [2]Proteomics Platform, CIC bioGUNE, CIBERehd, ProteoRed. Vizcaya Technology Park, Building 800, 48160, Derio.

54 COMPARATIVE TEAR FLUID PROTEOMIC STUDY OF DRY EYE, BLEPHARITYS AND CONTROLS PATIENTS AS A TOOL FOR DIFERENTIAL DIAGNOSIS AUTHORS. Javier Soria[1], Jaime Echevarria[2], Iñaki Rodriguez-Agirretxe[3], Arantxa Acera[1], Nerea Gonzalez[1], Tatiana Suárez[1] Bioftalmik Applied Research S.L. Vizcaya Technology Park, Building 800, 48160, Derio, Spain 1. Hospital de Cruces, Baracaldo, Plaza Cruces-gurutzeta, 12 Vizcaya, Spain 2. Hospital de Donostia, San Sebastian, Paseo Doctor Begiristain 115, Guipuzcoa, Spain

55 TEAR PROTEIN LEVELS IN KERATOCONUS. Sivaraman A. Balasubramanian[1,2,] David C. Pye[2], Mark D.P.Willcox[1, 2] [1]Brien Holden Vision Institute, [2]School of Optometry and Vision Science, University of New South Wales, Sydney, Australia

56 ADVANCED GLYCATION END PRODUCT (AGE) MODIFIED PROTEINS IN TEARS OF DIABETIC PATIENTS. Zhenjun Zhao,[1,3] Jingfang Liu,[1,2] Bingyin Shi,[2] Shuixiang He,[2] Xiaoli Yao,[2] and Mark D.P. Willcox[1,3], Brien Holden Vision Institute,[1] Sydney, Australia; First Hospital Affiliated to Medical College, Xi'an Jiaotong University,[2] Xi'an, China; The School of Optometry and Vision Science, University of New South Wales,[3] Sydney, Australia

57 BLOOD COAGULATION FACTOR XIII IN TEARS. Zsuzsanna Z. Orosz,[1] Éva Katona,[1] Andrea Facskó,[2] László Módis,[2] László Muszbek,[1,3]András Berta.[2] Clinical Research Center,[1] Department of Ophthalmology[2] and Thrombosis, Hemostasis and Vascular Biology Research Group of the Hungarian Academy of Sciences,[3] University of Debrecen, Medical and Health Science Center, Debrecen, Hungary

58 THE EFFECT OF EYE DROP WHICH COMBINES SODIUM HYALURONATE AND CARBOXY METHYL CELLULOSE IN TREATING DRY EYE Hungwon Tchah, Jae Yong Kim, Myoung Joon Kim, Jae Hyung Kim, Jooen Lee. Department of Ophthalmology, University of Ulsan, Asan Medical Center, Seoul, Korea

59 EFFICACY OF SODIUM HYALURONATE AND CARBOXYMETHYLCELLULOSE IN TREATING MILD TO MODERATE DRY EYE DISEASE. Tae-im Kim,1 Ji Hwan Lee,1 Ji-Won Kwon,2 Hyun Suk Ahn,1 Eung Kweon Kim,1 1The Institute of Vision Research, Department of Ophthalmology, Yonsei University College of Medicine, Seoul, Korea 2 Department of Ophthalmology, Seoul National University Hospital, Seoul, Korea

60 **Discussion:** LUBRICIN AS AN OCULAR SURFACE-CONTACT LENS BOUNDARY LUBRICANT: DOSE-DEPENDENT & SYNERGISTIC EFFECTS. S. Morrison[1], B. Snider[1], B.D. Sullivan[2], E. Truitt III[3], D.A. Sullivan[4], T. Schmidt[1] [1] University of Calgary, Calgary, Canada; [2] TearLab Corp., San Diego, CA; [3] Singularis, Inc., San Diego, CA; [4] Schepens Eye Research Institute and Harvard Medical School, Boston, MA

61 EFFECTS OF THE COMBINATION OF HYALURONIC ACID AND TAMARIND SEEDS POLYSACCHARIDE IN THE MANAGEMENT OF DRY EYE. Stefano Barabino, Cristiana Valente, Guia Corsi, Maurizio Rolando. Ocular Surface Research Center, Department of Neurosciences, Ophthalmology, and Genetics, University of Genoa, Genoa, Italy.

62 PREPARATION OF A CORD BLOOD SERUM EYE DROPS FOR TOPICAL USE IN SEVERE CORNEAL EPITHELIOPATHY. M. Buzzi, A. Stancari, C. Vaselli, C. Coslovi, A. Terzi, A. Abenavoli, G. Bersani P. Versura , EC Campos Emilia Romagna Cord Blood Bank-Transfusion Service, Pharmacy Service S.Orsola-Malpighi Hospital, Ophthalmology Unit Alma Mater Studiorum University of Bologna

63 CORD BLOOD SERUM EYE DROPS IN THE TREATMENT OF SEVERE CORNEAL EPITHELIAL DEFECTS IN GVHD AND SS-I PATIENTS: A PILOT STUDY. EC Campos, P. Versura, V. Profazio, L. Foroni, C. Schiavi, M. Arpinati, N Malavolta - Ophthamology Unit, Hematology Department, Rheumatology Service, Alma Mater Studiorum University of Bologna, Italy

64 CELL TARGETING BY TEAR PROSECRETORY MITOGEN – LACRITIN. Gordon W. Laurie, Yinghui Zhang Cell Biology, University of Virginia

65 EFFICACY OF NOVEL THIOLATED BIOPOLYMER IN THE TREATMENT OF DRY EYE SYNDROME. Leopold Schmetterer, Sonja Hoeller, Margit Hornof Medical University of Vienna, Croma Pharma

66 THERAPEUTIC EFFECT OF ELEDOISIN IN OCULAR PHATOLOGICAL MANIFESTATIONS SJOGREN'S SYNDROME. Capra Piera. Ophthalmological Clinic University La Sapienza Roma, Italy

67 HOW ARTIFICIAL TEAR PRODUCTS CAN MODIFY & RESTORE THE PHYSICAL/CHEMICAL CHARACTERISTICS OF THE HUMAN TEAR FILM. D. Meadows[1], H. Ketelson[1], Robert Baier[2], Gerald G. Fuller[3], Donald Korb[4], Tom Millar[5], Robert Pelton[6], [1]Alcon Research Inc, Ft. Worth, TX, [2]SUNY Buffalo, [3] Stanford University [4]Korb Associates, [5]Univ. of Western Sydney, [6]McMaster University.

68 AUGMENTATION OF TEAR FILM LIPID LAYER BY AN NEW ARTIFICIAL TEAR EMULSION. Howard Ketelson[1], Robert Baier[2], Anne Meyer[2], Jonathan Prindle[2], Michael Christensen[1], and Michelle Senchyna[1].[1]Alcon Research Ltd;[2]SUNY Buffalo

69 SEVERE DRY EYES NOT AMENABLE TO CONVENTIONAL TOPICAL LUBRICATION: WHAT IS NEXT? Boboridis G. K., Mikropoulos G. D., Ziakas G. N., Toumanidou V., Lake S., Georgiadis S. N. 1st Ophthalmology Department, Aristotle University of Thessaloniki.

70 THE EFFECT OF OCULAR SURFACE LUBRICANT EYEDROPS ON LID PARALLEL CONJUNCTIVAL FOLDS (LIPCOF) AND OTHER SIGNS AND SYMPTOMS OF TEAR FILM DYSFUNCTION. Igor Petriček[1], Snježana Lovrinčević[2], Sanja Njirić[3], Goranka Petriček[4], Petar Rašregorac[5], Iris Urlić[6], Martina Tomić[7] Zagreb University Hospital Eye Department, Zagreb, Croatia[1] Croatia insurance, Zagreb, Croatia[2] Ophthalmology Polyclinic "dr Luciana Pavićević", Rijeka, Croatia[3] Zagreb University Medical School Family Medicine Department, "Andrija Štampar" School of Public Health, Zagreb, Croatia[4] Private Ophthalmology Practice, Samobor, Croatia[5] Ghetaldus Ophthalmology Polyclinic, Zagreb, Croatia[6] Clinical Hospital for Diabetes „Vuk Vrhovac", Zagreb, Croatia[7]

71 CLINICAL AND HISTOLOGICAL CHANGES CAUSED BY SUGAR CANE BURNING EMISSIONS ON THE OCULAR SURFACE OF SUGAR CANE WORKERS. Priscila Novaes[1A], Monique Matsuda[1A], Maristela P. Rangel[1A], Ubiratan P. Santos[1B], Newton Kara-José[1A], Alejandro Berra[2], Paulo H. N. Saldiva[1CA]Ophthalmology, [B]Pneumology- INCOR, [C]Pathology, [1]University of São Paulo, São Paulo, Brazil; [2]Pathology, University of Buenos Aires, Buenos Aires, Argentina

72 THE EXPRESSION AND FUNCTION OF RIG-I AND MDA-5 IN HUMAN OCULAR SURFACE EPITHELIUM. Mayumi Ueta[a,b], Norihiko Yokoi[a], Satoshi Uematsu[c], Taro Kawai[c], Shizuo Akira [c], and Shigeru Kinoshita[a] [a]Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan [b]Research Center for Inflammation and Regenerative Medicine, Faculty of Life and Medical Sciences, Doshisha University, Kyoto, Japan [c]Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan

73 TEMPERATURE-SENSING BY THE HUMAN CONJUNCTIVAL EPITHELIUM THROUGH ACTIVATION OF TRANSIENT RECEPTOR POTENTIAL VANILLOID (TRPV) CHANNELS. Fabian Garreis[1,2], Monika Valtink[3], Friedrich Paulsen[1],[2], Uwe Pleyer[4] and Stefan Mergler[4] [1]Department of Anatomy and Cell Biology, Martin Luther University, Halle-Wittenberg, Halle, Germany; [2]Department of Anatomy II, Friedrich Alexander University Erlangen-Nürnberg, Erlangen, Germany [3]Department of Anatomy, Medical Faculty "Carl Gustav Carus" TU Dresden, Dresden, Germany; [4]Department of Ophthalmology, University Medicine Berlin, Campus Virchow Hospital, Berlin, Germany

74 ENVIRONMENTAL BIOMECHANICS GOVERNS CELL BEHAVIOUR OF CORNEAL KERATINOCYTES. Eberwein P, Steinberg T, Schulz S, Tomakidi P, Beck D, Reinhard. T University Eye Hospital Freiburg;Department of Oral Biotechnology

75 SOURCES OF VARIABILITY IN MORPHOMETRIC CLASSIFICATION OF CORNEAL EPITHELIAL CELLS. Gemma Julio[1], Mª Dolores Merindano[1], Sara Lluch[1], Carme Caum[2] [1]Department of Optics and Optometry, Universitat Politècnica de Catalunya (UPC), Spain. [2]Faculty of Mathematics and Statistics, Universitat Politècnica de Catalunya (UPC), Spain

76 MORPHOMETRIC DESCRIPTION OF CORNEAL EPITHELIAL CELLS WITH LOW DENSITY OF MICROVILLI IN DIFFERENTS DRYING TIMES Gemma Julio[1], Mª Dolores Merindano[1], Sara Lluch[1], Carme Caum[21]Department of Optics and Optometry, Universitat Politècnica de Catalunya (UPC), Spain. [2]Faculty of Mathematics and Statistics, Universitat Politècnica de Catalunya (UPC), Spain

FRIDAY, SEPTEMBER 24, 2010

Session II

Late Breaking News: Sjogren's Syndrome

Chairpersons - Esen K. Akpek (USA), Ammon B. Peck (USA) & Yuichi Uchino (Japan)

8:00 **Keynote Address:** RECENT ADVANCES TOWARDS UNDERSTANDING THE GENETIC BASIS OF SJöGREN'S SYNDROME. Christopher Lessard and Kathy L. Moser. Arthritis and Immunology Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA

8:20 **Keynote Address:** SJOGREN'S SYNDROME: FROM SLIT LAMP TO CYTOPLASM. Barbara Caffery. University of Waterloo, School of Optometry Waterloo, Ontario Canada

8:45 **Keynote Address:** REVERSAL OF END-STAGE SJÖGREN'S SYNDROME AND DIABETES IN THE NOD MOUSE: CURRENT CLINICAL TRIAL PROGRESS AND BIOMARKER DESIGN. Denise L. Faustman, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

9:00 **Keynote Address:** QUALITY OF LIFE IN PRIMARY SJÖGREN'S SYNDROME. Simon Bowman, Consultant Rheumatologist, Selly Oak Hospital Birmingham, UK and Honorary Senior Clinical Lecturer, University of Birmingham, UK

9:20 **Keynote Address:** THE INTERNATIONAL SJÖGREN'S SYNDROME REGISTRY–CURRENT STATUS AND FUTURE OBJECTIVES. Troy Daniels,[1] Caroline Shiboski,[1] Lindsey Criswell,[1] Stephen Shiboski,[1] John Whitcher,[1] Morten Schiødt,[2] Hector Lanfranchi,[3] Hisanori Umehara,[4] Zhao Yan,[5] Stephen Challacombe,[6] M. Srinivasan,[7] Fred Vivino,[8] Alan Baer,[9] John Greenspan,[1] for the Sjögren's International Collaborative Clinical Alliance (SICCA). [1]University of California, San Francisco, USA; [2]Copenhagen University, Rigshospitalet, Denmark; [3]University of Buenos Aires and German Hospital, Argentina; [4]Kanazawa Medical University, Ishikawa, Japan; [5]Peking Union Medical College Hospital, Beijing, China; [6]King's College London, UK; [7]Aravind Eye Hospital, Madurai, India; [8]University of Pennsylvania, Philadelphia, USA; [9]Johns Hopkins University, Baltimore, USA

9:40 **Poster Session II (with Coffee & Tea)**

Visual & Optical Effects of Tear Film Instability

Chairpersons - Christine Purslow (UK), Eric Xiaojia Wei (Australia) & Norihiko Yokoi (Japan)

10:15 **Keynote Address:** THE EFFECTS OF TEAR FILM INSTABILITY ON VISION. Carolyn G. Begley, Indiana University School of Optometry, Bloomington, IN, USA

10:40 **Keynote Address:** MEASURING THE OPTICAL EFFECTS OF TEAR FILM INSTABILITY. Larry N. Thibos, Indiana University School of Optometry, Bloomington, IN, USA

11:05 POST BLINKING SERIAL MEASUREMENTS OF DYNAMIC WAVEFRONT ABERRATIONS AND FUNCTIONAL VISUAL ACUITY IN NORMAL AND DRY EYES. Suk Kyue Choi, M.D., Hae Won Seo, M.D., Jin Hyoung Kim, M.D., Do Hyung Lee, M.D., Ph.D

11:20 CHARACTERISTICS OF DRY EYES WITH SHORT TEAR FILM BREAK-UP TIME. Seika Den[1], Dogru Murat[1,2], Kazunari Higa[1], Jun Shimazaki[1,2] 1; Department of Ophthalmology, Tokyo Dental College. 2; Keio University School of Medicine

11:35 EVALUATION OF TEAR FILM QUALITY WITH A DOUBLE-PASS SCATTERING INDEX. Pisella Pj, Habay T, Nochez Y. CHU Bretonneau, Tours, France Faculté de Médecine François Rabelais, Tours, France.

11:50 **Poster Viewing & Lunch**

Poster Discussion II

Chairpersons - Fabian Garreis (Germany), Winston W. Kao (USA) & Eduardo M. Rocha (Brazil)

13:20 EFFECT OF PUNCTAL OCCLUSION ON LIPID-LAYER SPREAD AND TEAR FILM STABILITY IN AQUEOUS-DEFICIENT DRY EYE. Norihiko Yokoi,[1] Rieko Sakai,[1] Anthony J. Bron,[2] John M. Tiffany,[2] Georgi As. Georgiev,[3] and Shigeru Kinoshita.[1] Kyoto Prefectural University of Medicine,[1] Kyoto, Japan; University of Oxford,[2] Oxford, UK; University of Sofia,[3] Sofia, Bulgaria

13:25 IMMUNE RESPONSE IN THE CONJUNCTIVAL EPITHELIUM AND OCULAR SURFACE DAMAGE IN PATIENTS WITH DRY EYE. Stefano Barabino[1], Cristiana Valente[1], Elisa Montaldo[2], Maria Cristina Mingari[2,3], Maurizio Rolando[1]. [1]Ocular Surface Research Center, Department of Neurosciences, Ophthalmology, and Genetics, University of Genoa; [2]Department of Experimental Medicine, University of Genoa; [3]Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy

13:30 QUANTIFICATION OF TEAR FILM INFLAMMATORY CYTOKINES IN SJOGREN'S DRY EYE. Michelle Senchyna,[1] Ravaughn Williams,[1] Nancy McNamara,[2] Michael Brubaker[1], Pavel Iserovich,[3] Robert Sack.[3] Alcon Research Ltd,[1] U California-San Fransisco,[2] SUNY School of Optometry.[3]

13:35 DRY EYE AND *DEMODEX* BLEPHARITIS. Jae Chan Kim, Jee Taek Kim, Seok Hyun Lee, Yeoun Sook Chun. Department of Ophthalmology, College of Medicine, Chung-Ang University, Seoul, Korea

Inflammation: a Cause or Consequence of Ocular Surface Disease

Chairpersons - Virginia L. Calder (UK), Laura Contreras Ruiz (Spain) & Alison M. McDermott (USA)

13:40 **Keynote Address:** INFLAMMATION: A CAUSE OR CONSEQUENCE OF MUCOSAL DISEASE? Richard S. Blumberg, Laboratory of Mucosal Immunology, Gastroenterology Division, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts

14:05 **Keynote Address:** INDUCTION OF CD4+ T CELL MEDIATED IMMUNITY IN DRY EYE DISEASE. Reza Dana. Schepens Eye Research Institute and Massachusetts Eye and Ear Infirmary, Harvard Medical School Department of Ophthalmology, Boston MA, USA

14:30 **Keynote Address:** ROLE OF INFLAMMATION IN HSV-1-INDUCED STROMAL KERATITIS. Robert L. Hendricks. Department of Ophthalmology, University of Pittsburgh, Pittsburgh, PA USA

14:55 INTERLEUKIN 33, A NOVEL EPITHELIUM-DERIVED CYTOKINE, LINKS INNATE IMMUNITY TO ALLERGIC INFLAMMATION ON OCULAR SURFACE. De-Quan Li, M.D., Ph.D., Lili Zhang, M.D., Xiaofen Zheng, M.D., Ph.D., Guiqiu Zhao, M.D, Ph.D., Matthew A. Cunningham, M.D., Cintia S. De Paiva, M.D., Stephen C. Pflugfelder, M.D. Ocular Surface Center, Cullen Eye Institute, Department of Ophthalmology, Baylor College of Medicine, Houston, Texas, USA

15:10 INFLAMMATORY CONDITIONS AFFECT TIGHT JUNCTION PROTEINS IN CORNEAL EPITHELIAL CELLS L. Contreras-Ruiz,[1,2] U. Schulze,[3] A. López, [1,2] F. Paulsen,[3,4] Y. Diebold.[1,2] Ocular Surface Group, IOBA-University of Valladolid, Valladolid, Spain; [1] Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER BBN), Valladolid, Spain; [2] Department of Anatomy and Cell Biology, Martin-Luther-University Halle-Wittenberg, Halle/Saale, Germany; [3] Department of Anatomy II, Friedrich Alexander University Erlangen, Germany. [4]

15:25 **Poster Session II (with Coffee & Tea)**

The Best Defense is a Good Offense: Ocular Surface Infection

Chairpersons - Danielle Augustin (USA), Gerald B. Pier (USA) & Fiona J. Stapleton (Australia)

16:00 **Keynote Address:** CORNEAL EPITHELIAL BARRIER FUNCTION AGAINST BACTERIA. Fleiszig SMJ[1], Tam C[1], Mun J[1], Evans DJ[2]. UC Berkeley, CA[1]. Touro University-CA[2].

16:25 **Keynote Address:** ELEVATED EXPRESSION OF TLRS, DECTIN-1, AND IL-1B IN HUMAN CORNEAS INFECTED WITH THE FILAMENTOUS FUNGI *ASPERGILLUS* AND *FUSARIUM*. R. Siva Ganesa Karthikeyan[1], Sixto M. Leal[2], Lalitha Prajna[1] and Eric Pearlman[2]. [1]Aravind Eye Hospital, Madurai, Tamil Nadu, India, [2]Department of Ophthalmology and Visual Sciences, Case Western Reserve University, Cleveland, Ohio

16:50 **Keynote Address:** CHARACTERIZATION OF THE PATHOGENIC MECHANISM OF ACANTHAMOEBA KERATITIS - THE PROTECTIVE ROLE OF TEAR FLUID. Noorjahan Panjwani, Departments of Ophthalmology and Biochemistry, and The New England Eye Center, Tufts University School of Medicine, Boston, Massachusetts

17:15 A METALLOPROTEINASE ZmpC SECRETED BY *STREPTOCOCCUS PNEUMONIAE* INDUCES MUC16 SHEDDING from oculr surface EPITHELIAL CELLS B. Govindarajan, B. B Menon, S. Spurr-Michaud, M. Gilmore, P. Argüeso, and I. K. Gipson. Schepens Eye Research Institute, Harvard Medical School, Boston, MA

17:30 MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) PROMOTES *P. AERUGINOSA*-INDUCED OCULAR KERATITIS. Jill Nagashima[1], Tanweer Zaidi[1], Robert A. Mitchell[2], Gerald B. Pier[1], and Mihaela Gadjeva[1] Channing Laboratory[1], Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115; James Graham Brown Cancer Center, University of Louisville[2], Louisville, Kentucky

17:45 - 18:45

Poster Session II (with Wine & Hors d'oeuvres)

Chairpersons - Fabian Garreis (Germany), Winston W. Kao (USA) & Eduardo M. Rocha (Brazil)

1 CONCENTRATION-BASED FLUORESCENT OBSERVATIONS OF TEAR FILM BREAKUP P. Ramamoorthy, P.E. King-Smith, J.J. Nichols The Ohio State University College of Optometry

2 THE PROTEINS AND THEIR INTERACTIONS IN HUMAN AND RABBIT TEARS: IMPLICATION ON TEAR FILM STABILITY. Eric Xiaojia Wei,[1,2] Zhenjun Zhao[1,2] and Mark DP Willcox.[1,2] Brien Holden Vision Institute, Sydney, Australia,[1] The School of Optometry and Vision Science, University of New South Wales, Sydney, Australia.[2]

3 EVALUATION OF EXTENDED TEAR STABILITY BY TWO EMULSION BASED ARTIFICIAL TEARS. Donald Korb1, Caroline Blackie[1], David Meadows[2], Mike Christensen[2], Marion Tudor [2] : [1] Korb Associates, Boston, MA; [2] Alcon Research LTD. Fort Worth, TX;.

4 SURFACE INTERACTIONS OF BENZALKONIUM CHLORIDE WITH MEIBOMIAN AND CORNEAL LIPIDS AND WITH WHOLE TEARS. Georgi As. Georgiev,[1] Norihiko Yokoi,[2] Krasimir Koev,[3] Slavyana Ivanova,[1] Elena Kutsarova,[1] Alexander Kyumurkov,[1] Rumen Krastev,[4] Zdravko Lalchev.[1] University of Sofia[1] and University hospital,[3] Sofia, Bulgaria, Kyoto Prefectural University of Medicine,[2] Japan, Max Planck Institute of Colloids and Interfaces,[4] Potsdam, Germany

5 THE INTERACTION BETWEEN EYE MAKE-UP REMOVERS AND THE TEAR FILM. Edward Ian Pearce, Madeline Harvey-Brown & Claire Higginson Glasgow Caledonian University, Glasgow, Scotland UK

6 ASSOCIATION OF TEAR FILM WAVEFRONT METRICS WITH GRADE OF PRE-LENS TEAR BREAK-UP AND VISION. Haixia Liu, C.G. Begley, N.L. Himebaugh, L.N. Thibos, Z. Wu, School of Optometry, Indiana University, Bloomington, IN

7 MEASURING LIGHT SCATTER DURING TEAR BREAK-UP WITH SHACK-HARTMANN WAVEFRONT ABERROMETER. Larry N. Thibos, Jayoung Nam, Nikole Himebaugh, Haixia Liu, Arthur Bradley. School of Optometry, Indiana University, Bloomington, IN, USA

8 CHANGES OF DYNAMIC WAVEFRONT ABERRATION AFTER PUNTAL OCCLUSION IN DRY EYE PATIENTS. Do hyung Lee, MD, PhD., Hyung seok Cho, MD, Jin Hyoung Kim, MD, Suk Kyue Choi, MD. Department of Ophthalmology, Ilsan Paik hospital, Inje University, Korea

9 **Discussion:** EFFECT OF PUNCTAL OCCLUSION ON LIPID-LAYER SPREAD AND TEAR FILM STABILITY IN AQUEOUS-DEFICIENT DRY EYE. Norihiko Yokoi,[1] Rieko Sakai,[1] Anthony J. Bron,[2] John M. Tiffany,[2] Georgi As. Georgiev,[3] and Shigeru Kinoshita.[1] Kyoto Prefectural University of Medicine,[1] Kyoto, Japan; University of Oxford,[2] Oxford, UK; University of Sofia,[3] Sofia, Bulgaria

10 A NEW PORTABLE DIGITAL MENISCOMETER. Stefan Bandlitz[1,2], Heiko Pult[1,3], Christine Purslow[1], Paul Murphy[1], Anthony J. Bron[4].[1]School of Optometry and Vision Sciences, Cardiff University, Cardiff, UK; [2]Cologne School of Optometry, Cologne, Germany; [3]Optometry and Vision Research, Weinheim, Germany; [4]Nuffield Laboratory of Ophthalmology, University of Oxford, Oxford, UK

11 MEASURING OF THE LOWER TEAR MENISCUS HEIGHT WITH TEARSCOPE® Krisztina Kosina-Hagyó, Amarilla Veres, Eszter Fodor, Béla Csákány, János Németh. SemmelweisUniversity, Department of Ophthalmology

12 TEAR MENISCUS AREA SOFTWARE (TMAS) FOR THE ASSESSMENT OF TEAR MENISCUS CHARACTERISTICS IN THE DIAGNOSIS OF DRY EYE DISEASE. Takashi Kojima[1,2], Osama M.A. Ibrahim[1,2], Tais Hitomi Wakamatsu[1,2], Koji Tonomura[3], Yukihiro Matsumoto[1,2], Murat Dogru[1], Kazuo Tsubota2.1. Johnson & Johnson Ocular Surface and Visual Optics Department, Keio University School of Medicine 2. Department of Ophthalmology, Keio University School of Medicine [3]. Konan Medical

13 MEASUREMENT OF TEAR MENISCUS IN DRY EYE PATIENTS WITH FOURIER-DOMAIN OPTICAL COHERENCE TOMOGRAPHY. David Huang, Pho Nguyen, Matthew C. Bujak, Ethan Tittler, Xinbo Zhang, Yan Li, Samuel Yiu.Doheny Eye Institute, University of Southern California, Los Angeles, CA, USA

14 MENISCOMETRY USING ANTERIOR SEGMENT OPTICAL COHERENCE TOMOGRAPHY Hiroaki Kato,[1,2] Norihiko Yokoi,[2] Anthony J Bron,[3] John M Tiffany[3] and Shigeru Kinoshita[2]. National Center for Geriatrics and Gerontology,[1] Aichi, Japan; Kyoto Prefectural University of Medcine,[2] Kyoto, Japan; University of Oxford,[3] Oxford, UK

15 COMBINATION OF PHENOL RED THREAD TEST AND SCHIRMER 1 TEST AS A RESCUE STRATEGY TO DETECT SEVERE OCULAR DRYNESS. De Monchy I, Mariette X, Pogorzalek N, Kaswin G, Gendron G, Labetoulle M Hopital Bicetre, Université Paris-Sud, 94275 Kremlin-Bicetre, FRANCE

16 SENSITIVITY AND SPECIFICITY OF A MODIFIED TEAR BASAL SECRETION TEST AND SCHIRMER'S I TEST IN SJÖGREN'S SYNDROME DIAGNOSIS Pasquale Aragona, Rosaria Spinella, Anna Roszkowska, Laura Rania, Elisa Postorino. Department of Ophthalmology, University of Messina, Italy

17 IS BLINKING ALTERED IN DRY EYE? Meredith E. Jansen, Carolyn G. Begley, Minhua Chen, Haixia Liu. Indiana University School of Optometry; Bloomington, IN USA

18 DRY EYE: A PRIMARY CHARACTERISTIC OF CYSTIC FIBROSIS? Katharine Evans, Rachel North, Christine Purslow School of Optometry & Vision Sciences, Cardiff University, UK

19 TEAR FERNING IN CYSTIC FIBROSIS. Katharine Evans, Rachel North, Christine Purslow.School of Optometry & Vision Sciences, Cardiff University, UK

20 INVESTIGATION OF TEAR FERNING IN NORMAL AND DRY EYES BEFORE AND AFTER USING ARTIFICIAL TEARS. Ali Masmali[1'2], Christine Purslow[1], Paul Murphy[1]. 1School of Optometry & Vision Sciences, Cardiff University, Cardiff, United Kingdom 2Optometry Department, School of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia

21 COMPARISON OF DRY EYE PATIENT SIGNS IN ENVIRONMENTAL CONDITIONS TO STATIC AND MOBILE CONTROLLED ADVERSE ENVIRONMENT(CAE) MODELS. Joel Naor[1], Donna Welch[1], Gail Torkildsen[2], George W. Ousler III[11]Ora, Inc, Andover, MA; [2]Andover Eye Associates, Andover, MA

22 LIPCOF IN THE DIAGNOSIS OF DRY EYE - MULTICENTER STUDY. Janos Nemeth, [1] Eszter Fodor, [1] Andras Berta, [2] Tímea Komar, [2] Igor Petricek, [3] Mohamed Higazy, [4] Pavel Nemec, [5] Marek Prost, [6] Galina Semak, [7] Hristina Grupcheva, [8] Ozlem Evren, [9] Petra Schollmayer, [10] Ameed Samaha, [11] Katarina Hlavackova. [12] Dept of Ophthalmology Semmelweis University Budapest, Hungary, [1] Debrecen, Hungary, [2] Croatia, [3] Egypt, [4] Czech Republic, [5] Poland, [6] Belarus, [7] Bulgaria, [8] Turkey, [9] Slovenia, [10] Lebanon, [11] Slovakia[12]

23 CONJUNCTIVAL FOLDS: SIGN OF AGE OR SIGNS OF LACRIMAL TEARS DYSFUNCTION. Johannes Nepp Ophthalmological Department, Medical University Vienna

24 THE LONGITUDINAL IMPACT OF SOFT CONTACT LENS WEAR ON LID WIPER EPITHELIOPATHY AND LIDPARALLEL CONJUNCTIVAL FOLDS. Heiko Pult[1,2,] Paul J Murphy[2], Christine Purslow[2] [1]Optometry and Vision Research, Weinheim, Germany, [2]School of Optometry and Vision Sciences, Contact Lens and Anterior Eye Research (CLAER) Unit, Cardiff University, Wales, UK.

25 COMMUNITY BASED STUDY IN ELDERLY POPULATION FOR THE ASSOCIATION BETWEEN DEPRESSIVE SCORE / DEMENTIA SCORE AND DRY EYE. Joon Young Hyon[1], Sang Beom Han[1], Ji Won Kwon[2], Se Joon Woo[1], Jung Jae Lee[3], Tae Hui Kim[4], Ki Woong Kim[4] 1 Department of Ophthalmology, Seoul National University Bundang Hospital, Seongnam, Korea 2 Seoul National University Healthcare System Gangnam Center, Seoul, Korea 3 Department of Psychiatry, Kyungbook National University Hospital, Daegu, Korea 4 Department of Neuropsychiatry, Seoul National University Bundang Hospital, Seongnam, Korea

26 THE IMPACT OF DRY EYE ON EVERYDAY LIFE (IDEEL) QUESTIONNAIRE: SATURATION, RELIABIILTY, VALIDITY AND DISCRIMINATIVE ABILITY COMPARED TO GENERIC MEASURES Linda Abetz MA[1], Robin Chalmers OD[2], Carolyn Begley OD[3], Polyxane Mertzanis MPH[1], Kitty Venkataraman PhD, Rod Barnes, MBA[4], and IDEEL Study group [1]Mapi Values, Cheshire, UK [2]Clinical Trial Consultant, Atlanta, GA, [3]Indiana University, School of Optometry, Bloomington, IN, [4]Alcon Research Ltd, Fort Worth, TX,

27 A REVIEW OF THE PATIENT-REPORTED OUTCOME INSTRUMENTS TO MEASURE THE IMPACT OF DRY EYE ON HEALTH-RELATED QUALITY OF LIFE. Isabelle Guillemin & Benoit Arnould Mapi Values, Lyon, France

28 TRANSCULTURAL ADAPTATION AND VALIDATION OF THE OCULAR SURFACE DISEASE INDEX (OSDI) IN PATIENTS OF AN UNIVERSITY HOSPITAL IN SÃO PAULO, BRAZIL. Felipe Ribeiro Ferreira[1], Ruth Miyuki Santo[1], Priscila Novaes[1] [1]Division of Ophthalmology, School of Medicine of the University of São Paulo , São Paulo, Brazil

29 IMPLEMENTATION OF A NEW QUESTIONNAIRE INTO RECENTLY REVISED JAPANESE DRY EYE DIAGNOSTIC CRITERIA. Miki Uchino[1,2], Murat Dogru[2], Yuichi Uchino[2], Samantha Ward[2], Tais Wakamatsu[2], Yoko Ogawa[2], Norihiko Yokoi[3], Kazuo Tsubota[2] [1]Ryogoku Eye Clinic, [2]Keio University School of Medicine, Tokyo, Japan, [3] Kyoto Prefectural University of Medicine, Kyoto, Japan

30 COMPARISON OF TWO DRY EYE QUESTIONNAIRES AND CLINICAL OBSERVATIONS IN NON-CONTACT LENS WEARERS. J. Enbuske, E. Blixt, K. Silfwerbrand, and J. P. Gierow. Schoolof Natural Sciences, Linnaeus University, Kalmar, Sweden

31 DRY EYE SYMPTOMATOLOGY OF NON CONTACT LENS WEARERS WITH THE ODSI QUESTIONNAIRE. Cécile Maissa, Michel Guillon, Caroline Flomet, Elisabeth Bolton. OTG Research & Consultancy London UK

32 CONTACT LENS DRY EYE QUESTIONNAIRE-8 (CLDEQ-8) REFLECTS STATUS OF AND RESPONDS TO CHANGE IN OVERALL OPINION OF CL PERFORMANCE. Robin L. Chalmers[1], Kurt Moody[2], Graeme Young[3], Sheila Hickson-Curran[2], Carolyn Begley[4], Chris Hunt[3] [1]Clinical Trial Consultants, Atlanta, GA, USA, [2]Vistakon, Inc., Jacksonville, FL, USA, [3]Visioncare Research Ltd., Farnham, Surrey, UK, [4]Indiana University, Bloomington, IN, USA

33 DRY EYE SYMPTOMATOLOGY OF CONTACT LENS WEARERS WITH THE OSDI QUESTIONNAIRE. Michel Guillon, Cecile Maissa, Elizabeth Bolton, Caroline Flomet. OTG Research & Consultancy, London, UK

34 DRY EYE-LIKE SYMPTOMS AND SIGNS AFTER CATARACT SURGERY. Stefano Barabino, Federico Solignani, Cristiana Valente, Maurizio Rolando Ocular Surface Research Center, Department of Neurosciences, Ophthalmology, and Genetics, University of Genoa, Genoa, Italy

35 **Discussion:** IMMUNE RESPONSE IN THE CONJUNCTIVAL EPITHELIUM AND OCULAR SURFACE DAMAGE IN PATIENTS WITH DRY EYE. Stefano Barabino[1], Cristiana Valente[1], Elisa Montaldo[2], Maria Cristina Mingari[2,3], Maurizio Rolando[1]. [1]Ocular Surface Research Center, Department of Neurosciences, Ophthalmology, and Genetics, University of Genoa; [2]Department of Experimental Medicine, University of Genoa; [3]Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy

36 HYPEROSMOLAR STRESS ENHANCES HLA-DR EXPRESSION IN HUMAN CONJUNCTIVA. P. Versura, V Profazio, C Coslovi, L Foroni, C. Schiavi, E C CamposOphthalmology Unit, Alma Mater Studiorum University of Bologna, Italy

37 CONJUNCTIVAL HLA-DR EXPRESSION AND SURGICAL OUTCOMES OF TRABECULECTOMY. João M. Furtado, Jayter S. de Paula, Edson G. Soares, Eduardo M. Rocha, Régia C. P. Lira, Ana M. da Rocha, Neifi H. S. Dhegaide, Eduardo A. Donadi, Maria de L. V. Rodrigues. Departments of Ophthalmology, Internal Medicine and Pathology, Medical School of Ribeirão Preto, University of São Paulo, Brazil

38 CONJUNCTIVAL INFLAMMATION IN PATIENTS UNDER TOPICAL GLAUCOMA TREATMENT. Maria L. Veronese Rodrigues, Joao Marcello F.Furtado, Jayter S.Paula, Régia P. Lira, Edson G. Soares, Eduardo A. Donadi, Eduardo M. Rocha Medical School of Ribeirão Preto, University of São Paulo, Brazil

39 DRY EYE AND SECONDARY SJÖGREN'S SYNDROME IN MIXED CONNECTIVE TISSUE DISEASE (MCTD). Fany S. Usuba [1], Priscila Novaes[1], Milton R. Alves [1 1] Division of Ophthalmology, School of Medicine of the University of São Paulo, São Paulo, Brazil

40 CHRONICALLY DISTURBED IP_3 RECEPTOR-MEDIATED CA^{2+} SIGNALING IN EXOCRINE GLANDS CAUSES SJÖGREN'S SYNDROME-LIKE AUTOIMMUNE DISEASE. Takaaki Inaba[1, 2], Chihiro Hisatsune[2], Yasumasa Sasaki[1], Yoko Ogawa[1], Taishin Akiyama[3], Etsuko Ebisui[2], Naoko Ogawa[2], Minoru Matsui[4], Tsutomu Takeuchi[5], Katsuhiko Mikoshiba[2] & Kazuo Tsubota[1] [1]Department of Ophthalmology, Keio University School of Medicine [2]Laboratory for Developmental Neurobiology, Brain Science Institute, RIKEN [3]Division of Cellular and Molecular Biology, Department of Cancer Biology, Institute of Medical Science, University of Tokyo [4]Department of Pharmacy, Chiba Institute of Science [5]Department of Rheumatology, Keio University School of Medicine

41 A CASE OF IgG4-RELATED CHRONIC SCLEROSING DACRYOADENITIS. Mi Sun Sung, Joo Hwa Lee Sanggy-Paik Hospital, Inje University, Seoul, Korea

42 KERATITIS SUPERFICIALIS AFTER SURGICAL THERAPY OF TRIGEMINUSNEURALGIA. I. Boldin,[1] M. Trummer,[2] D. F. Rabensteiner,[1] J. Horwath-Winter.[1] Medical University Graz, Department of Ophthalmology, [1] Department of Neurosurgery, [2] Austria

43 A GENETIC ASSOCIATION OF IL 6 AND IL 6R GENES IN KOREAN DRY EYE PATIENTS. Kyung-Sun Na,[1, 2, 3,] Jee-Won Mok,[1,2] Choun-Ki Joo.[1, 2, 3]Laboratory of Ophthalmology and Visual Science, The Catholic University of Korea, [1]Korea Eye Tissue and Gene Bank, [2]Department of Ophthalmology and Visual Science, St. Mary's Hospital[3]Seoul, Korea

44 TH17 PROMOTING ENVIRONMENT IN THE LACRIMAL GLAND OF THROMBOSPONDIN-1 DEFICIENT MICE WITH OCULAR SURFACE DISEASE. Sharmila Masli, Bruce Turpie. Schepens Eye Research Institute, Harvard Medical School, Boston, MA

45 BONE MARROW MESENCHYMAL STEM CELLS TRIGGER PATHOGENIC FIBROSIS IN CHRONIC GRAFT VERSUS HOST DISEASE. Yoko Ogawa[1,3], Shigeto Shimmura[1], Satoru Morikawa[2,4] Yo Mabuchi[2], Tomonori Yaguchi[3], Sadafumi Suzuki, Takaaki Inaba[1], Yutaka Kawakami[3], Hideyuki Okano[2], Yumi Matsuzaki[2] , Kazuo Tsubota[1] [1]Department of Ophthalmology, [2]Department of Physiology, [3]Institute for Advanced Medical Research, Division of Cellular Signaling, [4]Department of Dentistry and Oral Surgery, Keio University, School of Medicine

46 CXCR4 AND CXCR7 – TWO POTENTIAL RECEPTORS FOR TFF3 AT THE OCULAR SURFACE. Dieckow J[1], Schulze U[1], Paulsen F[2], [1]Department of Anatomy and Cell Biology, Martin Luther University Halle-Wittenberg, Halle, Germany, [2]Department of Anatomy II, Friedrich Alexander University Erlangen- Nuernberg, Germany

47 CYTOKINE CHANGES IN THE TEAR FLUID OF KERATOCONUS PATIENTS. Shukti Chakravarti, Leslie Cope and Albert Jun. Johns Hopkins School of Medicine

48 TEAR CYTOKINE PROFILES IN SJÖGREN SYNDROME AND IN NON-SJÖGREN DRY EYE. Sang Beom Han, MD, [1]Joon Young Hyon, MD,[1,2] Ji-Won Kwon, MD,[3] Won Ryang Lee, [2,4] MD,[1] Jin Hak Lee, MD.[1,2] Seoul National University Bundang Hospital[1] Seoul National University College of Medicine,[2] Seoul National University Hospital Healthcare System Gangnam Center,[3] Seoul National University Hospital, [4] Seoul, Korea

49 **Discussion:** QUANTIFICATION OF TEAR FILM INFLAMMATORY CYTOKINES IN SJOGREN'S DRY EYE. Michelle Senchyna,[1] Ravaughn Williams,[1] Nancy McNamara,[2] Michael Brubaker[1], Pavel Iserovich,[3] Robert Sack.[3] Alcon Research Ltd,[1] U California-San Fransisco,[2] SUNY School of Optometry.[3]

50 ELEVATED TEAR INTERLEUKIN-17 LEVELS IN SJÖGREN'S SYNDROME DRY EYE PATIENTS. Kyoung Yul Seo, Jong-Hyuck Lee, Sang Yep Lee, Sang Min Nam Yonsei university college of medicine, department of ophthalmology

51 DIURNAL, DIFFERENTIAL CONTROL OF BIOACTIVITY OF PR0-INFLAMMATORY CYTOKINES AND CHEMOKINES. R Sack, B Cooper, S Sathe, A Beaton, P Iserovich. SUNY

52 IS INFLAMMATION INVOLVED IN THE "TIRED EYE" RESPONSE? Mark DP Willcox, Percy Lazon de la Jara, Eric Papas, Jennie Diec, Zhenjun Zhao Brien Holden Vision Institute, Sydney, Australia

53 ENDOGENOUS SECRETORY GROUP IIA PHOSPHOLIPASE (sPLA2-IIa) AMPLIFIES INFLAMMATION AT THE OCULAR SURFACE. Penny Asbell, Yi Wei, Seth Epstein. Department of Ophthalmology, Mount Sinai School of Medicine of New York University, New York, NY 10029, USA

54 THE EFFECT OF CONTACT LENS WEAR ON THE DIURNAL PROFILE OF MATRIX METALLOPROTEINASE-9 AND ITS INHIBITOR IN THE TEAR FILM. Maria Markoulli,[1,2] Eric Papas,[1,2] Nerida Cole,[1,2] Brien Holden.[1,2 1] Brien Holden Vision Institute, Sydney, Australia [2] School of Optometry & Vision Science, University of New South Wales, Australia

55 TEAR MITOGEN LACRITIN RAPIDLY COUNTERS INFLAMMATORY STRESS IN HUMAN CORNEAL EPITHELIAL CELLS. Ningning Wang, Gordon W. Laurie Cell Biology, University of Virginia

56 OCULAR SURFACTANT PROTEINS AND THEIR REGULATION IN DRY EYE DISEASE.Martin Schicht , Andreas Posa , Friedrich Paulsen and Lars Bräuer Department of Anatomy and Cell Biology, Martin Luther University Halle-Wittenberg, Germany; Department of Anatomy II, Friedrich Alexander University Erlangen-Nürnberg, Germany

57 ALLERGIC MEDIATORS IN TEAR FROM CHILDREN WITH SEASONAL AND PERENNIAL ALLERGY. Tatiana Suárez [1], Ricardo Martinez[2], Javier Soria [1], Nerea Gonzalez [1], Arantxa Acera [1] 1. Bioftalmik Applied Research S.L. Technology Park, Building 800, 48160, Derio, Vizcaya, Spain.2. Hospital de Cruces, Baracaldo, Plaza Cruces-gurutzeta, 12, Vizcaya, Spain

58 IgE and ECP AS MARKERS OF SEVERITY IN DIAGNOSIS OF ATOPIC KERATOCONJUNCTIVITIS. Ayako Igarashi, Tais Hitomi Wakamatsu,[2]Yoshiyuki Satake, Yoji Takano, Osama Ibrahim, Naoko Okada, Kazumi Fukagawa, Murat Dogru, Jun Shimazaki, Kazuo Tsubota, Hiroshi Fujishima[5]. Tokyo Dental College Ichikawa Hospital, Chiba, Japan [2] Keio University School of Medicine,Tokyo,Japan [3] Kitasato University School of Medicine,Tokyo, Japan [4] Ryogoku Eye Clinic,Tokyo,Japan [5] Saiseikai Central Hospital, Tokyo, Japan

59 THERAPEUTICAL USE OF A NEW BIODEGRADABLE DRUG DELIVERY SYSTEM FOLLOWING CORNEAL TRANSPLANTATION. J Schwartzkopff[1], A Hyatt[2], L Bredow[1], C Noack[1], P Eberwein[1], K Martin[2], T Reinhard[1] 1University Eye Hospital, Freiburg, Germany [2]Cambridge Centre for Brain Repair, University of Cambridge, United Kingdom

60 **Discussion:** DRY EYE AND *DEMODEX* BLEPHARITIS. Jae Chan Kim, Jee Taek Kim, Seok Hyun Lee, Yeoun Sook Chun. Department of Ophthalmology, College of Medicine, Chung-Ang University, Seoul, Korea

61 SEX DIFFERENCE IN INNATE ANTI MICROBIAL FACTORS IN RAT LACRIMAL GLAND. Lilian Eslaine Costa Mendes da Silva, Ana Carolina Dias, Carolina Maria Módulo, Stella Felippe de Freitas, a, Leonardo Tannus Malki, Eduardo Melani Rocha Department of Ophthalmology, Faculty of Medicine of Ribeirão Preto, USP, Ribeirão Preto, Brazil

62 DRY EYE MODULATES THE EXPRESSION OF ANTIMICROBIAL PEPTIDES ON THE OCULAR SURFACE. R. L. Redfern[1], W. Farley[2], C. S. De Paiva[2], S. C. Pflugfelder[2] and A. M. McDermott.[1] College of Optometry, University of Houston, Houston, Texas,[1] Baylor College of Medicine, Ocular Surface Center, Cullen Eye Institute, Houston, Texas[2]

63 TEAR FLUID REGULATION OF GENE EXPRESSION IN CORNEAL EPITHELIAL CELLS. J. Mun[1], C. Tam1, D. Evans[1,2] and S. Fleiszig[1]. UC Berkeley, CA[1]. Touro University, CA[2]

64 TRAVERSAL OF CORNEAL EPITHELIAL CELLS BY *P. AERUGINOSA*: BACTERIAL ADAPTATION REVEALS GENES THAT CONTRIBUTE TO THE PROCESS. Danielle Augustin[1], David Evans[1,2], Suzanne Fleiszig[1]. University of California, Berkeley, Berkeley, CA, USA[1]; College of Pharmacy, Touro University, Vallejo, CA, USA[2].

65 BACTERIAL INFECTION IN PRESUMED VIRAL INTERSTITIAL (STROMAL) KERATITIS. Suksri Chotikavanich[1], Pinnita Prabhasawat[1], Nattaporn Tesavibul[1], Amornrat leelaporn[2], Mongkol Uiprasertkul[3]. 1Department of Ophthalmology, 2Department of Microbiology, 3Department of Pathology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand.

66 SPECIFIC SITE-DIRECTED MUTATIONS IN THE *STREPTOCOCCUS PNEUMONIAE* CORNEAL VIRULENCE FACTOR PNEUMOLYSIN ABROGATE LYTIC ACTIVITY AND CORNEAL EROSIONS. Sidney Taylor, Justin Thornton, Larry S. McDaniel, Melissa E. Sanders, Mary E. Marquart. Department of Microbiology, University of Mississippi Medical Center, Jackson, MS, USA

67 TOLL-LIKE RECEPTOR 2 IS INVOLVED IN CORNEAL DISEASE SEVERITY OF *STREPTOCOCCUS PNEUMONIAE* KERATITIS IN THE ABSENCE OF PNEUMOLYSIN. Nathan Tullos, Erin Norcross, Sid Taylor, Quincy Moore, Melissa Sanders, Mary E. Marquart. Department of Microbiology, University of Mississippi Medical Center, Jackson, MS, USA

68 ASSESSMENT OF *STREPTOCOCCUS PNEUMONIAE* CAPSULE IN CONJUNCTIVITIS AND KERATITIS *IN VIVO*: NEURAMINIDASE ACTIVITY INCREASES IN NONENCAPSULATED PNEUMOCOCCI FOLLOWING CONJUNCTIVAL INFECTION. Erin W. Norcross,[1] Nathan A. Tullos,[1] Sidney D. Taylor,[1] Melissa E. Sanders,[1] and Mary E. Marquart[1] Department of Microbiology[1], University of Mississippi Medical Center, Jackson, MS, USA

69 MOXIFLOXACIN AND CHOLESTEROL COMBINED TREATMENT OF PNEUMOCOCCAL KERATITIS. Melissa E. Sanders[1], Nathan A. Tullos[1], Sidney D. Taylor[1], Erin W. Norcross[1], Lauren B. King[1], Isaiah Tolo[1], and Mary E. Marquart[1]. [1]Department of Microbiology, University of Mississippi Medical Center, Jackson, MS 39216.

70 ANTIMICROBIAL SUSCEPTIBILITY OF OCULAR BACTERIAL PATHOGEN TO LEVOFLOXACIN, MOXIFLOXACIN, GATIFLOXACIN, AND CIPROFLOXACIN. Joon Young Hyon, Hung Won Tchah. Korean Corneal Disease Study Group

71 EFFICACY AND SAFETY ASSESSMENT OF A NOVEL UVC DEVICE IN TREATING CORNEAL BACTERIAL INFECTIONS. SJ Dean[1,] A Petty[1,] S Swift[1,] J McGhee[1,] A Sharma[2,] J Moore[3,] S Shah[4], JP Craig[11] Univeristy of Auckland, New Zealand; [2] Moorfields Bedford, UK; [3] Univeristy of Ulster, UK; [4] Birmingham Midlands Eye Centre, UK

72 CORNEAL ANTIMICROBIAL PEPTIDE EXPRESSION IN RESPONSE TO CANDIDA ALBICANS AND FUSARIUM SOLANI. Satya Sree Kolar, Hasna Baidouri, Wanyu Zhang, Alison M McDermott. University of Houston, College of Optometry, Houston, TX, 77204

73 DONOR-RELATED CANDIDA KERATITIS AFTER DESCEMET STRIPPING AUTOMATED ENDOTHELIAL KERATOPLASTY. Katsuya Yamazoe, Seika Den, Yoichi Tanaka, Kazuki Hotta, Jun Shimazaki 1)Kameda Medical Center 2)Tokyo Dental College

74 THE CORNEAL PROTECTIVE EFFECTS OF SILICON HYDROGEL SOFT CONTACT LENS WEAR FROM UV-B EXPOSURE AND OXIDATIVE STRESS Murat Dogru, [1,2], Ibrahim Osama,[1,3] Tais Wakamatsu,[1,3] Takashi Kojima, [1,3],Yukihiro Matsumoto,[1,3], Kazuno Negishi, [3], Jun Shimazaki,[2]Yasuo Matsumoto,[4] Hiroshi Sasaki,[4] Kazuo Tsubota[3] 1) J&J Ocular Surface and Visual Optics Dept, Keio University School of Medicine 2) Dept. of Ophthalmology, Tokyo Dental College School of Medicine 3) Dept. of Ophthalmology, Keio University School of Medicine 4) Dept. of Ophthalmology, Kanazawa Medical University

75 DO CONTACT LENSES ELEVATE TEAR OSMOLARITY IN THE TYPICAL LENS WEARER? Alan Landers, Mary Mowrey-McKee, Walter Nash, Robert Scott, CIBA VISION Corporation, Atlanta, GA.

76 THE EFFECT OF RIGID GAS PERMEABLE AND SOFT CONTACT LENS WEAR ON OCULAR SURFACE TEMPERATURE. Sachiko Nishimura[1, 2], Paul J Murphy[1], Christine Purslow[1] [1]CardiffUniversity, School of Optometry and Vision Sciences, Cardiff, UK, [2]Menicon, Japan

77 ADHESION OF TRANSFERRIN AND ALBUMIN TO FDA GROUP II OMAFILCON CONTACT LENSES. Darshan Solanki, Sophia Cuprillnilson, Brooke Liberman, Andrea Janoff, Edward O. Keith Nova Southeastern University

78 LIPID PENETRATION INTO CONTACT LENSES: A CONFOCAL MICROSCOPY VIEW. J. Jacob, J. Guinn, T. Edwards Louisiana State University Health Sciences Center, Dept of Ophthalmology, New Orleans, LA

SATURDAY, SEPTEMBER 25, 2010

Session III

Ocular Surface Regeneration & Reconstruction

Chairpersons - Dimitri T. Azar (USA), Victor L. Perez (USA) & Jose Ricardo (Brazil)

8:00 **Keynote Address:** MOUSE LACRIMAL GLAND IS A REGENERATABLE ORGAN. Masataka Ito, Department of Developmental Anatomy and Regenerative Biology, National Defense Medical College, Saitama, Japan

8:25 **Keynote Address:** REGENERATIVE MEDICINE OF THE OCULAR SURFACE. Paolo Rama, M.D., Stanislav Matuska, M.D., Giorgio Paganoni, M.D., Alessandra Spinelli, M.D., Michele De Luca, M.D., and Graziella Pellegrini, Ph.D. San Raffaele Scientific Institute, Ophthalmology Unit, Milan (P.R., S.M., G.P., A.S.); and the Center for Regenerative Medicine Stefano Ferrari, University of Modena and Reggio Emilia, Modena, Italy (M.D.L., G.P.).

8:50 **Keynote Address:** OCULAR SURFACE RECONSTRUCTION. Shigeto Shimmura. Department of Ophthalmology, Keio University School of Medicine

9:15 MICRO-ENGINEERED SILK BIOMATERIALS FOR OCULAR SURFACE RECONSTRUCTION. MI Rosenblatt, BD Lawrence, Z Pan Margaret M. Dyson Vision Research Institute, Department of Ophthalmology, Weill Cornell Medical College, New York, NY

9:30 ANALYZATION OF LONG TERM CULTURED LACRIMAL GLAND STEM CELLS. Philipp Ackermann,[1] Anja Richter,[2] Friedrich Paulsen [1,3] Department of Anatomy and Cell Biology, Martin Luther University Halle-Wittenberg, Halle, Germany [1]; Fraunhofer Insitute of Marine Biotechnology, Fraunhofer Institute, Lübeck, Germany [2]; Department of Anatomy II, Friedrich Alexander University Erlangen-Nürnberg, Erlangen, Germany [1,3]

9:45 **Poster Session III (with Coffee & Tea)**

Drug, Preservative and Contact Lens Solution Interactions with the Tear Film & Ocular Surface

Chairpersons - Maria Markoulli (Australia), Teruo Nishida (Japan) & John L. Ubels (USA)

10:20 **Keynote Address:** EFFECTS OF TOPICAL DRUG PRESERVATIVES ON THE TEAR FILM AND OCULAR SURFACE. Christophe Baudouin, Quinze-Vingts National Ophthalmology Hospital, Vision Institute, University Paris 6, Paris, France

10:45 **Keynote Address:** CONTACT LENS SOLUTIONS: WHERE NEXT? Lyndon Jones, School of Optometry Associate Director, Centre for Contact Lens Research University of Waterloo Ontario, Canada

11:10 SUITABILITY OF A LIPOSOMAL DRY EYE SPRAY FOR USE IN SILICONE HYDROGEL CONTACT LENS WEAR. Jennifer P. Craig[1], Trisha Albuquerque[2], Chee Seang Loh[2], Varny Ganesalingam[2], Suhaila Al-Kanani[2], Stuti Misra[1] New Zealand National Eye Centre, Departments of [1]Ophthalmology and [2]Optometry and Vision Science, University of Auckland, New Zealand

11:25 IN VITRO CYTOTOXICITY OF HYDROGEN PEROXIDE TO CORNEAL EPITHELIAL CELLS JL Ubels, DS Mlnarik, BJ Konynenbelt Department of Biology, Calvin College, Grand Rapids, MI, USA

11:40 MODIFICATION OF THE TEAR FILM OSMOLARITYWITH THE USE OF CONTACT LENSES IN OMAFILCONA AND METHAFILCONA MATERIALS. Montani Giancarlo, University Of Salento Formazione Continua In Medicina, Lecce, Italy

11:55 **Poster Viewing & Lunch**

Poster Discussion III

Chairpersons - Darren G. Gregory (USA), Maryam Mokhtarzadeh (USA) & E. Ian Pearce (UK)

13:25 TRANSPLANTATION OF CONJUNCTIVAL EPITHELIAL CELLS CULTIVATED EX VIVO INPATIENTS WITH TOTAL LIMBAL STEM CELL DEFICIENCY. Jose RS Ricardo[1,2,] Jose AP Gomes[1,2,] Ocular Surface Advanced Center (CASO),[1] Cornea and External Disease Service, Department of Ophthalmology, Federal University of São Paulo, São Paulo, Brazil[2]

13:30 IN VIVO CONFOCAL MICROSCOPY OF MEIBOMIAN GLANDS IN SJOGREN'S SYNDROME. Edoardo Villani, Michela De Capitani, Silvia Beretta, Daniela Galimberti, Francesco Viola, Roberto Ratiglia. Clinica Oculistica Università degli Studi di Milano. Fondazione IRCCS Ca'Granda Ospedale Maggiore Policlinico, Milan, Italy.

13:35 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.

13:40 TOPICAL JAK INHIBITOR, TASOCITINIB (CP-690,550), MODULATES OCULAR SURFACE INFLAMMATION IN DRY EYE. Jing-Feng Huang, Rolla Yafawi, Min Zhang, Michael McDowell, Kay D Rittenhouse, Frederick Sace, Melissa Liew, Scott R Cooper, Eve H Pickering. Pfizer Inc., San Diego, CA, USA

Macro to Micro: New Imaging Approaches for Understanding the Ocular Surface

Chairpersons - Shukti Chakravarti (USA), Thomas Fuchsluger (USA) & Jianhua Wang (USA)

13:45 **Keynote Address:** OCULAR SURFACE IMAGING. Nathan Efron, Nicola Pritchard, Munira Al-Dossari. Institute of Health and Biomedical Innovation, and School of Optometry, Queensland University of Technology, Kelvin Grove, Queensland, Australia

14:10 **Keynote Address:** EXOCYTIC MACHINERY REVEALED BY HIGH RESOLUTION INTRAVITAL MICROSCOPY IN LIVE ANIMALS. Roberto Weigert Intracellular membrane Trafficking Unit, Oral and Pharyngeal Cancer Branch National Institute for Dental and Craniofacial Research National Institute of Health, Bethesda, MD USA

14:35 EPITHELIAL IRREGULARITY FACTOR (EIF): A NEW DIAGNOSTIC CRITERION FOR THE DIAGNOSIS OF DRY EYE SYNDROME. Victor L. Perez, Mohamed Abou Shousha, William Feuer, Anat Galor and Jianhua Wang. Bascom Palmer Eye Institute, University of Miami Miller School of Medicine

14:50 NON-INVASIVE IMAGING OF KEY PLAYERS IN OCULAR SURFACE INFLAMMATION. P. Steven[1,2], S. Siebelmann[1], A. Gebert[2], G. Huettmann[3], R. Orzekowsky-Schroeder[3], N. Koop[3], U. Gehlsen[1,2] [1]Department of Ophthalmology, [2]Institute of Anatomy, [3]Institute of Biomedical Optics, University of Luebeck, Germany

15:05 A UNIQUE OCULAR SURFACE INTERFEROMETER (OSI) TO MEASURE DYNAMIC LIPID LAYER THICKNESS (LLT). T.Willis[1], S.M. Grenon[1], D.R. Korb[1,2], C.A. Blackie[1,2], W. Weber[3], R. Chinnock[3]. [1]TearScience, Morrisville, NC; [2]Korb Associates, Boston, MA; [3]Optimum Technologies, Southbridge, MA

15:20 **Poster Session III (with Coffee & Tea)**

New & Emerging Diagnostics & Treatments

Chairpersons - José Alvaro P. Gomes (Brazil), Stefan Schrader (UK) & Jun Shimazaki (Japan)

15:55 **Keynote Address:** EMERGING PARADIGMS FOR CORNEAL REPLACEMENT: THE FUTURE OF KERATOPROSTHESIS SURGERY. James Chodosh, MD, MPH Massachusetts Eye and Ear Infirmary – Harvard Medical School, Boston, MA, USA

16:20 **Keynote Address:** RELEVANCE OF TEAR FILM PROTEOMICS IN THE DIAGNOSIS OF DISEASE. F. Grus. Experimental Ophthalmology, Dept. of Ophthalmology, University Medical Center, Johannes-Gutenberg-University Mainz, Germany

16:45 **Keynote Address:** POLYMER NANOMATERIALS FOR THERAPEUTIC DRUG DELIVERY. Alexander V. Kabanov. Center for Drug Delivery and Nanomedicine, College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska, USA

17:10 PREDICTORS OF SJÖGREN'S SYNDROME IN PATIENTS WITH DRY EYE Esen K. Akpek, M.D. [1]; Ramya Swamy, MPH[1]; Canan Asli Utine, M.D., M.S. [1, 2], Jennifer Thorne, M.D.[1], Alan N. Baer, M.D.[3], [1]The Wilmer Eye Institute, The Johns Hopkins University School of Medicine, Baltimore, Maryland. [2] Yeditepe University Eye Hospital, Istanbul, Turkey. [3]Division of Rheumatology, Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland

17:25 DISTRIBUTION OF AQUEOUS DEFICIENT AND EVAPORATIVE DRY EYE IN A GENERAL PATIENT POPULATION. Benjamin D. Sullivan[1], Michael A. Lemp[7]. [1]TearLab Corp., San Diego, CA; [2]Georgetown University Department of Ophthalmology, Washington DC, USA

17:40 – 18:40 **Poster Session III (with Wine & Hors d'oeuvres)**

Closing Session

Chairperson: Carlos Belmonte (Spain)

18:40 Academic perspective: Sarah F. Hamm-Alvarez (USA)
18:45 Clinical perspective: Jason J. Nichols (USA)
18:50 Industry perspective: Melissa Liew (USA)

Closing Remarks

18:55 *Carlos Belmonte (Spain)*

Poster Session III

Chairpersons - Darren G. Gregory (USA), Maryam Mokhtarzadeh (USA) & E. Ian Pearce (UK)

1 DRY EYE AFTER 20 AND 25 GAUGE VITRECTOMY C.Fabiani, S.Barile, JM Rakic, M. De Zanet. CHU, LIEGE - BELGIUM

2 A CASE OF GRANULOMA FORMATION AFTER CORNEAL REFRACTIVE SURGERY IN STEVENS-JOHNSON SYNDROME Eung Kweon Kim, MD, PhD,[1-3]Kyung Eun Han, MD,[1] Jae Hoon Kim, MD,[1] Sang Min Nam, MD,[1] Tae-im Kim, MD, PhD,[1] Kyung Ryul Seo, MD, PhD[1]. [1]Corneal Dystrophy Research Institute, Department of Ophthalmology, Yonsei University College of Medicine, Seoul, Korea, [2]Severance Medical Research Institute, Yonsei University College of Medicine, [3]Brain Korea 21 Project for Medical Science, Yonsei University, Seoul, Korea

3 THE OPHTHALMOLOGIC EVALUATION AND MANAGEMENT OF ACUTE STEVENS-JOHNSON SYNDROME: A COMPREHENSIVE APPROACH. Darren G. Gregory. University of Colorado, Denver, USA

4 EFFICACY OF SURGERY FOR CONJUNCTIVOCHALASIS WITH SUBJECTIVE AS WELL AS OBJECTIVE SYMPTOMS. Hitoshi Watanabe[1,2]Sizuka Koh[2], Yuichi Hori[1] Kansai Rosai Hospital[1], Osaka University Medical School[2]

5 AMNIOTIC MEMBRANE TRANSPLANTATION: OUR EXPERIENCE. Soniya Bhala, Sudesh Kumar Arya, Archana Malik, Sunandan Sood Department of Ophthalmology, Government Medical College and Hospital, Chandigarh, India

6 LONG TERM CLINICAL RESULTS OF LIMBAL CONJUNCTIVAL AUTOGRAFT VERSUS AMNIOTIC MEMBRANE TRANSPLANTATION IN PTERYGIUM SURGERY. Hyung Joon Kim, Sin Hoo Kim. Department of Ophthalmology, Catholic University of Daegu, Daegu, Korea

7 EFFECTS OF AMNIOTIC MEMBRANE SUSPENSION IN HUMAN CORNEAL WOUND HEALING IN VITRO. Choun-Ki Joo. Department of Ophthalmology and Visual Science, Seoul St. Mary\'s Hospital, College of Medicine, the Catholic University of Korea

8 **Discussion:** TRANSPLANTATION OF CONJUNCTIVAL EPITHELIAL CELLS CULTIVATED EX VIVO INPATIENTS WITH TOTAL LIMBAL STEM CELL DEFICIENCY. Jose RS Ricardo[1,2,] Jose AP Gomes[1,2,] Ocular Surface Advanced Center (CASO),[1] Cornea and External Disease Service, Department of Ophthalmology, Federal University of São Paulo, São Paulo, Brazil[2]

9 OUTCOME OF TRANSPLANTATION OF CULTIVATED ORAL MUCOSAL EPITHELIAL SHEETS PREPARED WITH FIBRIN-COATED CULTURE DISHES. Jun Shimazaki, Masatoshi Hirayama, Takefumi Yamaguchi, Yoshiyuki Satake INSTITUTIONS: Department of Ophthalmology, Tokyo Dental College

10 DEVELOPMENT OF A SERUM FREE AND XENOBIOTIC FREE SURROGATE CULTURE SYSTEM FOR HUMAN LIMBAL EPITHELIAL STEM CELL THERAPY. Schrader S[1,2,3,] Tuft SJ[2], Beaconsfield M[2], Geerling G[4], Daniels JT[1,2], Notara M[1]. [1]UCL Institute of Ophthalmology, London, UK, [2]Moorfields Eye Hospital NHS Foundation Trust, London, UK, [3]Department of Ophthalmology, University of Luebeck, Germany, [4]Department of Ophthalmology, Julius-Maximilian-University Wuerzburg, Germany

11 CLUSTERIN PROMOTE CORNEAL/LIMBAL EPITHELIAL GROWTH THROUGH EPITHELIAL-MESENCHYMAL INTERACTION. [1]Naoko Okada, [1,3]Tetsuya Kawakita,[2]Kenji Mishima,[2]Ichiro Saito,[1,3]Hideyuki Miyashita, [1,3]Shigeto Shimmura, [1,3]Kazuo Tsubota[1]Department of Ophthalmology, Keio University School of Medicine, Tokyo, JAPAN and [2]Department of Pathology, Tsurumi University, [3]Department of Ophthalmology, Tokyo Dental College, Chiba, JAPAN

12 POTENTIAL LOCALIZATION OF PUTATIVE STEM/PROGENITOR CELLS IN HUMAN BULBAR CONJUNCTIVAL EPITHELIUM Hong Qi[1,2], Xiaofen Zheng[1], Xiaoyong Yuan[1], Stephen C. Pflugfelder[1],De-Quan Li[1*] [1]Ocular Surface Center, Cullen Eye Institute, Department of Ophthalmology, Baylor College of Medicine, Houston, Texas [2]Peking University Third Hospital, Department Ophthalmology,Beijing, China

13 ISOLATION AND PROPAGATION OF MESENCHYMAL STEM CELLS FROM THE LACRIMAL GLAND. Samantha You, Claire Kublin and Driss Zoukhri Tufts University School of Dental Medicine and Departments of Neuroscience, Tufts University School of Medicine, Boston, MA

14 CELLULAR FACTOR XIII, A TRANSGLUTAMINASE, IS PRESENT IN THE CORNEAL STROMA. Zsuzsanna Z. Orosz,[1] Helga Bárdos,[2] Andrea Facskó,[3] András Berta,[3] Róza Ádány,[2] László Muszbek.[1,4] Clinical Research Center,[1] Department of Preventive Medicine,[2] Department of Ophthalmology[3] and Thrombosis, Hemostasis and Vascular Biology Research Group of the Hungarian Academy of Sciences,[4] University of Debrecen, Medical and Health Science Center, Debrecen, Hungary

15 TRANSGLUTAMINASE-2 DEPENDENCE IN HYPEROSMOLARITY-INDUCED MITOCHONDRIAL DYSFUNCTION. Evelyn Png,[1] Shyam S. Chaurasia,[1] Louis Tong.[1,2,3] Singapore Eye Research Institute,[1] Singapore National Eye Center,[2] Duke-NUS Graduate Medical School, Singapore[3]

16 EFFECT OF MELATONIN AND ANALOGUES ON CORNEAL WOUND HEALING: INVOLVEMENT OF MT_2 MELATONIN RECEPTOR. Assumpta Peral, Ana Guzmán-Aranguez, Almudena Crooke and Jesús Pintor University Complutense of Madrid, School of Optics

17 POTENTIAL ROLE OF TFF3 IN CORNEAL WOUND HEALING. U. Schulze,[1] L. Contreras Ruiz,[2] A. López,[2] N. Barker,[3] Y. Diebold,[2] F. Paulsen[1],[4]. Department of Anatomy and Cell Biology , Martin Luther University Halle, Germany,[1] IOBA University of Valladolid, Spain,[2] GI Company, Framingham, USA,[3] Department of Anatomy II, Friedrich Alexander University Erlangen, Germany[4]

18 RELAXIN 2 AND INSL3 PROMOTE WOUND HEALING AT THE OCULAR SURFACE. Ulrike Hampel,[1] Thomas Klonisch,[2] Saadettin Sel,[3] Friedrich Paulsen.[1] Departments of Anatomy and Cell Biology,1 Ophthalmology,[3] Martin Luther University of Halle-Wittenberg, Halle/Saale, Germany; Department of Human Anatomy and Cell Science and Department of Medical Microbiology and Infectious Diseases[2], University of Manitoba, Winnipeg, Manitoba, Canada

19 DIFFERENTIAL EFFECT OF INDIVIDUAL ELECTROLYTES ON CORNEAL EPITHELIAL BARRIER FUNCTION DURING HYPEROSMOTIC STRESS. Ashley Woodward,[1] Michelle Senchyna,[3] Pablo Argüeso.[1,2] Schepens Eye Research Institute,[1] Harvard Medical School,[2] Boston, MA, USA, Alcon Research, Ltd.,[3] Fort Worth, TX, USA

20 SELENOPROTEIN P CONTROLS OXIDATIVE STRESS IN CORNEA. Akihiro Higuchi[1], Kazuhiko Takahashi[2], Kazuo Tsubota [1,3]. [1]Center for Integrated Medical Research, School of Medicine, Keio University, Tokyo, Japan. [2]Department of Nutritional Biochemistry, School of Pharmacy, HokkaidoPharmaceutical UniversityHokkaido, Japan. [3]Department of Ophthalmology, Schoolof Medicine, KeioUniversity, Tokyo, Japan

21 PROTECTIVE EFFECT OF AMINO ACIDS AND COMMERCIAL OPHTHALMIC INGREDIENTS ON 4-HYDROXYNONENAL-INDUCED CYTOTOXICITY IN HUMAN CORNEAL EPITHELIAL CELLS. Takahiro Kurose[1, 2], Kazuhiro Tsuji[2], Takayuki Miyano[1, 2], Yoichi Honma[2], Norihiko Yokoi [1] and Shigeru Kinoshita[1] Kyoto Prefectural University of Medicine, [1] Kyoto, Japan; Rohto pharmaceutical co., Ltd, [2] Kyoto, Japan

22 TREATMENT OF PERSISTENT CORNEAL EPITHELIAL LESIONS AFTER VITREOUS SURGERY BY PUNCTAL PLUG OCCLUSION. Miki Sakata, [1,2] Hirotsugu Ogura[2], Wakita Eye Clinic[1] ,Tokyo, Kozawa Eye Hospital and Diabetes Center [2], Mito, JAPAN

23 EXPRESSION OF MATRIX METALLOPROTEINASES 7 AND 14 IN UV IRRADIATED CORNEA Taras Ardan, Jitka Cejkova. Institute of Experimental Medicine, Academy of Sciences of the Czech Republic

24 INHIBITION OF UVB ACTIVATION OF SEK1/MKK4 AND JNK1 IN CORNEAL EPITHELIAL CELLS BY ELEVATED EXTRACELLULAR K^+ JL Ubels, MP Schotanus, LR Koetje, JL Louters Department of Biology, Calvin College, Grand Rapids, MI, USA

25 LONGTERM CHANGES OF BUT AND CORNEAL SENSITIVITY FOLLOWING LASIK AT MIDDLE AGE. Woo Chan Park, Jae Kwan Park, Ki Sung Park, Byung Moo Min. Dept. of Ophthalmology, Dong-A University, Busan, Korea

26 CORNEAL SENSATION AND LACRIMAL SECRETION BEFORE AND AFTER DESCEMET STRIPPING AUTOMATED ENDOTHELIAL KERATOPLASTY. Yumiko Tamari,Yukari Imai,Takefumi Yamaguchi,Kenji Konomi,Seika Den,Yoshiyuki Satake,Jun Shimazaki Department of Ophthalmology,Tokyo Dental Collage Ichikawa General Hospital

27 ALTERATIONS IN TEAR SECRETION, CORNEAL SENSITIVITY AND WOUND HEALING IN DIABETIC RATS. Fu-Shin Yu, Jia Yin and Keping Xu Kresge Eye Institute, Wayne State University School of Medicine

28 MANAGEMENT OF CORNEAL MELTING IN BOSTON KERATOPROSTHESIS WITH BIOCOMPATIBLE MATERIALS Arturo E. Grau (MD)[1], Jaime Etxebarria (MD) [1, 2] INSTITUTIONS:[1]Instituto Clínico-Quirúrgico de Oftalmología, [2] Hospital de Cruces, Bilbao, Vizcaya, Spain

29 TOXICOLOGICAL COMPARISON OF TRAVOPROST BAK-FREE, TRAVOPROST BAK-PRESERVED, AND LATANOPROST BAK-PRESERVED OPHTHALMIC SOLUTIONS IN HUMAN CONJUNCTIVAL EPITHELIAL CELLS. Brignole-Baudouin F,[1-5]Riancho L,[1-3] Liang H,[1-4] Baudouin C.[1-4] 1VISION INSTITUTE INSERM, U968, [2]UPMC Paris 6, [3]CNRS, UMR_7210, [4]QUINZE-vINGTS NATIONAL HOSPITAL, [5]Université Paris 5, Paris, France

30 IN VIVO ASSESSMENT OF THE OCULAR SURFACE EFFECTS OF TRAVOPROST BAK-FREE VERSUS BAK-PRESERVED TRAVOPROST AND LATANOPROST OPHTHALMIC SOLUTIONS. Liang H[1,2,3,4], Brignole-audouin F[1,2,3,4,5], Riancho L[1,2,3,] Baudouin C[1,2,3,4.] [1]INSERM, U968, [2]UPMC Paris 06, [3]CNRS, UMR_7210, [4]CHNO des XV-XX, [5]Université Paris Descartes,Paris, France

31 IN VIVO CONFOCAL MICROSCOPY ANALYSIS OF EFFECTS OF SYSTEMIC ISOTRETINOIN TREATMENT ON CORNEAL INNERVATION AND MORPHOLOGY Yonca A Akova, Sevda Metindoğan, Aylin Karalezli, Department of Ophthalmology, Baskent University, TURKEY

32 IN VIVO VISUALIZATION OF PRE-CORNEAL TEAR FILM IN DRY EYE PATIENTS. Jianhua Wang, MD, PhD,[1,2] Lele Cui, MD[1,3] Victor L. Perez, MD, Meixiao Shen, MSc,[1] Michael R. Wang, PhD[2] [1]Bascom Palmer Eye Institute, University of Miami Miller School of Medicine [2]Electrical and Computer Engineering, University of Miami [3]School of Ophthalmology and Optometry, Wenzhou Medical College

33 IN VIVO IMAGING OF TEAR FILM AND OCULAR SURFACE IN MEIBOMIAN GLAND DYSFUNCTION USING ULTRA HIGH RESOLUTION ANTERIOR SEGMENT OPTICAL COHERENCE TOMOGRAPHY (UHR-OCT). Mohamed Abou Shousha, Jiahuang Wang and Victor L. Perez Bascom Palmer Eye Institute, University of Miami, Miller School of Medicine, USA

34 MORPHOLOGIC EVALUATION OF MEIBOMIAN GLANDS IN CHRONIC GRAFT-VERSUS-HOST DISEASE USING IN VIVO LASER CONFOCAL MICROSCOPY Yumiko Ban,[1,2]Yoko Ogawa,[1]Osama M.A. Ibrahim,[3]Yukako Tatematsu,[1]Murat Dogru,[3]Kazuo Tsubota[1]. Department of Ophthalmology, School of Medicine, Keio University, Tokyo, Japan[1] Department of Ophthalmology, Hino Municipal Hospital, Tokyo, Japan[2] Johnson and Johnson Ocular Surface Visual Optics Department, School of Medicine, Keio University, Tokyo, Japan[3]

35 **Discussion:** IN VIVO CONFOCAL MICROSCOPY OF MEIBOMIAN GLANDS IN SJOGREN'S SYNDROME. Edoardo Villani, Michela De Capitani, Silvia Beretta, Daniela Galimberti, Francesco Viola, Roberto Ratiglia. Clinica Oculistica Università degli Studi di Milano. Fondazione IRCCS Ca'Granda Ospedale Maggiore Policlinico, Milan, Italy.

36 PERFORMANCE OF MEIBOMETRY IN ASSESSING MEIBOMIAN GLAND DYSFUNCTION. P. Versura, A. Bron*, V. Profazio, M. Ortolani, C. Coslovi, EC Campos Ophthalmology Unit, University of Bologna, Italy and *Nuffield Laboratory of Ophthalmology, Oxford University, UK

37 SODIUM FLUORESCEIN STAINING OF CORNEAL EPITHELIAL CELLS IN RESPONSE TO WOUNDING: AN *IN-VITRO* EVALUATION. Kalika Bandamwar[1,2], Qian Garrett[1,2] and Eric B Papas[1,2] Brien Holden Vision Institute.[1] School of Optometry and Vision Science, University of New South Wales, Sydney, Australia.[2]

38 WHAT STAINS WITH FLUORESCEIN IN PUNCTATE EPITHELIAL EROSIONS? Maryam Mokhtarzadeh[1], Richard Casey[1], Ben J. Glasgow[1]. [1]Jules Stein Eye Institute, University of California Los Angeles

39 QUANTITATIVE ANALYSIS OF CORNEAL STAINING. Montani Giancarlo, Romano Francesco Institutions: Università Del Salento Formazione Continua In Medicina, Lecce, Italy

40 REPEATABILITY OF GRADING OF REAL EYES VERSUS GRADING OF PHOTOGRAPHS. Heiko Pult[1,2,] Christine Purslow[2], Paul J Murphy[2], Russels L Woods[3] [1]Optometry and Vision Research, Weinheim, Germany, [2]Contact Lens & Anterior Eye Research Unit (CLAER), School of Optometry and Vision Sciences, Cardiff University, UK, [3]Schepens Eye Research Institute, Harvard Medical School, Boston, USA

41 THE ENHANCED CONTROLLED ADVERSE ENVIRONMENT (ECAE) SYSTEM INCREASES WITHIN-SUBJECT RELIABILITY. George W. Ousler III, Joel Naor, Donna Welch, Patrick Johnston, Keith J. Lane. Ora, Inc

42 SCREENING MODEL FOR NOVEL THERAPEUTICS FOR DRY EYE SYNDROME IN A NON-HUMAN PRIMATE. Crawford, KS[1], Torkildsen, G[1], Ousler, GW[1], Lawrence, M[2], Goody, R[2], Campion BK[1], Naor, J[1] [1]Ora, Inc., [2]RxGen Inc.

43 A NEW MODIFIED FLUORESCEIN STRIP: IT'S REPEATABILITY AND USEFULNESS IN TEAR FILM BREAK-UP TIME ANALYSIS. Heiko Pult[1,2], Britta Riede-Pult[1] [1]Optometry and Vision Research, Weinheim, Germany [2]Contact Lens & Anterior Eye Research Unit (CLAER), School of Optometry and Vision Sciences, Cardiff University

44 NORMAL VALUES FOR LISSAMINE GREEN STAINING OF THE OCULAR SURFACE. Christine Purslow & Rachel Tinsley School of Optometry & Vision Sciences, Cardiff University, Cardiff, United Kingdom

45 VARIABILITY OF THE SCHIRMER TEST RESULTS. Hiroko Yamagami, Ayumi Ota, Nozomi Kinoshita, Fumihiko Toyoda and Akihiro Kakehashi. Department of Ophthalmology Jichi medical university, Saitama Medical Center, Saitama, Saitama, Japan

46 EVALUATION OF METHODS EMPLOYED FOR THE QUANTITAION OF TEAR SECRETION. Michelle Senchyna,[1] Ravaughn Williams,[1] Carolyn Begley,[2] Kelly K. Nichols,[3] Sruthi Srinivasan,[3] Jenny Devenport,1 Michael Brubaker.[1] Alcon Research Ltd,[1] Indiana University School of Optometry, [2] The Ohio State University College of Optometry.[3]

47 LACK OF CORRELATION OF COMMONLY USED TESTS FOR THE ASSESSMENT OF SEVERITY OF DRY EYE DISEASE. Benjamin D. Sullivan[1], Anthony J. Bron[2], Christophe Baudouin[3], Gary N. Foulks[4], Kelly K. Nichols[5], Alan Tomlinson[6], Michael S. Berg[1], Michael A. Lemp[7]. [1]TearLab Corp. [2]University of Oxford [3]Quinze-Vingts National Ophthalmology Hospital [4]University of Louisville [5]The Ohio State University [6]Glasgow Caledonian University [7]Georgetown University

48 EVALUATION OF CORNEAL STAINING IN A HEALTHY, NON-DRY EYE POPULATION. Ravaughn Williams,[1] Judy Vittitoe,[1] Michael Brubaker,[1] Michelle Senchyna,[1] Gary Foulks.[2] Alcon Research Ltd,[1] Fort Worth, TX, USA; University of Louisville,[2] Louisville, KY, USA

49 GENDER DIFFERENCES IN DRY EYE DISEASE IMPACT, MANAGEMENT, PATIENT SATISFACTION, AND COMORBID CONDITIONS. Debra A. Schaumberg,[1] Jim Li[2] [1]Div of Preventive Med, Brigham & Women's Hospital, Harvard Medical School, Boston MA; [2] Outcomes Research, Pfizer, Inc., San Diego CA

50 DOES OCULAR IMPRESSION TAKING CAUSE DISTORTION OF THE OCULAR SURFACE? Jennifer Turner, Matthew Dobson, Paul J Murphy, Christine Purslow School of Optometry & Vision Sciences, Cardiff University, United Kingdom

51 THE ENVIRONMENTALLY INDUCED DRY EYE – EXISTING FINDINGS AND CURRENT ASPECTS. Dieter F. Rabensteiner, Jutta Horwath-Winter, Otto Schmut. Department of Ophthalmology, Medical University of Graz, Austria

52 QUANTIFICATION OF FORCES OF MEIBOMIAN GLAND EXPRESSION RELATED TO TYPE OF EXPRESSION AND PAIN. D.R. Korb[1,2], C.A. Blackie [2,1]. [1]TearScience, Morrisville, NC; [2]Korb Associates, Boston, MA

53 A NOVEL THERMAL PULSATION AND INNER EYELID HEAT APPLICATION FOR THE TREATMENT OF OBSTRUCTIVE MEIBOMIAN GLAND DYSFUNCTION. D.R. Korb[1,2], LipiFlow Study Group[2]. [1]Korb Associates, Boston, MA; [2]TearScience, Morrisville, NC

54 INCREASING THE BLINKING RATE USING THE "PISC" DEVICE FOR PATIENTS WITH EVAPORATIVE DRY EYE. Danielle L. Miura, Rossen M. Hazarbassanov, Camila K.N. Yamasato, Jose A.P. Gomes. Department in Ophthalmology and Visual Science, Federal University of Sao Paulo, Sao Paulo, SP, Brazil

55 **Discussion:** 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.

56 EFFICACY OF AZITHROMYCIN 1.5% EYE DROPS IN CHILDHOOD OCULAR ROSACEA. Serge Doan, Melissa Touati, Muriel Catanese, Isabelle Cochereau, Eric Gabison, Hopital Bichat and Fondation A de Rothschild, Paris, France

57 RANDOMIZED STUDY OF THE EFFICACY OF 0.05% CYCLOSPORINE OPHTHALMIC EMULSION IN THE TREATMENT OF MEIBOMIAN GLAND DYSFUNCTION. Pinnita Prabhasawat, Nattaporn Tesavibul, Wannaree Mahawong, Department of Ophthalmology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand

58 MULTICENTER, RANDOMIZED, CONTROLLED, DOUBLE-MASKED, CROSSOVER STUDY ON EFFICACY AND SAFETY OF CYCLOSPORINE A EYE-DROP TREATMENT IN VERNAL KERATOCONJUNCTIVITIS (VKC). M Sacchetti MD, PhD[1], A Lambiase MD, PhD [1], A Leonardi MD[2], V Deligianni MD, PhD[2], F. Mantelli MD[1], S Bonini MD1. [1] Dept. Ophthalmology, University of Rome Campus Bio-Medico, Italy [2] Dept. Ophthalmology, University of Padua, Italy

59 CYCLOSPORINE A PREVENTS ENHANCED NEURAL ACTIVITY OF CORNEAL COLD SENSORY NERVE TERMINALS IN CHRONIC DRY EYE. Illés Kovács[1,2], Susana Quirce[1], Carolina Luna[1], M. Carmen Acosta[1], Carlos Belmonte[1], Xavier Gasull[3], Juana Gallar1.1Instituto de Neurociencias, Universidad Miguel Hernandez-CSIC, San Juan de Alicante, Spain [2]Dept. of Ophthalmology, Semmelweis University, Budapest, Hungary [3]Dept. of Physiology-IDIBAPS, University of Barcelona, Barcelona, Spain

60 RESOLVINS FOR THE TREATMENT OF FRONT OF THE EYE DISEASES. Per Gjorstrup, Resolvyx Pharmaceuticals, Inc., Bedford, MA, USA

61 **Discussion:** TOPICAL JAK INHIBITOR, TASOCITINIB (CP-690,550), MODULATES OCULAR SURFACE INFLAMMATION IN DRY EYE. Jing-Feng Huang, Rolla Yafawi, Min Zhang, Michael McDowell, Kay D Rittenhouse, Frederick Sace, Melissa Liew, Scott R Cooper, Eve H Pickering. Pfizer Inc., San Diego, CA, USA

62 SELECTIVE ANDROGEN RECEPTOR MODULATORS (SARMs) AMELIORATE TEAR LIPID COMPOSITION IN A RABBIT MODEL OF MEIBOMIAN GLAND DYSFUNCTION (MGD). James T. Dalton[a], Jeetendra R. Eswaraka[a], Anand Giddabasappa[a], Jeffrey D. Kearbey[a], France Landry[b], Juhyun Kim[a], Monica M. Jablonski[c]. [a]Preclinical Research and Development, GTx Inc., [b]Merck-Frosst, Montreal, Canada and [c]University of Tennessee Health Science Center, Memphis, TN

63 NEW FORMULATION BASED ON LIPOSOMES LOADED WITH MEDROXYPROGESTERONE FOR DRY EYE TREATMENT. Rocío Herrero-Vanrell[1] ,Marta Vicario[1] ,José Manuel Benitez del Castillo[2], Beatriz de las Heras[3], Manuel Guzmán[4], Irene T. Molina-Martinez.[1,] [1]Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, Complutense University, Madrid, Spain. [2] Unidad Superficie e Inflamacion Ocular (USIO), Hospital Clínico San Carlos, Madrid, Spain. [3]Department of Pharmacology, School of Pharmacy, Complutense University, Madrid, Spain. [4]Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, Alcalá University, Alcalá de Henares, Madrid, Spain

64 EFFICACY AND SAFETY OF DIQUAFOSOL TETRASODIUM OPHTHALMIC SOLUTION IN DRY EYE PATIENTS: A PHASE 2 , RANDOMIZED, DOUBLE-MASKED, PLACEBO-CONTROLLED CLINICAL TRIA.L Yukihiro Matsumoto[1)], Yuichi Ohashi[2)], Hitoshi Watanabe[3)], Kazuo Tsubota[1)] 1) Department of Ophthalmology, Keio University School of Medicine 2) Department of Ophthalmology, Ehime University School of Medicine 3) Department of Ophthalmology, Kansai Rosai Hospital

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72 EFFECTIVENESS OF SINGLE ORAL PILOCARPINE ADMINISTRATION IN PATIENTS WITH SJÖGREN SYNDROME. Aoi Komuro[1, 2], Norihiko Yokoi[2], andShigeru Kinoshita. [1]Department of Ophthalmology, Nishijin Hospital, Kyoto, Japan, [2]Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan

73 THE THERAPEUTIC EFFECT OF EYELID BOTULINUM TOXIN A INJECTION FOR THE DYSFUNCTIONAL TEAR SYNDROME. Jee Taek Kim, Hyun Koo, Jae Chan Kim. Yong-san Hospital, Chung-Ang University

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75 GENE THERAPY TO MODULATE APOPTOSIS AND TO PROTECT ENDOTHELIAL CELLS AGAINST DEATH DURING STORAGE. Fuchsluger TA[1, 2], Jurkunas U[1], [3], Kazlauskas A[1], Dana R[1,3] [1]Schepens Eye Research Institute, Depart. of Ophthalmology, Harvard Medical School, Boston MA, USA; [2]Center of Ophthalmology / Institute of Anatomy, Essen University Hospital Essen, Germany; [3]Mass. Eye and Ear Infirmary, Depart. of Ophthalmology, Harvard Medical School, Boston MA, USA

76 LACRIMA®: THE ITALIAN REGISTER OF PATIENTS WITH TEAR DYSFUNCTION. M. Rolando,[1] S. Bonini,[2] P. Aragona,[3] G. M. Modorati[4] and S. Barabino[1] University of Genoa[1], Genoa, Italy; Biomedical Campus of Rome[2], Rome, Italy; University of Messina[3], Messina, Italy; San Raffaele Institute[4], Milan, Italy

77 EXTERNAL EYE DISEASES GROUP. Petricek Igor[1], Andras Berta[2], Janos Nemeth[3], Mohamed T Higazy[4], Marek Prost[5], Pavel Nemec[6]Head of Electrophysiology and Ultrasound Laboratory, Department of Ophthalmology, Zagreb University Hospital, Zagreb, Croatia[1] Professor, Chairman, Department of Ophthalmology, University of Debrecen, Debrecen, Hungary[2] Professor, Chairman, Department of Ophthalmology, Semmelweis University, Budapest, Hungary[3] Professor of Ophthalmology, Benha University, Heliopolius, Cairo, Egypt[4] Professor, Chairman, Department of Ophthalmology, Military Institute of Aviation Medicine, Warsaw, Poland[5] Department of Ophthalmology, Faculty of Medicine, Charles University, Prague, Czech Republic[6]

6th International Conference on the Tear Film and Ocular Surface: Basic Science and Clinical Relevance

Abstracts

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THE IMPACT OF DRY EYE ON EVERYDAY LIFE (IDEEL) QUESTIONNAIRE: SATURATION, RELIABIILTY, VALIDITY AND DISCRIMINATIVE ABILITY COMPARED TO GENERIC MEASURES Linda Abetz MA[1], Robin Chalmers OD[2], Carolyn Begley OD[3], Polyxane Mertzanis MPH[1], Kitty Venkataraman PhD, Rod Barnes, MBA[4], and IDEEL Study group [1]Mapi Values, Cheshire, UK [2]Clinical Trial Consultant, Atlanta, GA, [3]Indiana University, School of Optometry, Bloomington, IN, [4]Alcon Research Ltd, Fort Worth, TX,

Purpose: To use standard techniques to develop and assess a patient-reported outcomes (PRO) instrument that focuses on the impact of dry eye on everyday life (IDEEL) **Methods**: We conducted focus groups with 45 dry eye (DE) subjects, performed item generation, assessed content validity and tested for saturation to ensure that all areas of relevant DE impact were included. Psychometric validation was conducted in 210 DE subjects, including 32 Sjogrens'and 48 controls. The IDEEL's ability to discriminate patient-rated DE severities was compared to the generic quality of life (QoL) measures, SF- 36 and EQ-5D. **Results**: Qualitative analysis yielded 112 items. 55 items were deleted due to poor psychometric performance and lack of relevance. Saturation was achieved and DE subjects understood all items, indicating content validity. The final 57-item IDEEL met standard psychometric criteria for item discriminant validity (>97% success), internal consistency (=0.7-0.97) and test-retest (ICC=0.82-0.94) reliability. IDEEL domains include: symptom bother, impact on daily activities, emotional impact, impact on work and treatment satisfaction (satisfaction with treatment effectiveness, treatment related bother & inconvenience, treatment frequency). When examining the strength of differences between patient-rated severity levels, IDEEL scales significantly discriminated between severity levels (Range: $F=7.24$, $p<0.01$ to $F=148.81$, $p<0.001$) and consistently outperformed SF-36 ($F=4.0$, $p<0.01$ to $F=8.64$, $p<0.0001$) and EQ5D ($F=9.34$ and $F=10.61$, $p<0.001$).**Conclusion**: The IDEEL is a comprehensive, reliable, and valid dry eye specific PRO measure that was developed using standard methodology and meets FDA guidelines. Among DE subjects, it outperforms other generic measures of health related QoL outcome.

ANALYZATION OF LONG TERM CULTURED LACRIMAL GLAND STEM CELLS. Philipp Ackermann,[1] Anja Richter,[2] Friedrich Paulsen [1,3] Department of Anatomy and Cell Biology, Martin Luther University Halle-Wittenberg, Halle, Germany [1]; Fraunhofer Insitute of Marine Biotechnology, Fraunhofer Institute, Lübeck, Germany [2]; Department of Anatomy II, Friedrich Alexander University Erlangen-Nürnberg, Erlangen, Germany [1,3]

Purpose: Aqueous tear deficiency due to lacrimal gland insufficiency is one of the major causes of dry eye syndrome. In severe cases such as Sjogren syndrome, Stevens-Johnson syndrome or ocular mucous membrane pemphigoid, the therapy with artificial tears can be insufficient to relieve severe discomfort. Aim of this study was to detect lacrimal gland stem cells. To get into the matter we started with an analysis of murine lacrimal glands for markers of pluripotency, self- renewal and differentiation. As the amount of stem cells in differentiated tissue is particularly low, it is needed to separate stem cells from adult cells e.g. lacrimal gland cells and fibroblasts to further show pluripotency. **Methods.** Using a unique technique of the Fraunhofer-Institut of Marine Biotechnology/Lübeck with mechanical and enzymatic digestion of adult tissues we were able to isolate potential lacrimal stem cells and culture them over a longer period. Lacrimal gland stem cells were then grown until confluence in 7-10 days. Cells were photographed to visualize changes in cell morphology. To demonstrate cell migration we used time-lapse microscopy. To analyze pluripotency of the cells RT-PCR and immunohistochemistry as well as Western blot analysis were used to demonstrate mRNA and protein of different pluripotency markers. **Results.** The isolated cell type could be cultured over more than 18 passages and is still be cultured. Nanog, Sox2 and C-Myc as markers for pluripotency were detected. Additionally, we analyzed early and late lineage markers, therefore BMP-4, smooth muscle Actin, GATA-4 and GATA-6, Nestin and other lineage markers were experimentally verified. **Conclusions.** We have isolated and cultured lacrimal gland stem cells successfully. These cells have the ability to differentiate into all 3 germ layers. The results are a first step to get deeper insights into lacrimal gland stem cell physiology.

IN VIVO CONFOCAL MICROSCOPY ANALYSIS OF EFFECTS OF SYSTEMIC ISOTRETINOIN TREATMENT ON CORNEAL INNERVATION AND MORPHOLOGY Yonca A Akova, Sevda Metindoğan, Aylin Karalezli, Department of Ophthalmology, Baskent University, TURKEY

Objective: To evaluate the ocular surface changes and tear film functions in patients treated with systemic isotretinoin. **Methods**: Twenty one subjects treated with 0.8 mg/kg oral isotretinoin were enrolled in this clinical trial. All patients underwent an ophthalmic examination at baseline, on the 30th and 90th days of treatment. In each patient, a complete ophthalmic examination was performed, including best-corrected Snellen visual acuity, slit-lamp examination,anesthetized Schirmer test, tear film break-up time (BUT), Lissamin green staining, conjunctival impression cytology, confocal microscopic evaluation and ophthalmoscopy through a fully dilated pupil. Subjective ocular complaints were scored with an Ocular Surface Disease Index (OSDI) questionnaire. **Results**: Mean anaesthetized Schirmer's test scores and BUT decreased significantly during treatment ($p<0.05$, $p<0.001$respectively).Mean impression cytology scores, OSDI scores and Lissamin green staining scores increased significantly during treatment ($p<0.01$, $p<0.001$, $p<0.01$, respectively). Blepharitis, meibomitis, hyperemia, and vascularization of the lid margins were significantly more frequent at days 90 (17 patients 80,95%) compared to the beginning of the study (2 patients 9,52%) ($p<0.01$). There were no cellular morphology changes of corneal epithelium and stroma by confocal microscopic evaluations. Dendritic cells were increased in subepithelial nerve plexus in 4 patients (19.04%) on day 30 and in 11 patients on day 90 (52.38%) respectively ($p<0.001$). **Conclusion**: Our data show that conjunctival epithelial cells, basal tear secretion, and tear quality are markedly affected in patients during treatment with systemic isotretinoin (0.8 mg/kg). Elevation of the number of dendritic cells in subepithelial plexus by confocal microscopic evaluation may be due to the inflammatory nature of changes induced by the isotretinoin treatment.

PREDICTORS OF SJÖGREN'S SYNDROME IN PATIENTS WITH DRY EYE Esen K. Akpek, M.D. [1]; Ramya Swamy, MPH[1]; Canan Asli Utine, M.D., M.S. [1, 2], Jennifer Thorne, M.D.[1], Alan N. Baer, M.D.[3], [1]The Wilmer Eye Institute, The Johns Hopkins University School of Medicine, Baltimore, Maryland. [2] Yeditepe University Eye Hospital, Istanbul, Turkey. [3]Division of Rheumatology, Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland.

Objective: To determine whether medical history, ocular exam characteristics, and serologic test results of individuals who present with symptoms of dry eye can be used to predict eventual diagnosis of Sjögren's Syndrome (SS). **Methods:** Medical records of patients with a primary diagnosis of dry eye syndrome (International Classification of Diseases code 375.15, 370.33 and 710.2) were

reviewed retrospectively. These individuals had presented to the Dry Eye clinic between January 2002 and May 2009. Among these individuals, those who underwent additional serologic work-up for SS were selected for the study. **Results:** Among 1590 individuals who were referred with symptoms of dry eye syndrome, 237 underwent additional serologic work-up for suspected SS and were selected for further analysis. Ninety two (38.8%) of these individuals were eventually diagnosed with SS based on the revised European-American criteria. In a univariate analysis, gender, age, duration of symptoms, and presence of other co-morbid rheumatologic conditions did not differ significantly between the SS and non-SS groups. Features significantly associated with an eventual SS diagnosis included presence of dry mouth (P=0.015), Raynauds (P = 0.023), corneal staining (P=0.003), family history of SLE (P = 0.044), diabetes mellitus type I (P = 0.033), anti-SSA (OR = 40.2), anti-SSB (OR = 35.4), and RF (OR = 5.6). ANA correlated in a dose responsive fashion with higher titers correlating with increasing likelihood of SS diagnosis (titer 1:640, OR = 7.4). **Conclusions:** Primary SS is a common cause of dry eye and should be the focus of diagnostic evaluations. The study identifies additional factors on review of systems, history, ocular exam and serology to identify individuals who have higher odds of being diagnosed with SS. The results of the study can be used as a guide to select for individuals with dry eye who should undergo additional serologic testing for diagnosis and to initiate treatment. Research has no financial relationships –only Research to Prevent Blindness (RPB) support

SENSITIVITY AND SPECIFICITY OF A MODIFIED TEAR BASAL SECRETION TEST AND SCHIRMER'S I TEST IN SJÖGREN'S SYNDROME DIAGNOSIS Pasquale Aragona, Rosaria Spinella, Anna Roszkowska, Laura Rania, Elisa Postorino. Department of Ophthalmology, University of Messina, Italy

Objective: Sjögren's syndrome (SS), is a systemic autoimmune disease characterized by decreased lacrimal gland function and dry eye. Schirmer's I is the test suggested to detect an objective involvement of the lacrimal gland in the criteria for SS diagnosis.Its usefulness is biased by a low sensitivity, which does not allow the diagnosis of lacrimal involvement in the early stages of the disease. Aim of the present work is to compare sensitivity and specificity of Schirmer's I test with that of a modified tear basal secretion test in SS patients. **Methods**: This is a retrospective, comparative evaluation of diagnostic test. Participants: Three hundred and thirty-four subjects (186 patients whose final diagnosis was both primary or secondary SS, according to the items of the American-European Consensus Criteria; 42 patients with systemic autoimmune disorders without SS and 106 patients with non autoimmune ocular surface diseases). Clinical records of patients were divided according to the diagnosis in: SS patients, patients with systemic autoimmune disorders without SS and patients with non autoimmune ocular surface diseases. Only the systemic parameters were used for the diagnosis of SS, without considering the ocular symptoms and signs. To assess lacrimal gland secretion Schirmer's I test and a modified tear basal secretion test were considered. Tear basal secretion was performed under a topical anesthesia of the ocular surface.Main Outcome Measures: Schirmer's I test with a cut-off value of ≤5 mm/5' and modified basal secretion test with cut-off values of both ≤5 mm/5' and ≤3 mm/5' were considered. **Results**: Schirmer's I test showed a sensitivity of 34.6% and a specificity of 96.6%. Basal secretion test (≤5 mm/5') showed a sensitivity of 74.2% and a specificity of 85.1%, while basal secretion test (≤3 mm/5') showed a sensitivity of 66.02%and a specificity of 97.3%. **Conclusions**: The modified basal secretion test, with a cut-off value of ≤5 mm/5', showed a better balance between sensitivity and specificity. Performing both Schirmer's I test and the modified basal secretion test can give a better assessment of both reflex and basal tear function in SS patients.

EXPRESSION OF MATRIX METALLOPROTEINASES 7 AND 14 IN UV IRRADIATED CORNEA Taras Ardan, Jitka Cejkova. Institute of Experimental Medicine, Academy of Sciences of the Czech Republic

Objective: Many ocular inflammatory diseases are associated with an increased incidence of matrix metalloproteinases (MMPs). Based on our previous findings dealing with the coincidence of the expression of proteolytic enzymes (MMP-2 and MMP-9) with the severity of corneal injury induced by UV irradiation, the aim of this study was to investigate the effect of UVA and UVB rays on expression of MMP-7 and MMP-14 in the corneal epithelium. **Methods:** In the first group of rabbits the corneas were irradiated with UVA rays (365 nm, once a day during 4 days, a daily dose 1.01 J/cm^2), in the second group with UVB rays (312 nm, once a day during 4 days, a daily dose 1.01 J/cm^2). MMP-7 and MMP-14 were examined immunohistochemically using mouse monoclonal anti-MMP-7 and anti-MMP-14 antibodies. **Results:** Results show that UVA rays does not change the expression of MMPs studied whereas UVB rays induce the increased expression of MMP-7 in corneal epithelial cells. MMP-14 was expressed in very low levels in all corneas studied.**Conclusion:** Comparing the effect of the same doses of UVA and UVB rays on the normal rabbit cornea, UVB rays /not UVA rays/ evoked the increased expression of MMP-7 in the corneal epithelium. To investigate the importance of these findings is the aim of our next study.

ENDOGENOUS SECRETORY GROUP IIA PHOSPHOLIPASE (sPLA2-IIa) AMPLIFIES INFLAMMATION AT THE OCULAR SURFACE. Penny Asbell, Yi Wei, Seth Epstein. Department of Ophthalmology, Mount Sinai School of Medicine of New York University, New York, NY 10029, USA

Objective: To examine the role of sPLA2-IIa in amplification of the inflammatory response of the compromised ocular surface. **Methods:** sPLA2-IIa activity was measured in tears of subjects with dry eye (DE) disease. sPLA2-IIa effects on the compromised ocular surface were evaluated in human conjunctival tissue cultures and in BABL/c mice with DE induced by scopolamine injection in an air-drying device. Quantitative (q) RT-PCR was performed in an ABI7900HT Sequence Detection System using RT^2 qPCR Master Mix and established primers from SABiosciences. Data represented an average of 6 repeats and normalized with -actin and GAPDH. **Results:** sPLA2-IIa activity was 2-fold higher in tears from patients with non-Sjogren's severe DE disease than in normal age-matched controls. PCR results indicated that elevated human tear sPLA2-IIa activity was the result of gene up-regulation within the conjunctival epithelia. The additon of sPLA2-IIa to cultures of human conjunctival epithelial cells caused minimal to null increase in PGE2 secretion in control but caused large increases when these cells were pre-treated with pro-inflammatory cytokines (TNF- or IL-1). The experimentally induced DE increased sPLA2-IIa gene transcription in conjunctival epithelia associated with inflammation of the ocular surface.**Conclusions:** sPLA2-IIa can play a significant role in amplification of the inflammatory process at the ocular surface. Understanding the role of sPLA2-IIa in ocular inflammation will help develop better strategies for the treatment of the chronic inflammation associated with dry eye conditions and/or acute inflammatory flares. The authors have no commercial relationships to be disclosed. This research is supported by NIH fund (1R34EY017626) and Martin and Toni Sosnoff foundation.

TRAVERSAL OF CORNEAL EPITHELIAL CELLS BY *P. AERUGINOSA*: BACTERIAL ADAPTATION REVEALS GENES THAT CONTRIBUTE TO THE PROCESS. Danielle Augustin[1], David Evans[1,2], Suzanne Fleiszig[1]. University of California, Berkeley, Berkeley, CA, USA[1]; College of Pharmacy, Touro University, Vallejo, CA, USA[2].

Purpose: Bacterial keratitis caused by the opportunistic pathogen *Pseudomonas aeruginosa* is a sightthreatening corneal disease. Factors that modulate bacterial traversal after bacteria adhere to epithelia are not well understood. Here we explored how *P. aeruginosa* penetration of corneal epithelial cell multilayers impacts bacterial gene expression, as a first step towards identifying virulence strategies relevant to disease initiation. **Methods.** Multilayered telomerase-immortalized human corneal epithelial cells (HCE) were grown on 3 µm pore-size Transwell filters and inoculated with *P. aeruginosa* (strain PA01, ~106 CFU) at the apical surface. After 8 h, traversed bacteria were collected from the basal compartment, and gene expression compared to naïve bacteria (grown in tissue culture media without cell exposure) using Affymetrix Genechip® *P. aeruginosa* Genome Arrays. Genes upregulated > 2.7-fold were selected for functional analysis. Transposon mutants in impacted genes were examined for capacity to replicate (eliminates auxotrophs), before comparing each mutant to wild-type for cell traversal. Intracellular bacteria were quantified by gentamicin survival assays. **Results.** 517 bacterial genes were upregulated by epithelial cell traversal. Of 100 transposon mutants in impacted genes screened, 11 showed loss of function, including one known virulence factor regulator. For one mutant, more bacteria were found intracellular 4 h, but not at 8 h, post-inoculation compared to wild-type bacteria (p= 0.0004). Interestingly, 4 mutants had *enhanced* traversal capacity, including one in an Exotoxin A repressor. **Conclusion.** Determining mechanisms by which involved genes modulate cell traversal will improve our understanding of *P. aeruginosa* infection at epithelial sites, and could reveal targets for novel therapies that interfere with disease initiation. Support: NEI grants RO1 EY011221 (SF) and F31 EY19456 (DA)

TEAR PROTEIN LEVELS IN KERATOCONUS. Sivaraman A. Balasubramanian[1,2,] David C. Pye[2], Mark D.P.Willcox[1,2] [1]Brien Holden Vision Institute, [2]School of Optometry and Vision Science, University of New South Wales, Sydney, Australia.

Purpose: Keratoconus is a degenerating disease of the eye which causes an irregularly shaped cornea. The early detection of keratoconus is difficult as slit-lamp corneal changes are absent or too subtle to detect and keratometry may be normal. This research was designed to determine whether there were changes to the tear film of people with keratoconus. **Methods:** A case-controlled study was performed studying the tear proteome of keratoconus patients (case) and normal subjects (control). Basal tears were collected using a capillary tube. Total protein in tears was estimated using BCA assay, and the amount of the regulated protein lactoferrin or constitutive protein sIgA were measured using specific ELISAs. **Results:** There was a two-fold (p<0.0001) decrease in total protein levels between keratoconus (3.86 +1.62 mg/ml) and normal (7.00+1.58 mg/ml) tears. The amount of lactoferrin (0.67 + 0.28 vs. 1.13 + 0.29 mg/ml) and secretory IgA (0.78 + 0.36 vs. 1.70 + 0.66 mg/ml) were also significantly (p<0.0001) reduced in keratoconus tears. The differences in tear proteins was not associated with contact lens wear, age, gender or atopy of subjects. The reduction in total protein was correlated to the degree of corneal asphericity. **Conclusions:** Thetears of keratoconics appear to have a profoundly altered protein profile, and one that might change with the severity of the disease. These findings may lead the way to understanding or monitoring disease progression.

MORPHOLOGIC EVALUATION OF MEIBOMIAN GLANDS IN CHRONIC GRAFT-VERSUS-HOST DISEASE USING IN VIVO LASER CONFOCAL MICROSCOPY Yumiko Ban,[1,2]Yoko Ogawa,[1]Osama M.A. Ibrahim,[3]Yukako Tatematsu,[1]Murat Dogru,[3]Kazuo Tsubota[1]. Department of Ophthalmology, School of Medicine, Keio University, Tokyo, Japan[1] Department of Ophthalmology, Hino Municipal Hospital, Tokyo, Japan[2] Johnson and Johnson Ocular Surface Visual Optics Department, School of Medicine, Keio University, Tokyo, Japan[3]

Purpose: To evaluate the morphological changes of the meibomian glands (MG) using in vivo confocal microscopy (CM) in dry eye (DE) patients with chronic graft versus host disease (cGVHD). **Methods:** Nine eyes of 5 patients diagnosed as DE associated with cGVHD (3 males, 2 females; median 48.9 years) and 10 eyes of 5 HSCT recipients without DE (5 males; median 44.6 years) were enrolled. CM was used to investigate the MG and MG acinar unit density (MGAUD), the fibrosis grading, MG acinar longest diameter (MGALD) and MG acinar shortest diameter (MGASD) were measured. Clinical findings in the lid margin were studied. Tear dynamics, ocular surface vital staining, meibography and MG expressibility were also examined. Data were compared between the 2 groups using the Mann-Whitney test. **Results:** The mean value of MGAUD was significantly lower in DE patients with cGVHD than HSCT recipients without DE. (p = 0.03, 36.9 ± 24.8 glands/mm^2, 79.2 ± 21.4 glands/mm^2, respectively) The mean fibrosis grading was significantly greater in DE patients with cGVHD than HSCT recipients without DE. (p = 0.001, 1.67 ±0.61 grade, 0.10±0.32 grade, respectively) The mean MGALD and MGASD were significantly shorter than in DE patients with cGVHD than HSCT recipients without DE. (p = 0.0004, 25.5 ± 14.3µm and 58.0 ± 13.7µm, p = 0.008, 14.3 ± 7.88µm and 25.3 ± 5.94µm, respectively) The clinical findings in the lid margin, tear dynamics and ocular surface findings were significantly worse in DE patients with cGVHD than HSCT recipients without DE. **Conclusion:** CM can effectively demonstrate the morphological changes of the MG in DE patients with cGVHD. The authors have no commercial interest. This study was supported by grant #22791690 from the Japanese Ministry of Education, Culture, Sports, Science, and Technology. (Tokyo, Japan)

SODIUM FLUORESCEIN STAINING OF CORNEAL EPITHELIAL CELLS IN RESPONSE TO WOUNDING: AN *IN-VITRO* EVALUATION. Kalika Bandamwar[1,2], Qian Garrett[1,2] and Eric B Papas[1,2] Brien Holden Vision Institute.[1] School of Optometry and Vision Science, University of New South Wales, Sydney, Australia.[2]

Purpose: In-spite of its widespread use, the precise staining mechanism of sodium fluorescein on the ocular surface is not yet clear. The general understanding is that normal healthy corneal epithelium doesn't stain unless it experiences stress. The purpose of this study was to determine
if the fluorescein staining properties of cultured epithelial cells differ under normal and stressed conditions. **Methods:** Human corneal limbal epithelial cells (HCLE) were grown to monolayer using standard cell culture technique and were transferred to specialized medium to allow
stratification. Selection of stress stimuli was made to mimic real life conditions resulting in a clinical staining response. Those selected were scratch wound, alkaline wound, hypotonicity, hypertonicity, and exposure to preservatives. After exposure to stress stimuli, cells were washed once with buffered saline (BS) and incubated with 1% fluorescein for 1 minute and rinsed vigorously thrice with BS before imaging by fluorescent microscopy. Propidium iodide (PI), a nucleic acid dye, was used to detect necrosis. **Results:** Both monolayer and stratified HCLE cells took up fluorescein under normal conditions. With either scratch or alkaline wounding, cells close to the wound

edge showed hyperfluorescence compared to the cells further away. Hypotonicity resulted in cell swelling and increased uptake of fluorescein. Hypertonic conditions resulted in cell shrinkage and necrosis; these cells did not stain with fluorescein. Exposure to preservatives resulted in cell rounding and detachment. Non-specific diffuse fluorescein staining was observed in this case. Irrespective of stress type, necrotic cells took up minimal fluorescein. **Conclusions:** When exposed to fluorescein, damaged cells show hyper-fluorescence compared to both necrotic and healthy cells. Fluorescein staining of corneal epithelial cells may indicate compromised membrane permeability, but not dead cells.

A NEW PORTABLE DIGITAL MENISCOMETER. Stefan Bandlitz[1,2], Heiko Pult[1,3], Christine Purslow[1], Paul Murphy[1], Anthony J. Bron[4].[1]School of Optometry and Vision Sciences, Cardiff University, Cardiff, UK; [2]Cologne School of Optometry, Cologne, Germany; [3]Optometry and Vision Research, Weinheim, Germany; [4]Nuffield Laboratory of Ophthalmology, University of Oxford, Oxford, UK.

Objective: Reflective meniscometry is a non-invasive method to measure the tear meniscus radius (TMR), useful in dry eye diagnosis. We developed a portable, slit-lamp mounted, digital device (PDM) and compared its accuracy and reproducibility with the standard video-meniscometer (VM), *in vitro* and *in vivo*. **Methods:** The medians of three consecutive measurements on 5 glass capillaries (radii 0.100 to 0.505 mm) were compared between VM and PDM at two different sessions. Also, the lower tear meniscus radius (TMR) in 20 normal subjects (10M, 10F; mean age 32.3 SD ± 9.3 years) was measured using both techniques. Differences between sessions and instruments were analyzed using Bland-Altman plots, coefficient of repeatability (CR) and paired t-tests. **Results:** The PDM and VM were accurate *in vitro* (95% CI of difference: PDM - 0.0134 mm to + 0.0074; p=0.468; VM - 0.0282 to + 0.0226; p=0.775), and reproducible between sessions (95% CR: 0.019 and 0.018 respectively). The mean difference between the PDM and VM was 0.0002 (CI – 0.0252 to + 0.0256; p=0.984). In human subjects, there was no significant difference between the mean TMR measured with the PDM (0.34 ± 0.10 mm) and the VM (0.36 ± 0.11) (p=0.124). **Conclusions:** This new slit-lamp mounted digital meniscometer appears accurate and reliable, and provided similar values for tear meniscus radius in human studies, to the existing video-meniscometer. The instrument appears suitable for use in both research and clinical practice.

TEAR FILM: EPITOPES FACING THE OUTSIDE WORLD. Sarah Baos[1,2], Terence McMastera, David Phillipsa, Monica Berry[1] [1]HH Wills Physics Laboratory and [2]Academic Unit of Ophthalmology, University of Bristol, Bristol, UK

Purpose: We used atomic force microscopy (AFM) to establish intermolecular bond energies, relative availability ofpitopes to the molecular probe, and whether the spatial distribution of sialic acids is different in isolated molecules and mucins within a gel. **Methods:** AFM tips were functionalised with *Maackia amurensis* and *Sambucus nigra* and antibodies against mucin peptide core epitopes of MUC5AC and MUC16. Force-volume maps, spatially-correlated topographic and force-spectroscopy data, were used to quantify the localization, number, rupture force and rupture distances of recognition bonds. The loading rate dependence of the rupture force – increasing bond strength paralleling the increase in rate of force applied – was used to determine koff rates for the interactions. Statistical analysis was performed to confirm the occurrence of nearest-neighbour interactions, and the non-randomness of the observed clustering of specific moieties on gels and single molecules. **Results:** The specificity of interactions was confirmed by a vast decrease in their frequency when blocking sugars or peptides were used. The median rupture forces were similar irrespective of sample origin and density. All epitopes showed levels of clustering significantly higher than randomly distributed events. MUC16 epitopes were presented in clusters at the ocular surface and on purified mucins. Four times more -2,3- than -2,6 sialic acids were encountered on purified mucins, while in impressions this ratio was reversed, confirming both previous biochemical results. The observed clustering of sialic acids is expected when probing a highly glycosylated region of the mucin molecule. Not all -2, 3 sialic acids are available to the probing lectin when mucins are part of a gel, as reflected in the smaller cluster size. **Conclusions:** Our results point towards a highly heterogeneous ocular surface, whose restricted islands of receptors are likely to restrict the number of adhering microbiota. This work was supported by the Leverhulme Trust. No commercial interests.

DRY EYE-LIKE SYMPTOMS AND SIGNS AFTER CATARACT SURGERY. Stefano Barabino, Federico Solignani, Cristiana Valente, Maurizio Rolando OcularSurface Research Center, Department of Neurosciences, Ophthalmology, and Genetics, University of Genoa, Genoa, Italy.

Objective: An unclear consequence of cataract surgery is ocular surface discomfort reported by a high number of patients. The aim of our project was to investigate symptoms of dry eye and signs of ocular surface damage in normal subjects after cataract surgery. **Methods**: Forty subjects who underwent phacoemulsification for cataract extraction were assessed by means of visual analogic scale, Schirmer I test, tear break-up time (BUT), lissamine green conjunctival staining (NEI scoring system), and fluorescein corneal staining before surgery and 1, 7, 30, and 60 days after surgical procedures with corneal access performed on the corneal temporal side with a width of 2.75 mm . The Dynamic Lipid Interference Pattern (DLIP) test was used to quantify tear film stability at each follow up. **Result:** Statistically significantchanges in symptoms, corneal fluorescein staining, lissamine green conjunctival staining, BUT and DLIP occurred in the study group at day 1 and 7. At day 30 changes were recorded compared to baseline for BUT and DLIP, and at day 60 for DLIP only. Schirmer test did not show any significant changes throughout the study. **Conclusion**: This study indicates that cataract surgery may induce a clinical picture similar to dry eye, pointing out possible risks in patients with previous ocular surface diseases. Further studies are necessary for a better comprehension of the consequence of cataract extraction on the ocular surface, and on the effect of dry eye-like therapy.

EFFECTS OF THE COMBINATION OF HYALURONIC ACID AND TAMARIND SEEDS POLYSACCHARIDE IN THE MANAGEMENT OF DRY EYE. Stefano Barabino, Cristiana Valente, Guia Corsi, Maurizio Rolando. Ocular Surface Research Center, Department of Neurosciences, Ophthalmology, and Genetics, University of Genoa, Genoa, Italy.

Purpose: Nuclear magnetic resonance spectroscopy demonstrated synergistic interactions between hyaluronic acid (HA) and tamarind seeds polysaccharide (TSP) inducing the formation of supramolecular aggregates able to stabilize the tear film. Our pilot study wanted to investigate the potential clinical benefit of the combination of HA and TSP in managing dry eye (DE). **Methods**: Ten patients with DE, confirmed by Schirmer-1 test (without anesthesia) < 10 mm/5', tear break-up time (BUT) < 8", fluorescein staining of the cornea with NEI/Industry workshop Scale grade > 3, positive lissamine green staining of the conjunctiva into the inter-

palpebral fissure (NEI score system) were treated 4 times a day with a HA 0.2% / TSP 0.2% solution for 28 days. Ten patients with the same diagnosis who underwent commercial tear substitutes 4 times a day were used as controls. **Results**: After 14 and 28 days of treatment the two groups of patients had the same subjective benefit in the relief of symptoms of DE and in the stabilization of the lachrymal film (BUT) compared to baseline. No significant changes were recorded in tear secretion. Results of Lissamine green staining of the conjunctiva indicate a statistical significant improvement in patients treated with HA 0.2% / TSP 0.2% compared to controls at the 14 ($p<0.05$) and 28 day ($p<0.01$) follow up. **Conclusions**: The results of this study suggest that HA 0.2% / TSP 0.2% solution might have a role in promoting the repairing processes of the damaged conjunctival epithelium in DE. Due to the small sample size of this study these observations need further confirmation. The authors have no commercial relationship.

IMMUNE RESPONSE IN THE CONJUNCTIVAL EPITHELIUM AND OCULAR SURFACE DAMAGE IN PATIENTS WITH DRY EYE. Stefano Barabino[1], Cristiana Valente[1], Elisa Montaldo[2], Maria Cristina Mingari[2,3], Maurizio Rolando[1]. [1]Ocular Surface Research Center, Department of Neurosciences, Ophthalmology, and Genetics, University of Genoa; [2]Department of Experimental Medicine, University of Genoa; [3]Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy.

Objective: To test the hypothesis that patients with dry eye have a significant degree of lymphocyte infiltration in conjunctival epithelium that correlates with ocular surface damage. **Method**: Impression cytology specimens were collected in 21 dry eye patients and 16 healthy controls and placed in culture medium containing 10% foetal calf serum (FCS). Cells were stained for the expression of CK19, CD3, CD4, CD8, CD56, CD19, CD20, CD14 and HLA-DR, and analyzed by flow cytometry. Schirmer test, tear break-up time (BUT), corneal fluorescein and conjunctival lissamine green staining were performed in both groups. **Result**: No statistically significant differences were present in the percentage of CD45+CK19- cells, CD3+ and CD4+ T cells, B and NK cells in dry eye patients compared to healthy controls. In the dry eye group there was a significant difference in the CD4/CD8 ratio respect to what observed in normal subjects, and an increased number of CD14+ cells. HLA-DR expression was increased only in CK19+ conjunctival epithelial cells of dry eye patients. Significant correlation was found between the number of CD14+ cells and lissamine green staining of the conjunctiva. **Conclusion**: This study indicates that immune cells isolated from the superficial layer of the conjunctiva may play a pivotal role in the pathogenesis of dry eye and ocular surface damage.

EFFECTS OF TOPICAL DRUG PRESERVATIVES ON THE TEAR FILM AND OCULAR SURFACE. Christophe Baudouin, Quinze-Vingts National Ophthalmology Hospital, Vision Institute, University Paris 6, Paris, France

There is large evidence from experimental and clinical studies that the long-term use of topical drugs may induce ocular surface changes, causing ocular discomfort, tear film instability, conjunctival inflammation, subconjunctival fibrosis, epithelial apoptosis, corneal surface impairment, and the potential risk of failure for further glaucoma surgery. Subclinical inflammation has also been widely described in patients receiving antiglaucoma treatments for long periods of time. The most frequently used preservative, benzalkonium chloride (BAK), has consistently demonstrated its toxic effects in laboratory, experimental, and clinical studies. As a quaternary ammonium, this compound causes tear film instability, loss of goblet cells, conjunctival squamous metaplasia and apoptosis, disruption of the corneal epithelium barrier, corneal nerve impairment and potential damage to deeper ocular tissues. Preservative-induced adverse effects are therefore far from being restricted to only allergic reactions, and side effects are often very difficult to identify because they mostly occur in a delayed or poorly specific manner, and result from complex and multifactorial interactions with the ocular surface. Indeed, mild symptoms should not be underestimated, neglected, or denied, because they may very well be the apparent manifestations of more severe, potentially threatening inflammatory reactions that may later cause major concerns. Even though the issues raised by preservatives are limited in the short-term, the use of preserved eye drops thus induces over the long-term side effects, which may affect the ocular surface. In glaucoma, these side effects deeply impact quality of life, may decrease treatment compliance and enhance the failure of glaucoma surgery, resulting in inadequate control of intraocular pressure. Alternatives to BAK are currently under development, including preservative-free solutions or new low-toxicity preservatives. Therefore, on the basis of all these experimental and clinical reports it is advisable to use BAK-free solutions for long-term treatments, especially in patients with already damaged ocular surface, such as in dry eye disease or allergic eyes.

THE EFFECTS OF TEAR FILM INSTABILITY ON VISION. Carolyn G. Begley, Indiana University School of Optometry, Bloomington, IN, USA

According to the 2007 DEWS report definition of dry eye (DE), visual disturbance is a symptom of dry eye. Many published reports note that blurry vision is common among DE patients of all types and these and other symptoms adversely affect quality of life. The most likely cause of the visual disturbances in DE is tear instability or break-up over the pupil, which greatly impacts vision due to the high refractive index difference between air and tear film. Another cause may be an irregular corneal surface in DE, which exerts an especially large visual effect when the tear film is disrupted over the defective corneal surface. Thus there is good reason to believe that tear instability, targeted by the 2007 DEWS report as a core mechanism of DE, is the main cause of the symptom of visual disturbances in DE. The exact nature of this effect of vision is transient and changeable because tear instability can develop very rapidly in DE, and its location and spatial distribution often changes with every blink. We propose that the visual symptoms in DE arise from two basic mechanisms that occur during tear instability or break-up. (1) Initially, an irregular tear film thickness leads to macro aberrations due to "waves" that have formed in the tear film during break-up. (2) If tear break-up continues and the breach in the tear film exposes the mucin covered corneal epithelium, rapid evaporation should expose a microscopically rough corneal surface that scatters light, producing micro aberrations. Therefore, we hypothesize that the visual disturbances in DE should vary with the spatial location, timing and the severity of tear break-up.

IMPACT OF DIFFERENT CONTACT LENS MATERIALS ON MUCIN FRAGMENTATION: RELATION TO SYMPTOMS. M. Berry[1], Paul Murphy[2], Christine Pursolow[2], H. Pult[2,3]; [1]Academic Unit of Ophthalmology, University of Bristol, Bristol Eye Hospital, Bristol, United Kingdom; [2]Contact Lens and Anterior Eye Research (CLAER) Unit, School of Optometry and Vision Sciences, Cardiff University, Cardiff, United Kingdom. [3]Optometry and Vision Research, Weinheim, Germany

Purpose: Mucins adhere to contact lenses (CL), reflecting the renewal of the preocular fluid and enzymatic activity at the ocular surface.. The study aimed to analyse mucin fragmentation on materials new to the ocular surface, and whether this correlates with wearing comfort.

Methods: Lenses were obtained from new CL-wearers after 2 weeks each of wearing vifilcon A, followed by senofilcon A, followed by vifilcon A lenses. Symptoms were evaluated by the Ocular Surface Disease Index. CL`s were extracted in a mixture of GuHCl and RIPA buffer. Mucin mobility was analysed after electrophoresis on 4-12% NuPage gels with MES buffer, Western blotting and visualisation with antibodies against mucin peptide core. Mobilities, normalised to total reactivity in the lane, were compared between visits for each subject, and expressed as shifts. **Results:**. MUC5AC polymers exceeding 260kDa were observed in agarose gels. NuPage resolved polymers from 260 to 3.5kDa: when large mucins were detected, the smallest fragments (under 15KDa) were missing. Fragmentation patterns were significantly different between lens types: MUC1 (ANOVA; p=0.006) and MUC4 (p<0.001) but not for MUC5AC or MUC16 (p>0.293). Mobility shifts of MUC1 and MUC4 were significantly negatively correlate (Pearson; r=-0.908, p=0.002). In OSDI score >15 mucin fragmentation was unchanged, while for OSDI scores <15 MUC4 and MUC5AC fragments were longer in vifilcon A lenses than senofilcon lenses (unpaired t-test, p<0.046), irrespective of direction of change (ANOVA, p>0.366). Changes in MUC1 break-down was significantly negatively correlated to overall OSDI scores (r=-0.891, p=0.001). **Conclusion**: In CL wearers without symptoms of dry eyes changes in mucin fragmentation in response to a new material were consistent and fast, irrespective of contact lens order.

AMNIOTIC MEMBRANE TRANSPLANTATION: OUR EXPERIENCE. Soniya Bhala, Sudesh Kumar Arya, Archana Malik, Sunandan Sood Department of Ophthalmology, Government Medical College and Hospital, Chandigarh, India

Purpose: To evaluate the clinical applications of amniotic membrane transplantation and its outcome in ocular surface disorders and forniceal reconstruction. **Methods**: Retrospective analysis of 79 eyes of 76 patients who underwent amniotic membrane transplant (AMT) for ocular surface disorders or infective keratitis was done. Cryopreserved AMT (-80 degrees) was used after thawing. The indication for AMT, visual acuity, size of epithelial defect, size of infiltrates, extent of superficial and deep vascularisation, any complication, discomfort at first and last visits, and surgical details were noted. The patients were followed up for minimum period of 3 months. **Results**: The mean age was 38.60 ± 13.9 yrs (range 9-78 yrs) and sex ratio was 4.8 : 1 (M=63; F=13). Single layered AMT was done in patients with post-keratoplasty persistent epithelial defects (PED) (24), infectious keratitis (13), neurotrophic corneal ulcer(12), PED after chemical injuries(4), conjunctival reconstruction after pterygium excision(4), repair of exposed Ahmed glaucoma valve (4); Biopore implant after enucleation (1), ocular surface neoplasia removal (2) and forniceal reconstruction after symblepharon (9) eyes. PED decreased in size and healed faster. Infectious keratitis showed decrease in size of infiltrates. Multilayered AMT done in 5 patients with perforated corneal ulcer and 1 patient with corneal fistula was helpful in healing of ulcer and fistula. Complications like dislodged AMT, loose sutures, loss of BCL, infection was observed in few patients. **Conclusion**: Amniotic membrane provides scaffolds that provides growth and differentiation factors. It helps in ocular surface disorders by promoting epithelialization and reducing inflammation. It is used in various corneal and conjuctival disorders with varying success rate.

PREVALENCE OF NON-OBVIOUS MEIBOMIAN GLAND DYSFUNCTION (NOMGD) IN A DRY EYE STUDY. C.A. Blackie[1,2], D.R. Korb[1,2] [1]Korb Associates, Boston, MA; [2]TearScience, Morrisville, NC.

Objective: To determine the prevalence of non-obvious meibomian gland dysfunction (NOMGD) in a group of patients with dry eye symptoms. **Methods:** (1) Subjects with dry eye symptoms were recruited for a dry eye study. Of the total number recruited, n = 80, a subset of subjects were selected for standardized diagnostic meibomian gland expression (SDMGE) based on the following criteria: 1) dry eye symptoms quantified on two questionnaires and 2) the absence lid findings indicative of MGD. MG functionality was assessed using SDMGE: A functional gland releases liquid secretion during expression. If the average number of functional glands from both lower eyelids was < 5, the diagnosis of NOMGD was made. **Results:** Twenty-six patients were diagnosed with NOMGD (mean age = 31.6±12.8 yrs; range = 21–62 yrs). Their mean symptom score was 12.3±4.2 (SPEED) and 26.0±14.1 (OSDI). The mean number of MGs yielding liquid secretion upon SDMGE was 2.7±1.8 OD and 2.0±1.4 OS. Correlation analysis revealed that as the number of functional glands decreased, symptoms increased. **Conclusion:** In this study, 33% of the patients recruited were diagnosed with non-obvious MGD. These results support that NOMGD is likely a major cause of dry eye across all age groups. Diagnostic meibomian gland expression should be performed on all patients symptomatic for dry eye, so as to avoid failure to recognize, diagnose and treat NOMGD.

INFLAMMATION: A CAUSE OR CONSEQUENCE OF MUCOSAL DISEASE? Richard S. Blumberg, Laboratory of Mucosal Immunology, 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 adjacent mesenchymal and hematopoietic cells for appropriate development as well as repair after injury. Further, it has recently become established that ECs can not only regulate the structure and function of the microbial architecture that lines the mucosal surface but also the microbiota and other environmental forces can modify the composition and function of the epithelium and its affiliated immune cells. 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. 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.

SEVERE DRY EYES NOT AMENABLE TO CONVENTIONAL TOPICAL LUBRICATION: WHAT IS NEXT? Boboridis G. K., Mikropoulos G. D., Ziakas G. N., Toumanidou V., Lake S., Georgiadis S. N. 1st Ophthalmology Department, Aristotle University of Thessaloniki.

Purpose: To evaluate the efficacy of a new artificial tear carboxymethylcellulose 0.5% (CMC) with osmoprotective compatible solutes Optive for the management of severe dry eye disease. **Methods:** Fourteen patients with symptomatic dry eyes for more than 1 year not amenable to conventional topical lubrication were enrolled in this study over 4 month period. They were switched to Optive 6 times daily for 4 weeks and then they were asked their preferred topical medication. We recorded ocular surface staining with fluorescein and Lissamine green, BUT and Schirmer I tests, the

ocular surface disease index (OSDI) score and the possible adverse effects over the minimum observation period of 8 weeks. **Results:** At the end of 4 weeks we recorded mean reduction of fluorescein by 1,3 points (from 1,7 to 0,4) and Lissamine green by 0,7 points (from 1,3 to 0,6). There was mean BUT increase by 4,1 sec (from 4,6 to 8,7 sec) and Schirmer test by 3,2 mm (from 6,1 to 9,3 mm). We recorded subjective improvement of the OSDI score from 71,3 to 22,9 points. With the completion of 4 week of treatment 12/14 (85,7%) chose to continue with the current topical medication. **Conclusions:** This study provides evidence of increased efficacy of the new osmoprotective artificial tear preparation on the signs of severe dry eye disease not amenable to conventional topical treatment. It mainly offers significant subjective improvement of symptoms and impact of the ocular surface disease. A larger scale prospective study is warranted for statistical evaluation of the results.

DRY EYES IN ACTIVE THYROID OPHTHALMOPATHY: THE ROLE OF OSMOPROTECTION. Boboridis G. K., Mikropoulos D., Ziakas G. N., Georgiadou I., Georgiadis S. N. 1st Ophthalmology Department, Aristotle University of Thessaloniki

Purpose: To evaluate the effect a new artificial tear preparation, carboxymethylcellulose 0.5% with compatible solutes (CMC-solutes) (Optive, Allergan, Inc., Irvine, California) used in combination with topical flouroetholone 0,1% (FML, Allergan, Inc., Irvine, California) for the management of symptomatic dry eyes due to active thyroid ophthalmopathy. **Methods:** Eighteen consecutive patients with active thyroid orbitopathy (clinical activity score CAS≥4) and symptomatic dry eyes not amenable to conventional topical lubrication were recruited over a nine month period. They were treated with CMC-solutes six times daily, in combination with topical fluorometholone eye drops three times daily for at least two weeks before the induction of any systemic medication. Outcome measures were ocular surface staining with Fluorescein and Lissamine green measured with the Shimmura and Toda method, Schirmer's II and break up time tests, subjective ocular discomfort measured with a visual analog scale and CAS changes.**Results:** The mean reduction of Fluorescein staining was 1,8 whereas with Lissamine green was 0,6. Schirmer's test improved by 4 mm and brake up time by 3 sec The mean reduction of pain was 3,1 units of the visual scale. CAS was reduced by 2 points in 3 indices (static pain, erythema and conjunctival chemosis) within three weeks of treatment in 13/18 (72,2%). There were no complications related to the topical medications. In total, there was significant subjective improvement in 14/18 (77,7%) patients and moderate improvement in one. **Conclusions:** Active thyroid ophthalmopathy is strongly related to tear film instability. Treatment with the osmoprotective artificial tear Optive adjunctively with fluorometholone eye drops was shown to improve signs and symptoms of dry eye in such cases. The improvement in tear film stability, pain, erythema and chemosis may be due to the lubrication and osmotic hydration of ocular surface epithelium in combination with the anti-inflammatory action of topical fluorometholone.

KERATITIS SUPERFICIALIS AFTER SURGICAL THERAPY OF TRIGEMINUSNEURALGIA. I. Boldin,[1] M. Trummer,[2] D. F. Rabensteiner,[1] J. Horwath-Winter.[1] Medical University Graz, Department of Ophthalmology, [1] Department of Neurosurgery, [2] Austria.

Purpose. We present a patient with unilateral keratitis superficialis of the whole cornea after trigeminal nerve decompression due to idiopathic trigeminal neuralgia. **Methods.** In 2002 the patient underwent microvascular decompression at the root entry zone of the right Vth nerve due to severe idiopathic trigeminal neuropathic pain. During this intracranial operation (Operation of Jannetta) a small goretex patch (3x1 mm) was placed between the vessel and the laterocaudal part of the Vth nerve to avoid chronic irritation. Two years later dry eye symptoms on the side of decompression started. In 2010 the Patient was referred to our dry eye unit. **Results.** Idiopathic trigeminal neuralgia on the right side was completely cured by the primary operation. Ophthalmic examinations revealed decreased corneal sensitivity, reduced tear break up time, and increased lissamingreenestaining on the right cornea compared to the left cornea. Subjective dry eye symptoms were reported only for the right eye. Schirmer test I values, sensitivity of the conjunctiva, and morphology of the bulbar conjunctiva investigated by impression cytology were similar in both eyes. **Conclusions**. Microvascular decompression of the trigeminal nerve can cause sensory deficiency of the cornea accompanied by superficial keratitis and subjective dry eye symptoms. Atrophy due to surgical preparation, chronic mechanic irritation of the goretex patch or dislocation of the patch might be the reasons. We do not have commercial relationships or grant support.

DRY EYE AND HUMAN TEAR LIPID COMPOSITIONAL, CONFORMATIONAL AND FUNCTIONAL RELATIONSHIPS USING SPECTROSCOPY. Douglas Borchman, Gary N Foulks, Marta C Yappert. University of Louisville

Knowledge of the relationships among composition, conformation and function of tear film lipids could facilitate the development of therapies to alleviate symptoms related to meibomian gland dysfunction (MGD) and to diagnose the disease. Toward this goal, we used spectroscopic approaches to assess tear lipid composition and conformation relationships with age, sex and meibomian gland dysfunction. Spectra of meibum from 41 patients diagnosed with MGD (Md) and 27 normal donors (Mn) were acquired. Lipid order and phase transition temperature were significantly higher for Md and similar to levels of Mn from 3 year old donors. For Mn and Md, the intensity of the IR amide I and II bands from proteins were linearly related to the meibum phase transition temperature and order and indirectly and linearly related to meibum delivery.^{1}H-NMR spectra showed the ratio, C=C/ester, increased with age and MGD which indirectly relates to tear film stability. With age, the amount of CH_2 groups relative to ester moieties increased twice as much as the C=C moieties and the C=C/$CH_2$and CH_3/CH_2 ratios were related to lipid order and indirectly related to meibum delivery. With the use of MALDI-TOF MS, we resolved and quantified 189 lipid peaks with a sensitivity of 9 pmoles for each analyte for Mn and Md. Wax and cholesterol esters, hydrocarbons and phospholipids were detected. Extra peaks in Md spectra may arise from increased lipid synthesis, bacteria or cellular debris. It is reasonable that as the lipids become more ordered and more viscous as observed with Md, less lipid flows out of the meibomian gland orifice and more casual lipid is present on the lid margin. The age- and disease-related changes in the physical and chemical characteristics of meibum lipids suggest that the C=C/CH_2 and CH_3/CH_2 ratios may be more important than meibum quantity in relation to tear film stability. Supported by NIH, EY017094-01, the Kentucky Lions Eye Foundation and an unrestricted grant from Research to Prevent Blindness Inc. No commercial relationships.

QUALITY OF LIFE IN PRIMARY SJÖGREN'S SYNDROME. Dr Simon Bowman,Consultant Rheumatologist, Selly Oak Hospital Birmingham, UK and Honorary Senior Clinical Lecturer, University of Birmingham, UK

The term 'Quality of Life' (QoL) is a general term that can be applied to individuals, groups or countries and may include a broad

range of measures including prosperity, environment, social cohesion, psychological well-being etc. In evaluating the quality of life relating to specific diseases, the term 'Health-Related QoL' is preferred. This is generally evaluated through patient-reported measures that can range from a single question (e.g. 'how satisfied are you with your health?' to complex questionnaires such as the Short-form Medical Outcomes 36-item questionnaire (SF-36) which has been widely used for this purpose in a range of conditions. A number of studies using the SF-36 have consistently demonstrated reduced HR-QoL in Primary Sjögren's Syndrome (PSS) comparable to other rheumatic diseases such as rheumatoid arthritis or systemic lupus erythematosus. Studies that have examined predictors of reduced HRQoL in PSS have identified fatigue and pain as major predictors of reduced scores in components of the SF-36, whereas mental fatigue and dryness are not major predictors. At least in part, however, this may reflect the focus of the SF-36, which includes vitality, bodily pain and physical function domains to which fatigue and pain would be expected to correlate with. Other approaches to measuring HRQoL may be worth investigating in order to better understand these relationships. A number of biological and other mechanisms may be involved in explaining why HRQoL is reduced in PSS and consequently both biological and physical therapies may have a role to play in treating PSS to improve patients' HRQoL and this will be discussed in more detail in the presentation. Conflict of Interests: Dr Bowman has consulted for Roche and UCB

ON COMPUTATIONAL MODELS OF TEAR FILM AND OSMOLARITY DYNAMICS. R.J. Braun,[1] P.E. King-Smith,[2] J.J. Nichols[2] and P. Ramamoorthy.[2] [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

Purpose: To develop models for simultaneous predictions of osmolarity and tear film dynamics for the ocular surface. **Methods**: A model is derived for the thin tear film for a spatially uniform (flat) film or in one spatial dimension. The model is solved numerically using spectral or finite difference methods for spatial derivatives and a Matlab function to integrate in time. A one-equation model for the tear film thickness with osmotic supply is verified by deriving corneal permeabilities and evaporation rates with new measurements based on decay of fluorescence due to quenching. The model is generalized to include a second equation for transport of osmolarity (solutes) in the tear film. **Results**: With prolonged eye opening after a blink, osmotic flow out of the cornea may increase to reach a dynamic equilibrium with evaporation (steady tear thickness). Our analysis is used to model fluorescent decay due to quenching, yielding permeability of (mean ± SD) 13.0 ± 4.7 um/s (8 subjects). Using van der Waals forces to express wettability of the ocular surface, evaporation and van der Waals forces balance at a dynamic equilibrium of constant thickness [Winter et al, Math. Med. Biol., doi:10.1093/imammb/dqp019, 2009]. Solute transport effects may require van der Waals or similar forces to arrest thinning under some conditions. Osmolarity dynamics in a breakup region are studied and are similar to those of a flat interface. **Conclusions**: The model captures aspects of tear film and osmolarity dynamics that are expected from theories of breakup due to dry eye. [Support: NSF 0616483 (Braun); R01 EY017951 (King-Smith).]

TOXICOLOGICAL COMPARISON OF TRAVOPROST BAK-FREE, TRAVOPROST BAK-PRESERVED, AND LATANOPROST BAK-PRESERVED OPHTHALMIC SOLUTIONS IN HUMAN CONJUNCTIVAL EPITHELIAL CELLS. Brignole-Baudouin F,[1-5]Riancho L,[1-3] Liang H,[1-4] Baudouin C.[1-4] 1VISION INSTITUTE INSERM, U968, [2]UPMC Paris 6, [3]CNRS, UMR_7210, [4]QUINZE-vINGTS NATIONAL HOSPITAL, [5]Université Paris 5, Paris, France

Purpose: Compare the *in vitro* cytotoxicology of a new formulation of travoprost ophthalmic solution, 0.004% (trav BAK-free), containing the preservative polyquaternium-1 (PQ), with the commercial formulations of benzalkonium chloride (BAK)-preserved travoprost ophthalmic solution, 0.004% (trav BAK) and BAK-preserved latanoprost ophthalmic solution, 0.005% (lat BAK). **Methods**: Under standardized conditions, human conjunctival epithelial cells were incubated with phosphate-buffered saline (PBS), BAK 0.015%, BAK 0.020%, PQ 0.001%, trav BAK-free, trav BAK 0.015%, or lat BAK 0.020%. Six toxicological assays were used to assess: cell viability (neutral red, Alamar blue), apoptosis (YO-PRO-1, Hoechst 33342), and oxidative stress (H2DCF-DA, hydroethidine). Apoptosis and oxidative stress were each reported according to cell viability as observed with neutral red and Alamar blue for a total of 10 analyses per treatment. **Results**: For all 10 analyses, there were no significant differences in toxicity between cells exposed to PBS and cells exposed to trav BAK-free. Similarly, for 9 of 10 analyses PQ 0.001% was not significantly different from PBS treatment. In contrast, BAK 0.015%, BAK 0.020%, and lat BAK produced significantly more cytotoxicity than PBS in all 10 analyses ($P < 0.0001$). For all 10 analyses trav BAK-free produced significantly less cytotoxicity than lat BAK ($P < 0.0001$), and produced significantly better cell viability and less apoptosis than trav BAK (6/6 analyses, $P < 0.0001$). Trav BAK was significantly less cytotoxic than lat BAK in 7 of 10 analyses ($P < 0.0001$). **Conclusions**: An *in-vitro* panel of cell viability, apoptosis, and oxidative stress assays showed that trav BAK-free may be better for ocular surface health than BAK-preserved lat or trav. Clinical studies are needed to validate these findings on the ocular surface of patients with open-angle glaucoma or ocular hypertension. [Research supported by an unrestricted grant from Alcon Research Ltd.]

A SOLUTE GRADIENT IN THE TEAR MENISCUS TO EXPLAIN MARX'S LINE, ITS FORWARD MIGRATION AND MEIBOMIAN GLAND DYSFUNCTION – A NEW HYPOTHESIS. AJ Bron[1], N Yokoi [2], E.A.Gaffney [3], JM Tiffany [1]..[1]. Nuffield Laboratory of Ophthalmology, University of Oxford, UK; [2]. Department of Ophthalmology, Kyoto Prefectural University of Medicine, Japan; [3]. Mathematical Institutes, University of Oxford, UK.

Purpose: There is a zone of increased permeability of conjunctival epithelial cells, directly behind the mucocutaneous junction (MCJ) of the lid margin. These cells take up stain with dyes such as rose bengal to cause the staining pattern called Marx's line. This contrasts with the lack of staining of the remainder of the normal ocular surface. We attempt to explain this paradox and its implications. **Methods:** We consider the influence of evaporation from the exposed ocular surface. **Results:** We hypothesise that: i. Marx's line is due to stresses at the MCJ, delivered by the apex of the tear meniscus. ii. this results from a differential effect of evaporation, concentrating solute at the apex. iii. Such solutes include ions and pro-inflammatory proteins and lead to hyperosmolar and inflammatory stress. iv. Accumulation of gel mucin at the apex could retard the diffusion of ions away from the apex. The following consequences are predicted: v. Solute stress (eg. hyperosmolarity) stimulates increased epithelial turnover in the Marx's line region and causes surface cell and glycocalyx immaturity. vi. The immature glycocalyx fails to exclude dye entry and results in the staining, characteristic of Marx's line. **Conclusions:** Stress delivered in this way could result in the forward migration of Marx's line which accompanies ageing and diffusion of various agents, whether hyperosmolarity, specific ions or inflammatory mediators, could initiate MGD by causing hyperkeratinisation the terminal Meibomian gland ducts. A similar

gradient, generated at the central apex of the meniscus is presumed to be diffused by eye movements. Commercial Interests: **AJB**: I,C, - Tear Lab; **N Y**: N; **EAG**: N; **JMT**: N

RECENT ADVANCES IN DEFINING AND CLASSIFYING DRY EYE DISEASE. A. J Bron. Nuffield Laboratory of Ophthalmology, University of Oxford, UK

In 2007 the international Dry Eye Workshop (DEWS) recognised dry eye disease as a multifactorial disorder causing tear film instability, ocular surface damage and inflammation and visual disability and ocular discomfort. The DEWS report spawned a further workshop into Meibomian gland dysfunction and its findings are reported at the current meeting. These two workshops have provided a clearer understanding of dry eye disease. DEWS recognised the pivotal role of tear hyperosmolarity in dry eye and importantly, identified how tear hyperosmolarity and inflammatory mediators, released at the ocular surface, could create a vicious circle of events which might perpetuate disease in the absence of the primary stimulus. This concept has influenced our understanding of preservative toxicity in patients on chronic medications. The major classification of dry eye into aqueous tear-deficient (ADDE) and evaporative (EDE) dry eye was upheld in the DEWS report. This approach is a clinical convenience allowing key etiological factors to be recognised and influence treatment. But evaporation is the predominant agent of dry eye and desiccating environmental stress, together with new immune approaches, have been used to create models of dry eye disease and identify how hyperosmolarity and inflammatory mediators lead to squamous metaplasia, a the hallmark of dry eye. While the taxonomy of dry eye has changed little in the past three years, the means to characterise dry eye and its severity have improved and have implications for treatment selection, outcome prediction, recruitment to clinical trials and an understanding of mixed phenotypes. This has been made possible by better standardisation and the introduction of new diagnostic techniques, some of which will be highlighted in this overview.

HUMAN TEARS AND MEIBUM LIPIDOMES: A ROADMAP FOR SUCCESSFUL ANALYSIS. Igor Butovich, Department of Ophthalmology, UT Southwestern Medical Center, Dallas, TX 75390

Objective: The goal of this report is to provide an overview of the existing approaches to the lipidomic analysis of complex lipid-rich samples such as human and animal meibum and aqueous tears (AT), with emphases on various types of chromatography (TLC, GC, HPLC) and spectrometry (MS, NMR, UV, fluorescence, IR and Raman) and their combinations. **Methods:** The strengths and weaknesses of current and previously used experimental techniques will be discussed. The report includes direct side-by-side comparison of the major analytical techniques used in the author's laboratory and/or reported in independent publications. **Results:** The wide diversity of the lipidomes, and the small size of the samples, require the use of different tools, the choice of which is overwhelming. The author will demonstrate the power and benefits of a combination of HPLC and MS (HPLC-MS) for qualitative and quantitative lipidomic analyses of intact lipid species found in meibum and AT. The usefulness of GC-MS for this purpose is limited, but it is an indispensible tool for structural characterization of fatty acids and alcohols on which many lipids are based. Previously popular TLC and HPLC-UV became niche methods whose past results need to be verified by modern techniques. MALDI-ToF MS is effective in mapping polar lipids, but is not considered a quantitative method, and can have difficulties in analyzing isobaric compounds. NMR is extremely informative when it comes to the direct evaluation of the geometrical features of individual lipids (including branching and *cis,trans* isomerization), but is poorly suitable for analyzing complex lipid mixtures, series of homologous compounds, or molecular weight determinations. IR and Raman spectroscopies are better suited for conformational characterization of lipids and their mixtures than for the compositional analyses of the latter. **Conclusions:** Currently, there is no singular analytical approach that would provide all the necessary information to completely characterize the lipidomes of meibum and AT both qualitatively and quantitatively. However, one can rationalize a strategy for successful analyses using a combination of various techniques.

PREPARATION OF A CORD BLOOD SERUM EYE DROPS FOR TOPICAL USE IN SEVERE CORNEAL EPITHELIOPATHY. M. Buzzi, A. Stancari, C. Vaselli, C. Coslovi, A. Terzi, A. Abenavoli, G. Bersani P. Versura , EC Campos Emilia Romagna Cord Blood Bank-Transfusion Service, Pharmacy Service S.Orsola-Malpighi Hospital, Ophthalmology Unit Alma Mater Studiorum University of Bologna

Objective: The purpose of this work was to optimize the preparation of cord blood (CB) serum-based eye drops for topical use. **Method:** The whole procedure was performed under laminar flow hood (BacT/Alert sterility tests at each batch) and chain-of-custody monitoring process. Serum was collected from CB units frozen at -80°C for six months to allow the quarantine period according to Italian regulation. Thawed CB serum (7,5 ml/each patient) was sent to pharmacy lab where it was filtered (Millex HV 0,45 µm) and diluted 1:5 with refrigerated sterile physiological saline then it was aliquotted into luer-lock cap 1 ml sterile syringes. Filled syringes were sealed and packed in sealed labelled envelopes. Finally, they were stored at -20°C until they were delivered to Ophthalmology Unit for delivery to patients. The CB serum levels of EGF (Epithelial Growth Factor), TGF(Transforming Growth Factor)-1 and IL-10 were tested in different step points, specifically: freshly collected CB serum, thawed after quarantine, after filtration, after dilution and after one or two months storage at –20°C. **Result:** Sterility tests demonstrated that all batches remained sterile after handling and storage. The CB serum levels of EGF , TGF-1 and IL-10 was maintained over the whole process. **Conclusion:** The collaboration among interdisciplinary professional figures overcame preparation critical points, providing patients with a safe product.

SJOGREN'S SYNDROME: FROM SLIT LAMP TO CYTOPLASM. Barbara Caffery. University of Waterloo, School of Optometry Waterloo, Ontario Canada

Purpose: To determine if Sjogren's syndrome (SS) dry eye can be differentiated from other forms of dry eye using standard clinical tests and biological analysis of tear samples and cells of the ocular surface. **Methods**: Clinical data was collected by chart review of all patients seen at the UHN Sjogren's Syndrome Clinic (n=398). SS patients were diagnosed by the American European consensus criteria of 2002. Keractoconjunctivitis sicca (KCS) was defined by symptoms and Schirmer scores of ≤ 10 mm in 5 minutes. Recursive partitioning was performed on 90 variables. Molecular analysis was conducted on 25 each of SS, KCS and non-dry eye (NDE) controls. Tears were collected using an "ocular wash" method and total protein, lipocalin and lysozyme concentrations were determined. Impression cytology was performed to collect conjunctival epithelia from which MUC1 and MUC16 protein and mRNA were extracted and analyzed. **Results**: Rose bengal staining of the temporal conjunctiva was the most important non-invasive test in differentiating SS from KCS. SS subjects had significantly lower total protein ($p<0.001$) and lipocalin ($p<0.0001$) concentrations compared with both KCS and NDE

groups. The SS group demonstrated significantly higher concentrations of soluble MUC1 ($p \leq 0.001$) and MUC1 mRNA ($p < 0.05$) compared to both KCS and NDE groups. The SS group had significantly higher concentrations of soluble MUC16 ($p \leq 0.004$) and MUC16 mRNA ($p \leq 0.01$) compared with both KCS and NDE groups.**Conclusions**: Eye care practitioners can best capture and classify various forms of dry eye through observations of rose bengal staining of the ocular surface. Tear composition and ocular surface cells differ in various forms of aqueous deficient dry eye. Tears of SS subjects have lower total protein and lipocalin concentrations than those of KCS and NDE subjects. SS patients have increased levels of soluble MUC1 and MUC16 in their tears as well as increased levels of MUC1 and MUC16 mRNA in their conjunctival cells compared with KCS and NDE subjects.

MORPHOGENESIS OF THE MOUSE MEIBOMIAN GLAND. Mindy K. Call[1], Chyong Jy Nien[2], James V. Jester[2], Winston W-Y Kao.[1] [1]Edith J. Crawley Vision Research Center, University of Cincinnati, Cincinnati, OH, USA. [2]Gavin Herbert Eye Institute, University of California Irvine, CA, USA

Purpose: The meibomian gland produces and secretes meibum that prevents evaporation of the tear film for the health of the ocular surface. To date, little is known about meibomian gland morphogenesis. Current thought is that the terminal region of the central duct is lined by cells from epidermal epithelium at the lid margin. Our study set out to identify the cell types contributing to and responsible for the proper formation of the meibomian gland. **Methods:** Eyelids were removed from C57BL6 mice at embryonic day 18.5 or postnatal (P) days 0-15 and processed for histology and immunohistochemistry. Eyelids *en bloc* were stained with phalloidin and analyzed by confocal microscopy. In addition, a panel of cytokeratin antibodies was used to identify the various epithelial phenotypes present in the meibomian gland and at the lid margin in frozen sections. **Results:** Our data show that the mouse meibomian gland begins to develop at E18.5 with extension of the epithelium into the eyelid through P3. Ductal branching was first observed at P5 and reached to an adult gland by P15, concurrent with eye open. The conjunctival marker K4/K13 was found in the conjunctiva as well as in the suprabasal epithelial cells lining the cord. K10 was present within the superficial layers of the skin epidermis lining the invaginating cord. At P7 a small subset of K10 positive cells was also present in the basal layer of the epidermis. **Conclusions:** These data provide information regarding the morphological changes that occur during mouse meibomian gland development. The keratin expression profile has highlighted unique differences from the current ideology. Namely, that during meibomian gland morphogenesis there is a contribution from the conjuctival epithelium rather than solely from the skin epidermis.

CORD BLOOD SERUM EYE DROPS IN THE TREATMENT OF SEVERE CORNEAL EPITHELIAL DEFECTS IN GVHD AND SS-I PATIENTS: A PILOT STUDY. EC Campos, P. Versura, V. Profazio, L. Foroni, C. Schiavi, M. Arpinati, N Malavolta - Ophthamology Unit, Hematology Department, Rheumatology Service, Alma Mater Studiorum University of Bologna, Italy

Purpose: To evaluate Cord Blood Serum (CBS) eyedrop efficacy in healing diseased corneal epithelium and reducing discomfort symptoms in severe dry eye, such as in Graft Versus Host Disease (GVHD) and primary Sjogren's syndrome (SS-I) patients. **Methods:** Eleven GVHD and four SS-I patients with severe dry eye (DEWS score 3 and 4), non responding to autologous serum therapy were enrolled. CBS eyedrops were prepared and optimized as described elsewhere (abstr …) and adiministered eight drops/eye/day for thirty days,. Corneal epithelial damage evaluated in mm^2/area, discomfort symptoms scored by OSDI, Schirmer test I, Break Up Time (BUT), tear osmolarity (Tearlab, Ocusense), corneal esthesiometry (Cochet Bonnet, Luneau) conjunctival scraping and imprint cytology were performed at baseline (V0), after fifteen (V1) and thirty (V2, endpoint) days of treatment. A satisfaction and tolerability questionnaire were evaluated at V1 and V2. Statistical analysis of data was performed by using SPSS 14.0 software **Results:** A significant reduction was shown at the endpoint vs baseline in corneal epithelial damage (media+SD: 14,9$\pm$13.5 vs 26.5$\pm$20.1 mm^2/area, respectively), discomfort symptoms (OSDI score 34,2$\pm$8,9 vs 52,8$\pm$7), scraping cytology score (4,6$\pm$0,7 vs7,8 $\pm$1,8), tear osmolarity (314,2$\pm$7,7 vs 323,6$\pm$8 mOsm/L) p always <0,0001 while a significant improvement was shown in corneal esthesiometry (48,7$\pm$2,3 vs 47,5$\pm$3,2 nylon/mm/lenght, p<0,05) and BUT (8,3$\pm$1 vs 7,8$\pm$1,3 sec, p<0,05)..All patients reported an high satisfaction degree upon drop instillation. No adverse event was reported up to two months after endpoint **Conclusions:** CBS eyedrops represent a promising therapeutic approach in patients with severe dry eyes non responding to conventional therapy. Study supported in part by a grant from Fondazione Cassa di Risparmio in Bologna to ECC

THERAPEUTIC EFFECT OF ELEDOISIN IN OCULAR PHATOLOGICAL MANIFESTATIONS SJOGREN'S SYNDROME. Capra Piera. Ophthalmological Clinic University La Sapienza Roma, Italy

Purpose: Demostrate the efficacy of eledoisin takychibnin as stimulator of lacrimal and salivary secretion. **Methods**: In a first control of 20 patients examination of subjective data as pain, photophobia etc. and objective data of conjunctiva and cornea as Schirmer test, fluoirescein test etc. Collyrium with eledoisin was topically administered in conjunctiva at dose of 5-20 micrograms 6 times/day for a period variable from 10 to 60 days. **Result**: In 20 cases was found better objective conditions of cornea with increase of visual acuity in some cases: 19 cases patients felt subjectively better. **Conclusions**:. Eledoisin present in collyrium is a very efficient drug as it stimulates M3 cholinergic receptors in lacrimal gland secreting cells:it can be administered under tongue as way too in presoaked contact lanes. It doesn't cause so heavy collateral negative effects as policaroine

ADENOSINE A2A RECEPTOR UP-REGULATION IN THE MALE NOD MOUSE DRY EYE MODEL. Stina K. Carlsson[1], Daniel Diez[2], Sarah F. Hamm-Alvarez[2], Kai-Jin Wu[2] and J. Peter Gierow.[1] School of Natural Sciences, Linnaeus University, Kalmar, Sweden[1] Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, School of Pharmacy, Los Angeles, USA[2]

Purpose: Adenosine has recently been shown to potentiate the effect of carbachol on lacrimal gland (LG) secretion in rabbit through the A1 (Exp. Eye Res 86: 110, 2008) and the A2 receptors (Curr. Eye Res. 2010, in press). Since adenosine also has been shown to have anti-inflammatory properties (Trends Immunol. 25:33-39, 2004), the presence of adenosine receptors in a dry eye model, the male non-obese diabetic (NOD) mouse, was of interest. **Methods:** LGs from NOD mice and controls, the BALB/c mice and NOD/SCID, a NOD strain lacking functioning lymphocytes, were used in this study. To detect adenosine receptor presence on mRNA level microarrays and quantitative real-time PCR (qPCR) were performed. Immunohistochemistry was used to detect protein expression. **Results:** The results revealed that the mRNA expression of the A2a receptor in lacrimal glands was higher in 12 weeks old NOD compared to BALB/c both in the microarray by 2.7-fold ($p < 0.01$)

and in the qPCR by 20-fold (p<0.001). The qPCR showed an up-regulation of the A2a receptor also in lacrimal glands of the 8 weeks old NOD by 12-fold (p<0.001), but not in the 4 weeks old. The A2b receptor showed a slight up-regulation in the microarray (1.3-fold) but no difference was seen in the qPCR. A comparison with the NOD/SCID showed that the A2a expression was 10-fold higher in NOD than in NOD/SCID (p<0.01), indicating a lymphocyte-dependent up-regulation of the A2a receptor. Similar patterns were seen in the immunohistochemistry, where A2a receptor staining was most intense in 12 weeks old NOD, whereas the A2b receptor appeared to be expressed in all samples. **Conclusions:** Our results show that both the protein and mRNA expression of the A2a receptor is up-regulated in the 8 and 12 week old male NOD mice compared to the Balb/c control, making it a potential target for a future treatment of Sjögren's syndrome. **Support:** University of Kalmar Faculty grant, Magnus Bergvall Foundation (JPG) and NIH Grant EY11386 (SHA).

HUMAN TEAR LIPID BREAKS UP BY DEWETTING C. Cerretani[1], C. J. Radke[1,2] [1]Chemical Engineering Department and [2]Vision Science Group University of California, Berkeley

Objective:Recently, King-Smith et al (ARVO, Poster 4162/D713, 2010) presented images of the human-tear-film lipid layer (TFLL) showing patterns of droplets, irregular lipid, and black spots. No explanation for the reported shapes was given. Based on comparison to *in vitro* lipid films, we hypothesize that the TFLL can break up into biconvex lenses at the tear interface by a dewetting process. **Methods**:100-nm thick layers of oxidized mineral oil (viscosity 30 mPa s) and human meibum were spread at the air/isotonic saline water interface in a miniature Langmuir trough. Color video under white light at near-normal incidence provided continuous images of the spreading and dewetting films.**Results**: Upon touching the interface, oil and lipid droplets initially spread. Within seconds, however, black holes or spots begin to appear and grow. When two black holes expand into each other, the oil between them ruptures; they coalesce. Enough coalescence of black holes results in continuous black regions on the film consisting of adsorbed oil submonolayers. In between the continuous black regions, excess oil collects into thick rivulets. These are unstable and retract into biconvex lenses. When two lenses at the interface collide, they coalesce into larger lenses. This film breakup process is coined "dewetting". The images of King-Smith et alof the TFLL are isolated time snapshots of the dewetting process. Comparison of our *in vitro* dewetting images to those of King-Smith et al shows almost exact replication. The black spots in Figs. 12-15 correspond to the growing black holes. Figs. 10 and 11 display the breakup of oil rivulets. Figs. 6 and 7 illustrate oil lenses surrounded by black oil monolayers. **Conclusions**: Thin-film dewetting follows well-known physical laws of capillarity. Since the human lipid layer displays the same behavior, we conclude that human lipid can break up by a dewetting process. The large uncovered areas in a dewetted lipid layer permit unimpeded tear evaporation. It remains to be determined what characteristics of human meibum, such as lipid composition, layer thickness, and viscosity lead to dewetting. Funding: Unrestricted Alcon grant

TEAR EVAPORATION REDUCTION BY MODEL THIN OILY FILMS C. Cerretani[1], C.J. Radke1,[2 1] Dept. of Chemical Engineering, Univ. of California, Berkeley, CA [2] Vision Science Group, Univ. of California, Berkeley, CA

Purpose: Reduction of tear evaporation through the human tear-film lipid layer (TFLL) is crucial to maintaining proper tear osmolarity and preventing dry-eye syndrome. Recent studies, however, claim that films of oily substances, including human and bovine meibum, play no measureable role in controlling water evaporation rates1,2. We hypothesize that oily films of thickness near that of human TFLL do reduce water evaporation rates by forcing water to dissolve in and diffuse through the oil film. **Methods:** Following Holly3, a model lipid film is studied consisting of oxidized mineral oil and bovine submaxillary mucin. Resulting oily films are spread mechanically in a blinking motion on an aqueous substrate in a Langmuir trough. Evaporation rates are measured gravimetrically under controlled temperature, humidity, and air flow for films of thickness ranging from 100 nm (thickness of TFLL) to 100 µm. To verify uniform coverage, films are visualized using white-light interferometry. **Results:** As film thickness increases from 100 nm to 100 µm (100, 250, 500, 750, 1000, 10,000, 100,000 nm), evaporation rate is reduced by 24% to 97% (24±6, 40±6, 42±6, 56±5, 61±5, 89±5, 97±2; n=7 at each corresponding thickness). The newly measured water evaporation rates are in agreement with water dissolution/diffusion theory. Theory fitting gives a water permeability in the model lipid films of 5x10-5 cm2/s. **Conclusions:** We have engineered in-vitro thin oily films of thickness similar to that of the human TFLL that reduce water evaporation rates. The mechanism of evaporation-rate reduction is the resistance afforded by water dissolving in and diffusing through the oil film. Additionally, the oil film must cover the entire aqueous substrate uniformly and remain stable against rupture. Our findings suggest that to provide an effective barrier against tear evaporation, the human TFLL should have both low water solubility and diffusivity. 1. Borchman D, Foulks GN, Eye & Cont. Lens 1:32 (2009) 2. Herok GH, et al, Curr. Eye. Res. 34:589 (2009) 3. Holly FJ, J. Coll. Interf. Sci. 49[2]:221 (1974) This project is supported by a grant from Alcon.

CYTOKINE CHANGES IN THE TEAR FLUID OF KERATOCONUS PATIENTS. Shukti Chakravarti, Leslie Cope and Albert Jun. Johns Hopkins School of Medicine

Objectives: Keratoconus is viewed as a non-inflammatory ectatic corneal thinning disease. However, recent tear film studies report increases in IL-6, TNF-alpha and MMP-9 suggesting inflammatory underpinnings. We investigated tear samples from keratoconus and control individuals for changes in TH1 and TH2 cytokines. **Methods**: 5-8 µl of tear/eye was collected by capillary flow from consented individuals according to a Johns Hopkins IRB-approved protocol. In a multiplex cytokine analysis 32 patient and 21 control samples were assayed for IL-1ß, IL-4, IL-6, IL-10, IL-12, IL-13, IFN-gamma, TNF-alpha and the chemokine RANTES. IFN-gamma and IL-4 were further tested by ELISA on 33 control and 29 keratoconus cases pooled to provide13 control and 10 keratoconus pooled samples. Statistical significance for categorical variances between groups was analyzed by Chi square tests, and univariate comparisons of means between groups by 2-tailed Mann-Whitney nonparametric test with $p \leq 0.05$. **Results:**The multiplex data showed a slight increase in IL-6 as reported by others. In addition, keratoconus as compared to control samples showed significant decreases in TNF-alpha (p=0.015), IFN-gamma (p=0.06), RANTES (p=0.016), IL-12 (p=0.0001). Decreases in IL-4 and IL-13 were not significant. ELISA on an independent sample set confirmed IL-4 decreases (p=0.04), but showed no changes in IFN-gamma.**Conclusions:**A wide spread decrease in TH1 and TH2 cytokines in keratoconus suggests perturbations in immune-related homoeostasis. IL-4 also regulates collagen and extracellular matrix (ECM) proteins, and potentially decreased IL-4 in keratoconus could affect the corneal ECM.

CONTACT LENS DRY EYE QUESTIONNAIRE-8 (CLDEQ-8) REFLECTS STATUS OF AND RESPONDS TO CHANGE IN OVERALL OPINION OF CL PERFORMANCE. Robin L. Chalmers[1], Kurt Moody[2], Graeme Young[3], Sheila Hickson-Curran[2], Carolyn Begley[4], Chris Hunt[3] [1]Clinical Trial Consultants, Atlanta, GA, USA, [2]Vistakon, Inc., Jacksonville, FL, USA, [3]Visioncare Research Ltd., Farnham, Surrey, UK, [4]Indiana University, Bloomington, IN, USA

Objective: To test the Contact Lens Dry Eye Questionnaire 8 (CLDEQ-8) for ability to reflect status of and change in Overall Opinion of contact lenses (CLs). **Methods:** Subjects with complete datasets (n=309) in an IRB-approved randomized treatment trial completed the short form CLDEQ-8 and an anchoring question on Overall Opinion ("Opinion") of CLs at baseline and 2 weeks after randomization to silicone hydrogel lenses ; senofilcon A (SA), or lotrafilcon B (LB). Healthy current CL wearers were enrolled. Sum CLDEQ-8 scores (possible 0 to 35) were tested for correlation with Opinion of habitual lenses (Spearman's) and then for responsiveness to change in Opinion after randomization by ANOVA. **Results:** The CLDEQ-8 scores were highly correlated with habitual lens Opinion at baseline (-0.44, $p<0.0001$) and responsive to change in Opinion status after randomization (-0.58, $p<0.0001$). Baseline Opinion scores were: Fair 17.4±9.2, Good 13.7±6.4, Very Good 9.1±4.8, and Excellent 6.4±3.6 (, ANOVA, $F=291.1$ $p<0.0001$). After 2 weeks, change in CLDEQ-8 scores ranged from: Much Improved: -16.7±10.0, Unchanged: -2.3±5.0, to Much Worse 8.5±5.8; (ANOVA, $F=16.5$, $p<0.001$). **Conclusions:** CL clinical practice is hampered by unresponsive and unrepeatable clinical tests, hindering assessment of changes in patients' CL experience. The CLDEQ-8 score (Frequency plus PM intensity of Dryness, Discomfort and Blurry Vision; plus Frequency of Closing Eyes to Rest Them and Removing CLs to Relieve Discomfort) significantly reflected baseline status and change in Overall Opinion after refitting with two types of SiHy lenses. The CLDEQ-8 could be an efficient outcome measure in CL clinical trials. Funded by Vistakon, Inc.

BROMFENAC OPHTHALMIC SOLUTION FOR TREATING THE SIGNS OF DRY EYE DISEASE. Simon P. Chandler,[1] Shari L. Rowen,[2] Neal A. Sher,[3] James A. Gow,[1] Timothy R. McNamara.[1] ISTA Pharmaceuticals®, Inc., Irvine, CA, USA[1]; Eye and Cosmetic Surgery Center, Lutherville, MD, USA[2]; Eye Care Associates, PA, Minneapolis, MN, USA.[3]

Purpose. To evaluate the efficacy of bromfenac ophthalmic solution (bromfenac) using lissamine green staining in subjects with Dry Eye Disease (DED). **Methods**. On Day –14 and Day 0, subjects were diagnosed with mild or moderate DED with a mean National Eye Institute lissamine green staining grade of ≥1 in the same eye and with a minimum of 1/6 regions graded ≥ 2 in the same eye. The same region must have retained that grade on Days -14 and 0 in the same eye. During the 14 screening days, eligible subjects received only Refresh Plus® eye drops OU qid. From Day 0 to Day 42, subjects administered bromfenac OU bid and Refresh Plus prn (≤qid). Subjects returned to the office on Days 14±2, 28±2, and 42±2 for evaluation of safety and efficacy. During a 10-day follow-up period, Refresh Plus was dosed prn (≤qid), followed by a visit on Day 52±4. **Results**. A total of 38 subjects were enrolled and analyzed for safety, 38 were analyzed for efficacy in ITT population, and 31 subjects were analyzed for efficacy in PP population. There was a significant improvement from baseline in the mean lissamine green staining at Day 42. Mean corneal staining also showed a significant improvement from baseline. There were no deaths, SAEs, or discontinuations due to AEs and 9 subjects (23.7%) experienced 18 AEs. Most AEs were either mild (6/9 subjects, 66.7%) or moderate (2/9 subjects, 22.2%); 1/9 subjects (11.1%) had a severe AE (sinusitis) considered not treatment related. Two subjects had AEs (eye discharge and eye pain) considered possibly related to treatment. One subject had a mild AE of foreign body sensation in eyes during the follow-up period. Visual acuity and IOP had no significant changes from baseline. **Conclusion**. The efficacy data of bromfenac ophthalmic solution for treating DED subjects were robust and consistent in showing improvement in sign of DED for 42 days and at 10 days follow-up when treatment was discontinued. Sponsored by ISTA Pharmaceuticals®, Inc.

CROSSING OF PATHS: A DEFECT IN A MAJOR REGULATORY PROTEIN OF THE SECRETORY PATHWAY INCREASES DEGRADATIVE PATHWAY ACTIVITY. Lilian Chiang[1], Tanya Tolmachova[2], Alistair N. Hume[2], Joel Schechter[3], Miguel C. Seabra[2], Sarah Hamm-Alvarez[1,3] University of Southern California [1]School of Pharmacy, [3]Keck School of Medicine , Los Angeles CA, USA, [2]Cell and Molecular Biology, Division of Biomedical Sciences, Faculty of Medicine, Imperial College, London

Purpose. The lacrimal gland acinar cell contributes critical components to the ocular surface fluid. We have shown that this exocytotic pathway is positively regulated by Rab27b. The purpose of this study was to determine how Rab27b's function in exocytosis is related to homeostasis of other critical pathways. **Methods.** Rab27b-enrichment associated with mature secretory vesicles was studied by immunofluorescence in reconstituted rabbit lacrimal gland acini. Lacrimal gland sections for EM and indirect immunofluorescence were prepared from a background strain, C57BL/6 mice, as well as from $Rab27a^{ash/ash}$ (*Ashen*), $Rab27b^{-/-}$ (Rab27bKO), and $Rab27a^{ash/ash}Rab27b^{-/-}$ double-knockout (DKO) mice (n=5 of each). **Results.** The Rab27b isoform was localized to resting secretory vesicles in acinar cells which were in particular abundance in the subapical region beneath the lumen. In lacrimal glands from C57BL/6 and *Ashen* mice, which have numerous secretory vesicles, acinar cells retained their normal organization and polarity. However, acinar cells from lacrimal glands from Rab27bKO and DKO mice showed significant decreases in secretory vesicles concurrent with loss of general cell polarity and increased detection of degradative pathway organelles such as lysosomes and autophagosomal-like structures. **Conclusions.** The isoform specific knockout of Rab27b not only affects the secretory pathway, but increases the expression of components of the degradative pathway. Two theories may explain this increased activity, although both are related to cell homeostasis: 1. Blockage of the secretory pathway creates excess of either products or organelles normally involved in maintaining the pathway, or 2. Rab27b is directly involved in the regulation of metabolic organelles, and its knockout activates the degradative pathway. **Grant Support:** EY011386 and EY010550.

EMERGING PARADIGMS FOR CORNEAL REPLACEMENT: THE FUTURE OF KERATOPROSTHESIS SURGERY. James Chodosh, MD, MPH Massachusetts Eye and Ear Infirmary – Harvard Medical School, Boston, MA, USA

Purpose: To summarize emerging paradigms for corneal replacement. **Methods:** This presentation will review potential methods of corneal replacement, including bioengineered corneal constructs and emerging keratoprosthesis designs. **Results:** Corneal replacement by full thickness allograft is considered the most successful of all solid organ transplant procedures, with commonly cited success rates of up to 90% at one year post transplantation. However, 50% of corneal transplants have failed by 15 years after surgery, and for those that lose clarity due to allograft rejection, the likelihood of success falls precipitously with each repeat surgery. Indeed, third corneal allografts have a 5 year survival rate approaching zero. Newly utilized anterior lamellar approaches that spare host corneal endothelial cells will likely

reduce visually significant allograft rejection rates, but for patients with corneal endothelial disorders, immune rejection of allogeneic endothelial grafts are likely to remain a critical clinical problem. The last decade has seen substantial progress in the development of artificial corneas (keratoprosthesis), now a common corneal procedure, and for bioengineered corneas, some of which are in preclinical or phase I clinical trials.**Conclusions:** Visual success and device retention rates with keratoprosthesis implantation continue to improve. Emerging methodologies in corneal bioengineering are expected to make a significant impact on therapeutic corneal replacement in the future. [Supported in part by an unrestricted grant to the Department of Ophthalmology, Harvard Medical School from Research to Prevent Blindness, NY, NY. JC is a salaried employee of the Massachusetts Eye and Ear Infirmary, a nonprofit hospital which produces and markets the Boston keratoprosthesis.]

POST BLINKING SERIAL MEASUREMENTS OF DYNAMIC WAVEFRONT ABERRATIONS AND FUNCTIONAL VISUAL ACUITY IN NORMAL AND DRY EYES. Suk Kyue Choi, M.D., Hae Won Seo, M.D., Jin Hyoung Kim, M.D., Do Hyung Lee, M.D., Ph.D.

Objective :To evaluate the dynamic properties of wavefront aberrations and functional visual acuity in normal and dry eyes. **Methods** : Forty dry eye patients and forty normal subjects participated in this study.Break up time(BUT) and schirmer I test were done. Ocular higher-order aberrations (HOAs) were measured sequentially by using a KR1W® (Topcon, Tokyo, Japan) dynamic wavefront aberration and PSF in 80 eyes of 40 subjects. During the measurement, subjects were forced to open one's eyes. **Result**: The schirmer I test and break up time were significantly different between dry eyes and normal eye($p< 0.05$).Ocular and cornea aberration were 0.452±0.015 μm, 0.450±0.022 μm, in normal eye, 0.766±0.048 μm, 0.607±0.102 μm in dry eye. So, The ocular and coma aberration in dry eye group were significantly higher than normal eye group($p<0.05$). Dynamic aberration performed post blinking during 10seconds tended to keep high in dry eye group. Point spread functions(PSF) were 0.343±0.006 μm in normal eye and 250±0.009 μm in dry eye. So, PSF of dry eye were significantly lower than normal eye group($p<0.05$).**Conclusion** : Dynamic aberration estimation are useful metrics for optical quality analysis of tear film changes and symptom severity in dry eye. No author has a financial or proprietary interest in any material or method mentioned.

BACTERIAL INFECTION IN PRESUMED VIRAL INTERSTITIAL (STROMAL) KERATITIS. Suksri Chotikavanich[1], Pinnita Prabhasawat[1], Nattaporn Tesavibul[1], Amornrat leelaporn[2], Mongkol Uiprasertkul[3]. 1Department of Ophthalmology, 2Department of Microbiology, 3Department of Pathology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand.

Purpose: To describe a novel observation of bacterial infection in interstitial (stromal) keratitis (IK). The findings were considered as secondary bacterial infection in presumed viral IK or a distinctive clinical entity of bacterial manifestation. **Method:** A retrospective analysis of 19 eyes in 15 patients referred to a tertiary eye-care center between the year 2004 and 2009 with chronic presumed herpetic IK not responded to standard treatment. Predisposing factors, corneal characteristics, organism profiles, and management modalities were studied **Results:** The common presenting corneal characteristics in all cases were multifocal infiltration at localized (central/paracentral) or diffuse (total) area with relatively normal or opaque intervening stroma. Alternatively, these had previously been described as nummular keratitis. Definite histories of topical corticosteroid uses were elicited in all cases. Systemic workup for autoimmune diseases were negative. Corneal biopsies were essentially undertaken in all cases. Gram stained smears of the tissues showed gram-negative bacilli in 12 eyes (63.2%), gram-variable coccobacilli in 4 eyes (21.0%) and gram-positive bacilli in 3 eyes (15.8%). PCR revealed *Stenotrophomonas maltophilia* in 1 eye, *coagulase negative Staphylococcus* in 2 eyes, and cytomegalovirus (CMV) in 2 eyes. After discontinuation of topical corticosteroids, all patients received antimicrobial therapy. The keratitis in eight eyes (42.1%) resolved over months. However, therapeutic penetrating keratoplasty was required in 11 eyes (57.9%). **Conclusion:** Bacterial infection should be a concern in prolonged chronic IK. Use of corticosteroids should be discontinued. Early recognition and appropriately aggressive therapy contribute to successful outcome. Corneal biopsy is always essential when corneal scraping is insufficient for the deep lesion. PCR is particularly useful when culture result is negative.

INFLAMMATORY CONDITIONS AFFECT TIGHT JUNCTION PROTEINS IN CORNEAL EPITHELIAL CELLS L. Contreras-Ruiz,[1,2] U. Schulze,[3] A. López, [1,2] F. Paulsen,[3,4] Y. Diebold.[1,2] Ocular Surface Group, IOBA-University of Valladolid, Valladolid, Spain; [1] Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER BBN), Valladolid, Spain; [2] Department of Anatomy and Cell Biology, Martin-Luther-University Halle-Wittenberg, Halle/Saale, Germany; [3] Department of Anatomy II, Friedrich Alexander University Erlangen, Germany. [4]

Purpose: To determine the effect of inflammatory conditions on the expression of tight junction (TJ) proteins in human corneal epithelial cells and consequently in the corneal epithelial barrier integrity. **Methods:** ZO-1, ZO-2, claudin-1 and 2 (CLDN-1 and CLDN-2) and occludin (OCLN) expression were analyzed in the HCE cell line in basal conditions and after stimulation with inflammatory cytokines (IL-10, TNFa, TGF!), using real time RT-PCR, Western blotting and immunofluorescence. Transepithelial electrical resistance (TER) and transepithelial fluorescein permability were measured as barrier integrity functional assays. **Results:** ZO-1, ZO-2, CLDN-1, CLDN-2 and OCLN were detected in HCE cell membranes in basal conditions. After IL-10 stimulation, TJ proteins showed an increased expression, with remarkable membrane localization and without changes in TER and permeability. TNF" produced a CLDN-1 increased mRNA expression and a significant slight increase in ZO-2, CLDN-2 and OCLN proteins. Gene and protein expression of ZO-2 and CLDN-1 was increased after the stimulation with TGF!, whereas the expression of OCLN and CLDN-2 was downregulated. The TJ complex seemed to be disrupted after TNF" and TGF! stimulation, showing a predominant cytoplasmatic localization of studied proteins, a significant decrease in TER measurements, and an increased permeability. **Conclusions:** Cytokines tested modify TJ composition and alter the epithelial barrier function. IL-10 seems to have a barrier protective effect, whereas TNF" seems have an opposite effect. Support: FEDER-CICYT MAT2007-64626-C02-01/C02-02, and FPU Scholarship Program, Ministry of Education, Spain. DFG grant PA738/9-2; BMBF Roux program grants FKZ 9/18,12/08,13/08, Germany. Bilateral Research Grant Spain/Germany DE2009-0085 and DAAD ID6234017.

COMPARISON OF THREE CONTEMPORARY THERAPIES FOR THE MANAGEMENT OF MEIBOMIAN GLAND DYSFUNCTION. Jennifer P. Craig[1], Stuti Misra[1], Elizabeth Robinson[2] [1]New Zealand National Eye Centre, Department of Ophthalmology and [2]Department of Epidemiology and Biostatistics, University of Auckland, New Zealand

Objective: Despite its high prevalence and association with chronic irritation symptoms, meibomian gland dysfunction (MGD) remains

relatively unsuccessfully managed in clinical practice. This prospective, randomised, investigator-masked study compared the short-term effects of three contemporary therapies; a latent heat device (LHD) (Blephasteam®), a warm compress treatment (WCT) (MGDRx EyeBag) and a phospholipid liposomal spray (PLS) (TearsAgain™), on tear film and ocular surface characteristics in patients with and without MGD. **Methods:** In a randomised manner, one of the three treatments was administered to 21 participants without MGD, 23 with mild MGD, 27 with moderate MGD and 10 participants with severe MGD. Outcome measures compared before and after treatment included non-invasive tear film stability (NIBUT), lipid layer thickness (LLT), high and low contrast glare acuity (GA), tear meniscus height (TMH), tear evaporation rate (TER) and temperature variation factor (TVF).**Results:** Data analysis included generalised linear mixed models, with time as a repeated measure and participant as a random effect. No statistically significant effect on TER, TMH, GA, or TVF was observed following single application of any of the treatments, in any of the groups ($p>0.05$). However, both LLT ($p=0.012$) and NIBUT ($p=0.047$) were found to improve significantly in all MGD groups (mild, moderate and severe) but not in the control group, with all three treatments. No significant difference between treatment-type was observed.**Conclusion:**A single application of a LHD, WCT or PLS significantly improved tear film lipid layer and stability without affecting vision. No significant difference between the treatments, nor correlation with MGD severity, was observed. Financial disclosure: None

HUMIDIFYING THE COMPUTER WORKSPACE – CAN A USB-POWERED DESKTOP HUMIDIFIER MAKE A DIFFERENCE? Jennifer P. Craig[1], Evon Chan[2], Linda Ea[2], Clifford Kam[2], Yvonne Lu[2], Stuti Misra[1] New Zealand National Eye Centre, Departments of [1]Ophthalmology and [2]Optometry and Vision Science, University of Auckland, New Zealand

Objective: Low relative humidity environments are recognised to exacerbate signs and symptoms of dry eye, yet are common for visual display unit (VDU) operators. Increasing periocular humidity by wearing goggles increases lipid layer thickness, improving tear film quality and comfort, but lacks cosmetic appeal in the workplace. Desktop USB-powered humidifiers are available commercially but no evidence has been published to support their efficacy. **Methods:**Thirty non-contact lens wearing VDU users (14M 16F), with a mean age of 21±4 years participated in this prospective, randomised, double-masked, cross-over study. On separate days, participants had their tear film assessed before and after one hour's continuous VDU use, with or without exposure to the desktop humidifier. Outcome measures included lipid layer thickness, non-invasive tear film stability, tear meniscus height and subjective comfort. **Results:**Continuous data were normally distributed (Kolmogarov-Smirnoff, $p>0.05$), except stability data which were positively skewed and underwent logarithmic transformation prior to parametric analysis. Comfort data and lipid layer grades were ordinally scored and underwent non-parametric analysis. The desktop humidifier effected a relative difference in humidity between the two environments of 5.8±5.9% ($p<0.001$). No difference in tear meniscus height (paired t-test, $p=0.993$), nor in lipid layer thickness grade (Wilcoxan, $p=0.578$) was found, but a statistically and clinically significant improvement in non-invasive break up time was observed ($p<0.001$), of 3.0 seconds on average. This was associated with a significant improvement in reported comfort in the humidified environment (Wilcoxan, $p=0.001$).**Conclusion:**Despite only a modest increase in relative humidity locally, desktop humidifiers show potential to improve tear film stability and subjective comfort during VDU use.

SUITABILITY OF A LIPOSOMAL DRY EYE SPRAY FOR USE IN SILICONE HYDROGEL CONTACT LENS WEAR. Jennifer P. Craig[1], Trisha Albuquerque[2], Chee Seang Loh[2], Varny Ganesalingam[2], Suhaila Al-Kanani[2], Stuti Misra[1] New Zealand National Eye Centre, Departments of [1]Ophthalmology and [2]Optometry and Vision Science, University of Auckland, New Zealand

Objective: Improvements in tear film quality have been identified in normal subjects, dry eye patients, and daily disposable contact lens (CL) wearers following application of a phospholipid liposomal spray (Tears Again®) to the closed eye. Deemed suitable for all CL wearers, this therapy should benefit wearers of silicone hydrogel (SiHy) CLs, however, SiHy lenses have a propensity to attract lipid deposits. The current study investigated the effect of the liposomal spray on tear film quality and CL surface deposition in daily wear SiHy CL wearers. **Methods:** In this prospective, randomised and investigator-masked study, 31 participants were fitted with SiHy CLs (Acuvue® Oasys™). Participants applied liposomal spray (Tears Again®) 4 times daily to one eye (randomly assigned) for two weeks. Visual acuity, including high and low contrast glare acuity, non-invasive break up time and lipid layer quality were graded at baseline, and at days 7 and 14. Subjective comfort was recorded at days 7 and 14. On day 14, CLs were removed and analysed for lipid deposition by spectrofluorimetry (SpectraMax M2 Microplate Reader). **Results:** Visual acuity, at high and low contrast, with or without glare, was not significantly different between treated and control eyes. Tear film stability and lipid layer thickness were greater in the treated eyes (t-test, $p=0.040$; Wilcoxan, $p=0.020$) and participants reported superior comfort in the treated eye (Wilcoxan, $p=0.003$). Lipid deposition after two weeks of wear was not significantly different between the CL of treated and control eyes ($p=0.607$). **Conclusion:** The liposomal spray demonstrated potential to benefit SiHy CL wearers with dryness symptoms without causing significant lipid surface deposition.

SCREENING MODEL FOR NOVEL THERAPEUTICS FOR DRY EYE SYNDROME IN A NON-HUMAN PRIMATE. Crawford, KS[1], Torkildsen, G[1], Ousler, GW[1], Lawrence, M[2], Goody, R[2], Campion BK[1], Naor, J[1] [1]Ora, Inc., [2]RxGen Inc.

Objective: To assess the suitability of a non-human primate (NHP) species for the development of a screening model to investigate therapeutic interventions for ocular surface diseases, in particular dry eye syndrome (DES). **Method:** St. Kitts Green Monkeys (*Chlorocebus sabaeus*, Vervets) were examined for ocular abnormalities, particularly as they relate to DES. A sub-population of animals, primarily middle-aged and elderly females, presented with surface inflammation that was evaluated more thoroughly by slit-lamp exam. Tear film break-up (TBUT), tear production (Schirmer's), blink rate, and fluorescein staining (NEI and Ora Scale) were determined in these animals as well as in age and sex-matched controls. Tear samples were collected by capillary tubes for osmolarity (TearLab) and cytokine panel (Luminex) determination. These parameters were also evaluated after drug treatment, and after exposure to a low-humidity, high airflow environment, comparable to the Controlled Adverse Environment (CAE) that is used in clinical studies. **Result:** The effects of CAE exposure and various treatments on the multiple parameters characterizing DES will be presented. Parallels between NHP and human DES findings will be discussed.**Conclusion:**The St. Kitts Green monkey is a non-human primate species that may better approximate human tear film physiology and be well suited as a screening model for therapeutic interventions for dry eye syndrome. These animals provide an efficient and cost-effective means of studying human disease and drug delivery

SELECTIVE ANDROGEN RECEPTOR MODULATORS (SARMs) AMELIORATE TEAR LIPID COMPOSITION IN A RABBIT MODEL OF MEIBOMIAN GLAND DYSFUNCTION (MGD). James T. Dalton[a], Jeetendra R. Eswaraka[a], Anand Giddabasappa[a], Jeffrey D. Kearbey[a], France Landry[b], Juhyun Kim[a], Monica M. Jablonski[c]. [a]Preclinical Research and Development, GTx Inc., [b]Merck-Frosst, Montreal, Canada and [c]University of Tennessee Health Science Center, Memphis, TN 38163.

Purpose: Androgen deficiency in men and women increases the risk of Sjögren\'s syndrome and evaporative dry eye. These ophthalmic disorders are characterized by reduced tear lipid composition and MGD. Use of steroidal androgens such as testosterone is limited by concerns related to hirsuitism and effects on sebum production (acne). We developed a series of nonsteroidal SARMs that have tissue selective anabolic effects without the androgenic side effects associated with testosterone. In this study, we examined the ability of four SARMs to restore tear lipid composition in a rabbit model of MGD. **Methods:** New Zealand white rabbits were castrated to reduce circulating androgens and induce MGD. After castration, rabbits were treated with vehicle, SARM or testosterone propionate (TP) subcutaneously (SQ) or topically around the margins of the eye, once daily for 2 weeks. Lipid composition was measured by GC/MS and histology was performed. **Results:** Tear concentrations of palmitate decreased significantly after castration, compared to intact animals. Treatment with GTx-835 or GTx-837 (SQ or Topical) or TP (SQ) increased the tear palmitate levels, while treatment with GTx-830 or GTx-838 had lesser effects. Histological evaluation and oil red-O staining revealed significant atrophy and reduced lipid levels in the meibomian glands of vehicle treated castrated animals. Treatment with GTx- 835, GTx-837 or TP restored the structure and lipid content of meibomian glands. **Conclusions:** Our study provides the first histological evidence of meibomian gland atrophy following androgen depletion by castration. Selected SARMs can modulate the saturated fatty acid composition of tears and have the potential to treat evaporative dry eye and MGD. **Disclosure:** This research was funded by GTx Inc.

INDUCTION OF CD4+ T CELL MEDIATED IMMUNITY IN DRY EYE DISEASE. Reza Dana. Schepens Eye Research Institute and Massachusetts Eye and Ear Infirmary, Harvard Medical School Department of Ophthalmology, Boston MA, USA

Purpose: The precise contribution of the different CD4+ subsets in the pathogenesis of dry eye disease (DED) remains poorly characterized. Our laboratory has been active in delineating the function of different T cell subsets in DED. **Methods**: Female C57Bl/6 mice were placed in the controlled desiccating environment to induce DED. **Results:** Mobilization of mature ocular surface antigen-presenting cells to the draining lymph nodes of DED mice leads to significant activation of interferon-gamma (IFN-g)-secreting (T helper-1 [Th1]) and interleukin-17 (IL-17)-secreting Th17 (but not Th2) cells in the lymphoid tissues. Expansion of Th17 (but not Th1) cells occurs with minimal Treg suppression, allowing for expansion and homing of pathogenic T cell subsets to the ocular surface through a coordinated expression of CC and CXC chemokines, which is potently regulated by the expression of programmed death ligand-1 (PD-L1) by the surface epithelium. **Conclusions**: There is clear evidence for the activation and functional relevance of both Th1 and Th17 cells in DED induction and progression. Blockade of pathogenic cytokines or over-expression of regulatory factors may provide novel therapeutic strategies for the management of DED. Research Support: NIH, Research to Prevent Blindness, Alcon, Allergan

THE INTERNATIONAL SJÖGREN'S SYNDROME REGISTRY– CURRENT STATUS AND FUTURE OBJECTIVES. Troy Daniels,[1] Caroline Shiboski,[1] Lindsey Criswell,[1] Stephen Shiboski,[1] John Whitcher,[1] Morten Schiødt,[2] Hector Lanfranchi,[3] Hisanori Umehara,[4] Zhao Yan,[5] Stephen Challacombe,[6] M. Srinivasan,[7] Fred Vivino,[8] Alan Baer,[9] John Greenspan,[1] for the Sjögren's International Collaborative Clinical Alliance (SICCA). [1]University of California, San Francisco, USA; [2]Copenhagen University, Rigshospitalet, Denmark; [3]University of Buenos Aires and German Hospital, Argentina; [4]Kanazawa Medical University, Ishikawa, Japan; [5]Peking Union Medical College Hospital, Beijing, China; [6]King's College London, UK; [7]Aravind Eye Hospital, Madurai, India; [8]University of Pennsylvania, Philadelphia, USA; [9]Johns Hopkins University, Baltimore, USA.

Purpose: Sjögren's syndrome (SS) lacks international and professionally accepted classification criteria essential to support prospective research into its autoimmune mechanisms and define clinical trial patients. This first comprehensive international registry for SS is funded by the US NIH in years 2003- 2013, called SICCA and charged with developing both new classification criteria and a clinical data and specimen bank to support future research on SS. **Methods**: Each SICCA participant is uniformly evaluated with protocol-driven questionnaires and examinations, at nine 9 participating sites in 6 countries, and provides serum, DNA, tear, conjunctival cell, saliva, and salivary gland specimens. In its 7th year, we have enrolled over 1900 participants for baseline evaluations and over 500 for 2 year follow-up. **Results**: SICCA has reported: 1) an absence of significant association between dry eye and dry mouth symptoms to objective features of SS (i.e. positive serum SS-A and/or – B, ocular staining with lissamine green-fluorescein and focal lymphocytic sialadenitis in minor salivary gland biopsies) and 2) the presence of two forms of keratoconjunctivitis sicca (KCS), i.e. KCS associated with objective features of SS, and KCS not associated with objective features of SS. SICCA now has sufficient data to propose new SS classification criteria and better define its phenotype and natural history. **Conclusions**: SICCA is providing significant advances in our knowledge of SS. NIH contract NO1-DE-32636 supports SICCA 2003-13

RESOLVINS RVD1 AND THE ASPIRIN-TRIGGERED RESOLVIN 17 (R)-RVD1 BLOCK HISTAMINE-STIMULATED INCREASE IN CA^{2+} AND ACTIVATION OF EXTRACELLULAR REGULATED KINASE (ERK)1/2 TO PREVENT CONJUNCTIVAL GOBLET CELL SECRETION Dartt DA[1, 3], Li D[1, 3], Hodges RR[1, 3], Shatos M[1, 3], and Serhan CN[2,] INSTITUTIONS:[1]Schepens Eye Research Institute, [2]Brigham and Womens Hospital, [3]Harvard Medical School, Boston, MA

Purpose: To determine the cellular mechanism used by pro-resolution compounds resolvin RvD1 and aspirin-triggered resolvin 17(R)-RvD1 to block histamine-stimulated secretion from cultured rat conjunctival goblet cells. **Methods:** Goblet cells were preincubated for 0.5 hrs with both resolvins (10^{-10}-10^{-8} M) and stimulated with histamine (10^{-5} M) for 2 hrs. The media was analyzed glycoconjugate secretion. Cells were incubated for 1 hr with 8mm fura-2 ester prior to addition the resolvins for 0.5 hrs. Intracellular [Ca^{2+}] ([Ca^{2+}]$_i$) was determined following histamine stimulation. Cells were also serum starved for 2 hrs, preincubated for 0.5 hrs with resolvins and phosphorylated (active) and total ERK1/2 was determined by western blot. **Results:** RvD1 inhibited histamine-stimulated secretion with a maximum inhibition of 70 ± 12 % while 17(R)-RvD1 decreased it a maximum inhibition of 80 ± 12 % both at 10^{-8} M. RvD1 at 10^{-10} and 10^{-8} M and 17(R)-RvD1 at all concentrations completely inhibited histamine-stimulated increase in [Ca^{2+}]$_i$. RvD1 and 17(R)-RvD1 both at 10^{-10} and 10^{-9} M completely

inhibited histamine-stimulated increase in ERK1/2 activity. **Conclusion:** Activation of the pro-allergic histamine receptors stimulates conjunctival goblet cell mucin secretion that can be actively terminated by both resolvins. Supported by NIH EY467778

CHARACTERIZATION OF MUCIN-TYPE GLYCOPROTEINS IN MARINE MAMMAL TEARS. Robin Kelleher Davis,[1,2] Pablo Argueso.[1,2] Schepens Eye Research Institute,[1] Harvard Medical School,[1,2] Boston, MA, USA

Objective. Marine mammals experience varying degrees of desiccation stress at the ocular surface depending on the phylogeny. For example, pinnipeds (e.g. sea lions) spend considerable time on land and ice, but cetaceans (e.g. dolphins) are almost entirely aquatic. Marine mammal tears contain proteins, but the tear film lacks the lipid layer found in humans. We hypothesize that mucin-type glycoproteins, in lieu of lipids, are protective components of the marine mammal tear film, with different species having unique compositions depending on degree of exposure to air. **Methods.** For biochemical analysis, tear secretions from cetaceans, pinnipeds, and humans were collected via capillary suction or absorption onto cellulose sponges. Protein concentration was determined with a bicinchoninic acid assay. After electrophoresis on 1% SDS-agarose gels, proteins were transferred to nitrocellulose membranes by vacuum blotting. To detect mucin-type O-glycans, membranes were probed for the T-antigen carbohydrate using Arachis hypogea agglutinin (PNA). Lectin binding was visualized using chemiluminescence. For O-glycan analyses, samples were subjected to normal-phase high pressure liquid chromatography (HPLC) after release of O-glycans by ammonia-based beta-elimination. **Results.** Lectin blot analysis revealed PNA binding to proteins of high molecular weight (consistent with the molecular weight of mucins) in human, sea lion, and dolphin tear samples. The size distribution of PNA-positive bands was different for each species analyzed, with dolphin bands migrating at the highest molecular weight. Separation of O-glycans by HPLC revealed similar profiles in chromatograms of tears from humans and sea lions, but a different pattern in dolphin tears. **Conclusions.** Similar to humans, mucin-type O-glycans are present in the tears of cetaceans and pinnipeds, but there are differences in size distribution and O-glycan profiles across species. Support: NIH grants EY05612 & EY014847, Arey's Pond Boat Yard, Inc. We thank Aquarium of Niagara (NY), and Dolphin Quest Oahu (HI) for contributions of marine mammal tears, and David A. Sullivan for advice.

COMBINATION OF PHENOL RED THREAD TEST AND SCHIRMER 1 TEST AS A RESCUE STRATEGY TO DETECT SEVERE OCULAR DRYNESS. De Monchy I, Mariette X, Pogorzalek N, Kaswin G, Gendron G, Labetoulle M Hopital Bicetre, Université Paris-Sud, 94275 Kremlin-Bicetre, FRANCE

Objective: To define a combination between Schirmer I and phenol red thread test (PRT) that improves the screening of patients with ocular sicca syndrome. **Methods:** The PRT test was performed before (PRT1) and after (PRT2) the Schirmer I test, in both eyes of 143 patients complaining of ocular dryness secondary to Sjögren's syndrome (SGS) or Sicca Asthenia Polyalgia Syndrome (SAPS) (72 and 71 patients respectively), and in 40 patients with no sign of dry eye. Groups were matched by age and gender. After determining the best cut-off values using the ROC procedure, several combinations of PRT and Schirmer I were assessed to improve the predictive values of the procedure. **Results:** The best cut-off value for PRT2, estimated at 15mm, provided a satisfying match between sensitivity and specificity indexes (68% and 90% respectively), similar to those obtained with the Schirmer I test. If PRT1 alone was ineffective to screen SGS from control patients, the comparison between PRT 1 and PRT2 ("delta-PRT") was found as a good marker to detect patients with persistent tear reflex. The combination of positive Schirmer I, PRT 2 and/or delta-PRT tests was found as highly predictive of severe ocular sicca syndrome. **Conclusion:** The combinationof Schirmer I and PRT test improves the screening procedure to detect patients with severe ocular dryness. Since PRT test is non-invasive and time-effective, it could be more widely used in daily clinical practice, besides Shirmer I test, to optimize the work-up of patients presenting with dry-eye subjective signs.

EFFICACY AND SAFETY ASSESSMENT OF A NOVEL UVC DEVICE IN TREATING CORNEAL BACTERIAL INFECTIONS. SJ Dean[1,] A Petty[1,] S Swift[1,] J McGhee[1,] A Sharma[2,] J Moore[3,] S Shah[4], JP Craig[11] Univeristy of Auckland, New Zealand; [2] Moorfields Bedford, UK; [3] Univeristy of Ulster, UK; [4] Birmingham Midlands Eye Centre, UK

Objective: A prototype solid-state Ultraviolet-C (UVC) device may be useful in the treatment of corneal microbial infections, as UVC is commonly used for eradicating bacteria, fungi and viruses in other settings. This study addresses the efficacy of the device with four different bacterial strains, and the potential safety of the device with human corneal epithelium in vitro. **Methods**: Four organisms (S. aureus, E. coli, P. aeruginosa and S. pyogenes) were exposed to 265nm UVC at a fixed intensity and distance, for 30, 5, 4, 2 and 1 second exposures. The presence or absence of growth inhibition was assessed. Human corneal epithelial cells were then cultured on glass cover-slips, and exposed to corresponding doses of UVC from the same device. Immunoflourescent live/dead staining was performed and results quantified. **Results**: There was 100% inhibition of growth in all bacteria, at all exposure times tested down to 1 second. In human corneal epithelium exposed to UVC, there was no statistically significant difference between control and the 30 second exposure for the ratio of dead to live cells (p=0.877). **Conclusion**: The data demonstrate a one-second exposure to germicidal UVC from this LED source was sufficient to inhibit microbial proliferation in the four bacterial strains tested in vitro. The literature suggests UVC at this dose could potentially be tolerated in treating corneal surface infections, without causing significant adverse effects, which supports our findings in human corneal epithelium exposed to UVC. Financial disclosure: S Dean, A Sharma, J Moore and S Shah have a joint patent application filed.

CHARACTERISTICS OF DRY EYES WITH SHORT TEAR FILM BREAK-UP TIME. Seika Den[1], Dogru Murat[1,2], Kazunari Higa[1], Jun Shimazaki[1,2] 1; Department of Ophthalmology, Tokyo Dental College. 2; Keio University School of Medicine

Objective: To investigate characteristics of dry eyes with short tear film break-up time (BUT) without aqueous deficiency. **Method:** Forty four eyes of 44 patients (41 females; mean age: 56.1±13.6 years; range: 21 to 76 years) with dry eye symptoms were divided into two groups, short BUT without aqueous deficiency (Schirmer value > 5mm, BUT ≤ 5s and vital staining of ocular surface (VS) < 3 points) (SB), and aqueous deficiency (Schirmer value ≤ 5mm, BUT ≤ 5s and VS ≥ 3 points) (AD) groups. Six healthy subjects were used as controls. The measurements as follows were performed and compared among three groups; subjective symptoms, tear lipid layer interferometry (TLLI), visual function using functional visual acuity (FVA) measurement, tear stability analysis system (TSAS), goblet cell density (GCD) and squamous metaplasia (SM) in bulbar conjunctiva obtained by impression cytology, and the mRNA expression of MUC5AC and MUC16 in the bulbar conjunctiva obtained by brush cytology using quantitative real-time PCR. **Result**: SB and AD group

was consisted with 20 eyes (17 females, mean age: 51.6±15.2 years) and 24eyes (24 females, 59.9±11.1 years), respectively. SB group was significantly younger than that in AD group (P=0.048). Symptom scores were comparable between the two groups. Visual maintenance ratio (VMR) calculated by FVA tests were comparably decreased in the SB and AD groups compared to controls (P=0.0036 and 0.0009, respectively). The grading of TLLI in SB and AD groups were comparably higher than that in controls (P=0.0005 and 0.0007, respectively). Indices of irregular astigmatism measured by TSAS were comparably higher in the SB and AD groups compared to controls. There were no differences in GCD, SM, and the mRNA expression of MUC5AC and MUC16 among the three groups. **Conclusion:** Despite less epithelial damages, eyes with short BUT without decreased tear secretion had strong irritating symptoms, and deteriorated visual functions comparably to aqueous deficiency eyes. Alteration of mucin expression was not associated with tear film instability.

CXCR4 AND CXCR7 – TWO POTENTIAL RECEPTORS FOR TFF3 AT THE OCULAR SURFACE. Dieckow J[1], Schulze U[1], Paulsen F[2], [1]Department of Anatomy and Cell Biology, Martin Luther University Halle-Wittenberg, Halle, Germany, [2]Department of Anatomy II, Friedrich Alexander University Erlangen- Nuernberg, Germany

Purpose: Trefoil factor family (TFF) peptides, and in particular TFF3, are characteristic secretory products of mucous epithelia promoting antiapoptosis, epithelial migration, restitution and wound healing processes. So far, a receptor has not yet been identified. However, recently the chemokine receptor CXCR4 was shown as a low affinity receptor for TFF2. Furthermore, CXCR7, which is able to form heterodimers with CXCR4, has also been discussed as a potential receptor for TFF2. Since there are distinct structure similarities between the three known TFF peptides, this study aims to evaluate whether CXCR4 and CXCR7 may also act as putative TFF3 receptors and thereby are able to enhance corneal wound healing processes. **Methods:** Expression of both CXCR4 and CXCR7 was evaluated in samples of human lacrimal gland, cornea, conjunctiva and nasolacrimal duct as well as in a human corneal epithelial cell line (HCE) and a human conjunctival epithelial cell line (HCjE). Studies were performed by means of RT-PCR, immunohistochemistry and Western blot analysis. Functional studies are currently ongoing. **Results:** So far, CXCR4 as well CXCR7 could be detected on mRNA level in all observed tissues and cell lines. Immunohistochemistry as well as Western blot analysis revealed protein expression of both receptors in all tissues and cell lines under investigation.**Conclusions:** Until now this study could reveal expression of the receptors CXCR4 and CXCR7 in tissues of the human ocular surface and lacrimal apparatus. Subsequent functional studies analyzing the potential interaction between TFF3 and CXCR4 as well as CXCR7 are currently ongoing.

CHANGES OF ION TRANSPORTERS AND AQUAPORINS IN RABBIT LACRIMAL ACINI AND DUCTS DURING PREGNANCY. Chuanqing Ding[1], Michael Lu[1], Yanru Wang[2] Cell & Neurobiology[1], Physiology & Biophysics[2], University of Southern California, Los Angeles, CA 90089, USA

Objective: To test the hypothesis that reduced basal lacrimal secretion during pregnancy results from changes of ion transporters and aquaporins (AQPs) in lacrimal gland (LG). **Method:** Purified acinar cells and cells from each duct segment, from both control and term pregnant rabbits, were collected by laser capture microdissection for real time RT-PCR analysis. LGs were processed for immunofluorescence following standard protocol. **Result:** NKCC1 mRNA level was unchanged during pregnancy in acini, intralobular and interlobar ducts, whereas its level was significantly higher in interlobular duct and decreased in intralobar duct. NKA_1mRNA abundance was unchanged in acini, interlobular and intralobar ducts during pregnancy, whereas its abundance was significantly lower in intralobular and interlobar ducts. In pregnant rabbits, CFTR mRNA abundance was significantly higher in acini, although no difference was observed in any duct segment. During pregnancy, NHE1 mRNA level was unchanged in acini, intralobular, intralobar and interlobar ducts, whereas its level was significantly lower in interlobular duct. In pregnant rabbits, mRNA level for AQP4 was significantly lower in every duct segment except interlobular duct and acini, both were unchanged. For AQP5, its mRNA abundance was significantly lower in acini during pregnancy, although its level was unchanged in any duct segment. Immunofluorescence data showed the diverse staining pattern of these transporters and AQPs, and generally accorded with the abundances of mRNA expression. **Conclusion:** The present data strongly support our notion that changes of ion transporters and AQPs in LGs could be the reason underlying reduced basal lacrimal secretion during pregnancy. Commercial relationship: None Grant Support: NIH EY017731, EY005801, EY010550, EY03040, DK048522.

EFFICACY OF AZITHROMYCIN 1.5% EYE DROPS IN CHILDHOOD OCULAR ROSACEA. Serge Doan, Melissa Touati, Muriel Catanese, Isabelle Cochereau, Eric Gabison, Hopital Bichat and Fondation A de Rothschild, Paris, France

Purpose.: To report the efficacy of topical azithromycin in childhood ocular rosacea. Patients and methods. We retrospectively studied the files of 18 children suffering from ocular rosacea who were treated with azithromycin 1.5% eye drops (Azyter®, Thea laboratories, France). **Results:**24 eyes of 8 boys and 10 girls, aged 10±5 years were treated. The disease was resistant to lid hygiene, oral erythromycin and topical steroids in 15, 2 and 3 cases, respectively. All patients suffered from phlyctenular keratoconjunctivitis with blepharitis. They received Azyter® bid for 3 consecutive days every 10 days, associated with lid hygiene. Ocular redness resolved within 2 months whereas phlyctenular keratoconjunctivitis healed within 3 to 6 months in all cases. Palpebral inflammation partially decreased in 14 patients. Treatment was stopped after 6 months without recurrence (follow-up, 4 to 10 months without treatment). **Conclusions:** Azithromycin 1.5% eye drops are a potent treatment for phlyctenular keratoconjunctivitis complicating childhood ocular rosacea.

METHODOLOGIES TO DIAGNOSE AND MONITOR DRY EYE DISEASE. Murat Dogru, M.D, Ph.D. Johnson and Johnson Ocular Surface and Visual Optics Department, Keio University School of Medicine, Tokyo, Japan

Objectives: The purpose of the 2007 International Dry Eye Workshop (DEWS) "Diagnostic Methodology Subcommittee" report was to review the literature and develop a resource of tests used in dry eye disease diagnosis and monitoring with the aim to facilitate standardization and validation. The tests were displayed as templates on the TFOS website (www.tearfilm.org). This presentation will focus on emerging technologies, and describe new advances in the understanding of methodologies to diagnose and monitor dry eye disease with special emphasis on the most important directions for future research since the publication of the DEWS report. **Methods:** A thorough PubMed, Medline and Index Medicus search has been performed and will be carried out until the presentation by the author to delineate the new advances in relation to diagnostic methodologies cited in the 2007 International DEWS report and methods which emerged beyond the 2007 report. **Results:** There appears to be substantial research on new diagnostic technologies and advances in

methodologies especially in ocular surface and tear meniscus imaging technologies including infrared meibography, evaluation of lipid layer thickness and rheology, in-vivo confocal microscopy, other optical coherence tomography anterior segment imaging devices and tear meniscus volume quantification. One of the striking developments is in the area of tear osmolarity assessment and its application into diagnosis of dry eye disease and evaluation of treatment responses. **Conclusions:** It is the presentor's perspective and impression that the future of dry eye diagnostics will be with new and minimally invasive techniques that sample the eye and preserve its steady state.

THE CORNEAL PROTECTIVE EFFECTS OF SILICON HYDROGEL SOFT CONTACT LENS WEAR FROM UV-B EXPOSURE AND OXIDATIVE STRESS Murat Dogru, [1,2], Ibrahim Osama,[1,3] Tais Wakamatsu,[1,3] Takashi Kojima, [1,3],Yukihiro Matsumoto,[1,3], Kazuno Negishi, [3], Jun Shimazaki,[2]Yasuo Matsumoto,[4] Hiroshi Sasaki,[4] Kazuo Tsubota[3] 1) J&J Ocular Surface and Visual Optics Dept, Keio University School of Medicine 2) Dept. of Ophthalmology, Tokyo Dental College School of Medicine 3) Dept. of Ophthalmology, Keio University School of Medicine 4) Dept. of Ophthalmology, Kanazawa Medical University

Objectives: To investigate the effects of ultraviolet B radiation on the cornea, tear functions and the oxidative stress status in mice wearing silicon hydrogel contact lens (SHCL) compared with non wearers **Methods:** C57BL6 strain male mice (n=5) were fitted with senofilcon A SHCL and exposed to UV-B (312nm) 10 minutes/day for 5 days for a total dose of 2.73J/ cm^2 . Tear function tests (BUT and cotton thread), blink measurement, corneal sensitivity, corneal fluorescein and Rose Bengal stainings were performed before and 5 days after UV-B exposure. Tear samples were collected before and after exposure for cytokine and oxidative marker (HEL) assays. Corneal specimens underwent immunohistochemical stainings (IHCS) forCD45, 8OhdG, and 4HNE.The results were compared with age and sex matched non SHCL wearing mice (n=5) exposed to same level of UV-B. **Results:** All mice not wearing SHCL developed a significant decline in corneal sensitivity, blink rate, and tear stability; significant corneal edema, ulcers or extensive epithelial damage compared to mice wearing SHCLs which escaped corneal damage after exposure to UV-B. Tear HEL and IL-6 levels significantly increased in the non SHCL wearers compared to SHCL wearing mice after exposure. IHCS showed significantly higher number of cells positively stained for CD45, 8OhdG and 4HNE in the corneas of mice which did not wear SHCLs compared to wearers after UV-B exposure. **Conclusion:**Our findings suggest that SHCL wear has protective effects against UV-B exposure and oxidative stress related membrane lipid and cellular DNA damage due to UV blocking properties.

ENVIRONMENTAL BIOMECHANICS GOVERNS CELL BEHAVIOUR OF CORNEAL KERATINOCYTES. Eberwein P, Steinberg T, Schulz S, Tomakidi P, Beck D, Reinhard. T University Eye Hospital Freiburg;Department of Oral Biotechnology

Objective: Biomechanics of the extracellular microenvironment is crucial for cell functions including growth and differentiation. The objective of this study was to create an experimental microenvironment for corneal keratinocytes, to analyze these functions at defined biomechanics.**Methods:** Corneal keratinocytes were cultured on micropillar interfaces for up to 72 hours. For defined biomechanics, interfaces comprised a micropattern range from 3 – 11µm. Cell specimens were proceeded for scanning electron microscopy (SEM), and indirect immunofluorescence (IIF) for keratins (K) K19, K12, involucrin, and fillagrin, indicating progressive differentiation. **Results:** In SEM, regular morphologenesis concomitant with proceeding cell growth was denoted at small pillar micropatterns, i.e. 3, 4, and 5µm, while progressively deranged morphology was obserbved at rising pillar distances, suggesting higher adhesion efficiency at small micropatterns. IIF revealed that terminal differentiation marker expression K12, fillagrin and involucrin increased with rising pillar distances, while early marker expression K19 showed inverse expression. This demonstrates that smaller micropatterns favour early, and large scale pillar patterns terminal differentiation of corneal keratinocytes. **Conclusion:** We show that morphologenesis, adhesion and growth of corneal keratinocytes in conjunction with expression of biomarkers indicating early and late stages of differentiation are clearly directed by the pillar micropattern, indicating that environmental biomechanics governs cell behaviour. This points to the importance of a special microenvironment, needed for each cell type and differentiation status, in order to have cells fulfil their function. Determination of the biomechanics of this microenvi

OCULAR SURFACE IMAGING. Nathan Efron, Nicola Pritchard, Munira Al-Dossari. Institute of Health and Biomedical Innovation, and School of Optometry, Queensland University of Technology, Kelvin Grove, Queensland, Australia

Purpose. The capability of laser scanning confocal microscopy (LSCM) for assessing the transparent human cornea in vivo at a cellular level is well established. This presentation explores the potential for examining the adjacent semitransparent structures of the bulbar and palpebral conjunctiva. **Methods**. LSCM was used to observe and measure morphological characteristics the bulbar and palpebral conjunctiva of 22 healthy human volunteer subjects, 11 of whom wore soft contact lenses. **Results**. The appearance of the bulbar conjunctiva is consistent with known histology of this tissue based on light and electron microscopy. The bulbar conjunctival epithelium of lens wearers (30.9 ± 1.1 µm) was thinner than that in controls (32.9 ± 1.1 µm) ($p < 0.0001$). Superficial and basal bulbar conjunctival epithelial cell density in contact lens wearers was 91% and 79% higher, respectively, than that in controls ($p < 0.0001$). No difference was observed in goblet and Langerhans cell density between lens wearers and controls. Conjunctival microcysts were observed in greater numbers, and were larger in size, than in non-lens wearers. A number of key histological features of the palpebral conjunctiva could be clearly imaged, including the tarsal plate and posteriorly projecting meibomian gland acini. **Conclusions**. These observations highlight the utility of LSCM for assessing the human bulbar and palpebral conjunctiva in vivo. Contact lens wear can induce changes in the bulbar conjunctiva such as epithelial thinning and increased epithelial cell density. Further studies will be required to determine the impact of contact lens wear on goblet and Langerhans cell density.

COMPARISON OF TWO DRY EYE QUESTIONNAIRES AND CLINICAL OBSERVATIONS IN NON-CONTACT LENS WEARERS. J. Enbuske, E. Blixt, K. Silfwerbrand, and J. P. Gierow. Schoolof Natural Sciences, Linnaeus University, Kalmar, Sweden

Introduction. Identification of patients with dry eyes and classification of the severity of the condition is important for the patient, but also for the clinician to assure that the patient receives the appropriate treatment. Rather limited correlations between objective signs and subjective symptoms have been reported and moderate conditions are particularly difficult to diagnose. Therefore, two different questionnaires were compared with each other and with clinical signs obtained with the use of Tearscope-plus. **Methods.** Thirty patients (15 m, 15 f), all non-contact lens wearers, were enrolled. Two questionnaires were used: the Texas Eye Research and Technology Center Dry Eye Questionnaire (TERTC) and the Ocular Surface

Disease Index (OSDI). Each patient responded to both questionnaires with a two-week interval, half of the patients received TERTC first and the other half received OSDI first. Using the Tearscope-plus, the following clinical signs were recorded: non-invasive tear break-up time (NITBUT), lipid layer type and tear meniscus height. **Results.** 11 patients were identified as having dry eyes according to the questionnaires; 9 with OSDI (4 mild, 4 moderate, 1 severe) and 9 with TERTC (5 moderate, 4 dry eyes). The 4 patients in the TERTC dry eye category were all in the moderate-severe OSDI categories. A significant correlation between the scores from the two questionnaires was obtained ($r=0.84$; $p<0.001$). NITBUT indicated that 15 patients had dry eyes (< 10 s), of which only 6 were identified by the questionnaires. Of the 11 identified by the questionnaires, 5 had dry eyes according to NITBUT-values. No significant correlations were observed between NITBUT and either one of the questionnaires, or between the questionnaires and lipid layer type or meniscus height. **Conclusions.** A significant correlation was observed between the two questionnaires. A higher number of patients with at least moderate symptoms was identified with TERTC compared to OSDI (9 and 5, respectively), indicating that the former is better at recognizing moderate forms of dry eyes. Further studies on a larger patient group are needed to verify this. Our study also underscores the difficulty in comparing subjective symptoms and clinical signs.

OCULAR SURFACE MUCINS IN ADULTS WITH CYSTIC FIBROSIS. Katharine Evans[1], Rachel North[1], Christine Purlsow[1], Monica Berry[2]. School of Optometry & Vision Sciences, Cardiff University[1]; Academic Unit of Ophthalmology, University of Bristol, Bristol Eye Hospital[2], UK

Purpose: Dry eye in Cystic Fibrosis is thought to be secondary to vitamin A deficiency (VAD). Tear film mucin distribution is altered in dry eye and decreased rMuc5AC levels have been recorded in VAD rats. Furthermore, the major airway epithelial mucins, MUC5AC and MUC5B, are significantly decreased in CF airway secretions. This study aims to investigate ocular surface mucins in CF subjects with known vitamin A status and disease severity. **Methods:** Tear samples were collected with Schirmer strips from 28 CF subjects (19m, 9f; 27 ± 7 years) and age-matched healthy controls. Mucins were extracted in RIPA buffer and reactivity to antibodies against mucin peptide core epitopes was assessed in dot-blots on PVDF membranes. Serum vitamin A levels, lung function and genotype were recorded for CF subjects. **Results:** Higher reactivity levels of MUC1, MUC4, MUC5AC and MUC7 were recorded in the CF group, with significant differences in MUC1 and MUC4 (Mann-Whitney; $p<0.005$ and $p<0.01$ respectively). Greater mucin reactivity (MUC1, MUC4 and MUC7) was observed in the CF DF508 heterozygotes compared to homozygotes, although differences were not significant (Mann-Whitney; $0.622<p<0.938$). MU5AC was reduced in the VAD CF subjects compared to the vitamin A sufficient CF subjects and healthy controls, but differences were not significant (Kruskal-Wallis; $p=0.777$). There was no correlation of mucin reactivity and serum vitamin A, disease severity or lung function. **Conclusions:** Cell surface associated mucin reactivity is altered in CF, and in particular in patients with a less severe CF genotype. VAD in CF does not significantly impair MUC5AC secretion from the conjunctival goblet cells. Further investigation is necessary to understand the nature of these alterations. Commercial relationships: none. Grant support: Cardiff University.

DRY EYE: A PRIMARY CHARACTERISTIC OF CYSTIC FIBROSIS? Katharine Evans, Rachel North, Christine Purslow School of Optometry & Vision Sciences, Cardiff University, UK

Objective: Dry eye in Cystic Fibrosis (CF) is presumed to be secondary to vitamin A deficiency (VAD). CFTR, the abnormal membrane protein causing CF, is present in the conjunctival and corneal epithelium. CFTR mediates chloride (Cl^-) secretion from the ocular surface facilitating epithelial fluid transport and contributing to basal tear production. Therefore, dry eye may be a primary characteristic of CF and severity could be related to genotype. This study aims to investigate if dry eye is a primary manifestation of CF. **Method:** Tear film stability, ocular surface hyperaemia, corneal staining (Efron grade) and ocular comfort (Ocular Comfort Index) was assessed in 28 CF subjects (19m, 9f; 27 ± 7 years) and matched healthy controls. Serum vitamin A levels and genotype were recorded for CF subjects. **Result:** Non-invasive and fluorescein tear break-up time (FBUT) were lower in the CF group compared to controls with differences reaching significance for FBUT (Mann-Whitney; $p<0.05$). Increased hyperaemia and staining was observed in the CF group although differences were not significant ($0.079 <0.474$). Ocular comfort was similar in the two groups ($p=0.491$). There were no differences when subjects were grouped according to genotype. CF subjects with VAD had greater levels of staining compared to sufficient CF subjects and controls (Kruskall-Wallis; $p<0.05$). There was no correlation of measures of dry eye with serum vitamin A concentration.**Conclusion:**Whilst VAD appears to adversely affect the ocular surface in CF, there is limited evidence that dry eye is actually a primary manifestation of CF. The results suggest that alternative apical Cl^- channels may compensate for defective efflux via CFTR, allowing normal basal tear secretion from the epithelia. Commercial relationships: none. Grant support: Cardiff University.

TEAR FERNING IN CYSTIC FIBROSIS. Katharine Evans, Rachel North, Christine Purslow.School of Optometry & Vision Sciences, Cardiff University, UK

Objective: Abnormal tear ferning (TF) in Cystic Fibrosis (CF) has been observed previously and could be secondary to vitamin A deficiency (VAD). CFTR, the abnormal membrane protein causing CF, is present in the conjunctival and corneal epithelium and thought to facilitate basal tear secretion. Therefore, dry eye and abnormal TF may be a primary manifestation of CF. This study aims to investigate TF in CF subjects of known vitamin A status and disease severity.**Method:** Tear samples were collected from 37 CF subjects (25m, 12f; 20 ± 9 years) and 39 healthy controls (25m, 14f; 22 ± 8 years) and TF graded with Rolando's scale. Tear film stability (non-invasive break-up time (NIBUT) and fluorescein break-up time (FBUT)), ocular surface hyperaemia and corneal staining (Efron grade) were assessed. Serum vitamin A level, genotype and lung function were recorded for CF subjects. **Result:** Higher TF grades were observed in the CF cohort, although differences were not significant (Mann-Whitney; $p=0.293$). Abnormal TF (> Type 2) was not recorded in any subject. Similar TF grades were observed when CF subjects were grouped according to vitamin A status (Kruskal-Wallis; $p=0.320$) and genotype ($p=0.687$) and compared to controls. In the CF cohort, TF correlated with lung function (Spearman; $r=-0.336$, $p<0.05$) but not serum vitamin A ($r=0.268$, $p=0.114$). In all subjects, TF negatively correlated with NIBUT and FBUT ($r=-0.583$, $p<0.005$; $r=-0.531$, $p<0.005$ respectively) and positively correlated with limbal and conjunctival hyperaemia ($r=0.310$, $p<0.01$; $r=0.473$, $p<0.005$ respectively). **Conclusion:** The lack of abnormal TF in this study, compared to earlier investigations, could reflect modern CF disease management. There is limited evidence that dry eye is a primary manifestation of CF however. Correlations of TF and other tear film and ocular surface tests are novel to this investigation. Commercial relationships: none. Grant support: Cardiff University.

DRY EYE AFTER 20 AND 25 GAUGE VITRECTOMY
C.Fabiani, S.Barile, JM Rakic, M. De Zanet. CHU, LIEGE - BELGIUM

Objective: 25 Gauge Vitrectomy is widely used to accelerate patient recovery after surgery, to preserve ocular surface integrity reducing postoperative ocular discomfort. The aim of this study was to determine the presence of symptoms and signs of ocular surface dryness in patients treated with Vitrectomy 20G (group A) and Vitrectomy 25G (group B). **Methods:** We studied 21 patients (21 eyes; mean age: 69±10 years). 10 eyes underwent 20G vitrectomy (group A) and 11 eyes 25G vitrectomy (group B). Each patient answered a dry eye questionnaire and underwent a complete ocular examination. Clinical measurements of tear function (BUT, Schirmer 1) were completed and ocular surface osmolarity was quantified. Corneal surface integrity was determined by means of fluorescein staining.Patients were evaluated before surgery, at day 7, 1 month and 3 months after surgery. **Result:** A significant increase in mean corneal fluorescein staining score was observed in group A if compared to group B at day 7 after surgery (P<.001) and after 1 month. After 1 week BUT score was lower than 5 sec in 50% group A eyes and in 33% group B eyes. Non-statistically significant difference in Schirmer test values and Osmolarity was found between the two groups. 90% of group A patients reported dry eye symptoms and only 41.2% group B. **Conclusion:** Our results indicate that tear film and ocular surface are less affected after small gauge Vitrectomy, if compared to traditional 20G. By preserving ocular surface integrity, 25G Vitrectomy significantly reduces corneal epithelial damage and ocular surface discomfort.

REVERSAL OF END-STAGE SJÖGREN'S SYNDROME AND DIABETES IN THE NOD MOUSE: CURRENT CLINICAL TRIAL PROGRESS AND BIOMARKER DESIGN.
Denise L. Faustman,[1,2] Massachusetts General Hospital,[1] Harvard Medical School[2] Boston, MA, USA

Purpose. Our laboratory's clinical translation efforts in autoimmunity are based on the targeted death of autoreactive T cells by tumor necrosis factor (TNF) induction, a new approach for autoimmune treatments that does not involve immunosuppression. Methods. Preclinical work in the NOD mouse by our lab and other's shows that targeted autoimmune therapies capable of eradicating the rare autoreactive immune cells not only
help reverse established autoimmunity, but can also unleash spontaneous regeneration of the end organ, including the salivary gland and pancreas in end-stage mice. Results. We have been able to rapidly advance this work to the clinic and recently completed an FDA approved Phase I study using bacillus Calmette-Guérin (BCG), a generic drug that induces the subject's own TNF in the setting of advanced type 1 diabetes, as well as new human biomarker methods and robotics efforts that allow us to track human autoimmune cells. In addition, we have shown that the NOD mouse defect allowing poor T cell education and generation of autoimmunity is, in part, a missing proteasome protein called LMP2, a defect that makes these cells susceptible to apoptosis in the presence of elevated TNF. This work is extremely relevant to Sjögren's syndrome, since work out of Europe now identifies this same T cell error in 100% of Sjögren's patients, allowing preclinical work in the mouse to be more thoughtfully applied to humans with this disease. Conclusions. We will present the current human biomarker methods and robotics efforts for tracking autoimmune cells, including in Sjögren's syndrome. We will also present the clinical trial design we are using in humans to advance this safer approach to autoimmunity. [This research to develop a generic low cost drug was supported by grants from The Iacocca Foundation, other family foundations and individual donors.]

TRANSCULTURAL ADAPTATION AND VALIDATION OF THE OCULAR SURFACE DISEASE INDEX (OSDI) IN PATIENTS OF AN UNIVERSITY HOSPITAL IN SÃO PAULO, BRAZIL.
Felipe Ribeiro Ferreira[1], Ruth Miyuki Santo[1], Priscila Novaes[1] [1]Division of Ophthalmology, School of Medicine of the University of São Paulo , São Paulo, Brazil

Objective: Perform the transcultural adaptation and validation of the Ocular Surface Diseases Index (OSDI) in patients of the Hospital of the University of São Paulo (HCFMUSP), Brazil. **Methods**: The original OSDI was translated into Portuguese, reviewed by experts, and successively applied to groups of 5 subjects until a final version was obtained. For validation, this version was applied to 101 subjects consulted at the Dry-Eye Outpatient Clinic. They also answered the SF-36 and the VFQ-25, to assure construct validity, and underwent biomicroscopy, TBUT, vital staining with Fluorescein and Lissamine Green, and Schirmer I test. The OSDI was applied again to 30 subjects, 7-15 days after the initial evaluation. All data underwent descriptive analysis, and for the OSDI we calculated Cronbach's , factorial analysis, and the intraclass correlation coefficient to assess test-retest reliability. Comparisons were done using the Mann-Whitney test and Spearman's correlation. **Results**: Mean age was 51.53±14.83 years, 82.2% were women, and 77.2% of the subjects had dry eye, 57.7% due to secondary Sjogren's Syndrome. OSDI: Cronbach's was 0.905; factorial analysis indicated 3 subscales, and the intraclass correlation coefficient was 0.801. There was a difference between OSDI scores in patients with dry eye (41.15±27.40) and without dry eye (17.08±17.09)(p=0.02). There was a negative association between OSDI and VFQ-25 scores (p<0.001), and between the OSDI and five SF-36 domains: role limitations due to physical health, vitality, social functioning, role limitations due to emotional problems, and mental health. OSDI scores correlated positively with OSDI and Lissamine Green and Fluorescein scores(p<0.001) and negatively with Schirmer I and TBUT values (p<0.001). **Conclusions**: This culturally adapted version of the OSDI in Portuguese proved to be a valid and reliable instrument for this population, and may be useful as an outcome measure and in assessing the impact of dry eye in the patients' quality of life.

CORNEAL EPITHELIAL BARRIER FUNCTION AGAINST BACTERIA.
Fleiszig SMJ[1], Tam C[1], Mun J[1], Evans DJ[2]. UC Berkeley, CA[1]. Touro University-CA[2].

Objective: *Pseudomonas aeruginosa* possesses a large genome encoding many virulence factors. It also encodes a plethora of regulators of virulence factor expression and many survival/adaptation factors that have potential to be excellent targets for therapeutics. With this endpoint in mind, it is important to determine which genes are critical to pathogenesis in the tissue of interest using models that accurately reflect the circumstances that predispose to infection in people. Indeed, our data show that interactions between bacteria and cells grown in vitro with culture media can be very different from how bacteria/cells interact in vivo. Further, many bacterial "virulence" factors identified in vitro, were subsequently found not to play a role in corneal pathology in vivo. **Method**: In our laboratory we have been working towards making in vitro models more "in vivo-like", and developing in vivo models that more closely resemble how infections happen in humans. We have also studied differences between in vitro and in vivo responses to gain insights into how the in vivo ocular surface remains healthy under normal circumstances. Finally, we have developed imaging methods that enable bacteria and epithelial cells to be imaged within the intact living eyeball. **Result**: The data showed that corneal epithelial defenses against bacterial traversal are separate from barrier function to fluorescein, and also from defenses against bacterial adhesion. Defenses against traversal are dependent on both calcium and MyD88. Both invasive and

cytotoxic strains of *P. aeruginosa* can overcome defense against traversal given sufficient exposure time, with roles played by the bacterial type III secretion system. **Conclusion**: Corneal defenses against bacteria during health include factors that prevent adherent bacteria from traversing the epithelium. The fact that *P. aeruginosa* can circumvent epithelial defenses against traversal given appropriate circumstances could relate to its status as a corneal pathogen of contact lens wearers. **Grant support:** NEI grant RO1EY011221, the Bill and Melissa Gates Foundation, and Alcon Laboratories.

ADVANCES IN THE DESIGN AND CONDUCT OF CLINICAL TRIALS IN DRY EYE DISEASE. Gary N. Foulks, MD, FACS. Kentucky Lions's Eye Center, Lexington, KY, USA

The 2007 International Dry Eye Workshop Report described both general and specific recommendations for design and conduct of clinical trials in dry eye disease and suggested possible enhancements to such studies. This presentation will discuss some of those recommendations and determine from literature review whether such adaptations have occurred in current clinical trial design for study of dry eye and evaluation of possible new therapies. Changes in federal regulatory recommendations also affect clinical trial design and conduct such that new outcome measures may be included in recent trials. Assessment of the recent improvements in clinical trial design and conduct is made with recognition that no new drug therapy beyond topical cyclosporine has been yet approved by the FDA. Future directions and goals in design and conduct of clinical trials for dry eye disease will be recommended.

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.

GENE THERAPY TO MODULATE APOPTOSIS AND TO PROTECT ENDOTHELIAL CELLS AGAINST DEATH DURING STORAGE. Fuchsluger TA[1, 2], Jurkunas U[1], [3], Kazlauskas A[1], Dana R[1,3] [1]Schepens Eye Research Institute, Depart. of Ophthalmology, Harvard Medical School, Boston MA, USA; [2]Center of Ophthalmology / Institute of Anatomy, Essen University Hospital Essen, Germany; [3]Mass. Eye and Ear Infirmary, Depart. of Ophthalmology, Harvard Medical School, Boston MA, USA

Purpose: Regardless of the inciting cause, EC loss is a common denominator of corneal graft failure. EC loss *during storage* results in significant loss of suitable tissue for grafting, EC loss *after transplantation* is a major cause of graft failure, regardless of the transplantation method (pKP, DSAEK, DMEK). The purpose of these studies is to investigate the role of apoptosis in EC in order to prevent EC loss during storage and transplantation. **Methods:** Gene transfer of Lenti-Bcl-xL or -p35 was accomplished in human donor corneas, primary cultured EC and an immortalized EC line and compared to untreated controls and EC expressing the parental vector. Cell death (apoptosis) was induced via extrinsic and intrinsic apoptotic pathway. In addition, EC loss during preservation was studied both during Optisol GS (4C) and organ culture storage (37C). EC were enumerated, apoptosis was detected by TUNEL staining and confocal microscopy. **Results:** The percentage of TUNEL-positive EC provoked by the apoptotic inducers was significantly reduced relative to controls (up to 73% reduction). Transfected corneas preserved an almost intact endothelial monolayer while controls nearly entirely lost vital EC. During long-term storage experiments at 4C and at 37C, EC counts in corneas expressing anti-apoptotic genes were significantly increased compared to the controls. **Conclusion:** Protection of EC by anti-apoptotic genes appears to be an effective method to reduce EC loss during storage. The application of this technique could increase the amount of high quality grafts in eye banking and further reduce graft failure following corneal transplantation, and is of specific interest as to precut corneas and DSAEK/DMEK procedures. [SERI & Univ. Duisburg-Essen filed a joint PCT; NIH R01EY012963 (R.D.), K24EY019098 (R.D.), Research to Prevent Blindness (R.D.), German Research Foundation (DFG/FU 726/1-1, T.F.), Eye Bank Association of America (T.F.)]

CONJUNCTIVAL HLA-DR EXPRESSION AND SURGICAL OUTCOMES OF TRABECULECTOMY. João M. Furtado, Jayter S. de Paula, Edson G. Soares, Eduardo M. Rocha, Régia C. P. Lira, Ana M. da Rocha, Neifi H. S. Dhegaide, Eduardo A. Donadi, Maria de L. V. Rodrigues. Departments of Ophthalmology, Internal Medicine and Pathology, Medical School of Ribeirão Preto, University of São Paulo, Brazil.

Purpose: Since inflammatory reaction in conjunctiva and subconjunctival tissue may compromise the results of antiglaucomatous surgery, the purpose of this study was to investigate the presence of HLA-DR in biopsies collected during trabeculectomy and correlate with surgical outcomes.
Methods: Among 28 patients with open angle glaucoma, 34 eyes with indication of fistulizing antiglaucomatous surgery were submitted to trabeculectomy. All subjects used anti-glaucomatous drugs until the day of the surgery. During the surgery, a biopsy of the inferior bulbar conjunctiva was collected, and processed for immunohistochemistry. Patients were divided in groups according to their surgery outcomes at six and 24 month of follow-up, and the conjunctival expression of HLA-DR were compared between these groups. Surgical success was defined as intra-ocular pressure (IOP) higher than 6 and lower or equal 20mmHg, under the use of antiglaucomatous drugs or not after the surgery. Surgical failure was defined as IOP higher than 20mmHg. The slides were classified as positive or negative for the

presence of HLA-DR. The data were analyzed statistically by the exact Fisher test or by the chi-square test, with the level of significance set at p≤0.05. **Results:** Of the 34 eyes, 15 (44.11%) were positive for the inflammatory marker HLA-DR. There was no statistically significant association between conjunctival expression of HLA-DR and surgical outcomes, at six months (p=0.5714) and at 24 months (p=1.0000) of follow-up. **Conclusions:** This study demonstrates that the conjunctival expression of HLA-DR in biopsies collected at the moment of the surgery doesn't alter the prognosis of trabeculectomy. Financial Disclosure: The authors have no proprietary or commercial interest in any materials discussed in this article. This research was supported by CNPq (Brazilian Council of Research) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior).

EFFICACY OF BROMFENAC SODIUM OPHTHALMIC SOLUTION FOR THE TREATMENT OF DRY EYE DISEASE.
Miki Fuseya[1], Hiroshi Fujishima[1], Masao Ogata[1], Murato Dogrue[2] Department of Ophthalmology, Saiseikai Central Hospital[1], J&J Ocular Surface and Visual Optics Department, Keio University School of Medicine, [2]

Objective: To evaluate the efficacy of Bromfenac sodium ophthalmic solution (BF) in subject with inadequately controlled Dry Eye Disease (DED) with artificial tears (AT). **Method**: 26 patients (male 3, female 23, average age 67.5 ±11.9) who did not show any improvement in the signs and symptoms of DED after the use of AT qid for one month were enrolled. BF bid was administered adjunctively with AT for one month. Then the treatment of BF was discontinued, and the treatment of single use of AT was continued for 3 months. Schirmer's score, tear film breakup time (BUT), and corneal staining were evaluated at the beginning of BF treatment (Pre), at the end of the combined use of BF and AT (BF1M), and one and three months after discontinuation of BF (Po1M and Po3M, respectively). In addition, the dryness was assessed using 5 grades. **Result**: The significant improvements in the scores of dryness were observed at BF1M and Po1M (1.27 ± 0.87 and 1.23 ± 1.03, respectively) compared with Pre (2.58 ± 0.95) (each p<0.001), but there was no notable improvement at Po3M. There were no changes in Schirmer's scores throughout each treatment period. There were remarkable improvements in BUT at BF1M and Po1M (4.42 ± 2.30, and 4.27 ± 1.87, respectively) compared with BUT at Pre (2.77 ± 1.80) (each p<0.001), but there was no significant improvement at Po3M. The considerable improvements in the evaluations of SPK were observed at BF1M and Po1M compared with Pre (p<0.001, p<0.05, respectively), but there was no significant improvement at Po3M. **Conclusion:**. BF was considered to ameliorate the signs of DED by its anti-inflammatory effect. The improvement of BUT was thought to contribute to the reduction of SPK. The combined use of BF and AT was an effective treatment for DED

TEMPERATURE-SENSING BY THE HUMAN CONJUNCTIVAL EPITHELIUM THROUGH ACTIVATION OF TRANSIENT RECEPTOR POTENTIAL VANILLOID (TRPV) CHANNELS.
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Purpose: The members of transient receptor potential vanilloid (TRPV) subfamily are non-selective cation ion channels and are important membrane sensors, responding to thermal, chemical, osmotic or mechanical stress. This is sustained by a number of different regulatory mechanisms and responses to various stimuli. Temperature changes may have a major impact on the physiology of the ocular surface epithelium.This study was undertaken to examine conjunctival epithelial (HCjE) cells as well as human conjunctival tissue for functional TRPV channel activity. **Methods:** Gene expression of putative TRPVs in cultured HCjE cells and human conjunctiva was investigated by RT-PCR. Responses from these cells to drugs and heat-stimuli were investigated by measurements of intracellular free Ca2+ ([Ca2+]i) with fura-2. TRP channel currents were detected with a novel high throughput patch-clamp system. **Results:** RT-PCR analysis of RNA isolates from HCjE cells revealed the expression of TRPV1, TRPV2 as well as the osmosensor TRPV4. In addition, TRPV1, -2 and -4 transcripts could also be detected in human conjunctiva from body donors. Furthermore, temperature rises from 25 °C to over 45 °C as well as application of TRPV channel activators (100 µM 2-APB, 5 µM 4a-PDD) always induced a transient increase of [Ca2+]i in HCjE cells. This Ca2+ transient was significantly reduced in the presence of the TRP channel blockers ruthenium-red (RuR) (10 µM) and lanthanum chloride (La3+) (100 µM). Finally, increasing the temperature over 45 °C induced reversible rises in nonselective cation currents in HCjE cells. **Conclusions:** Functional expression of TRPV channels could be demonstrated in HCjE cells and also in human conjunctiva for the first time. These findings may have direct physiological and clinical implication (e.g. dry eye, conjunctivitis).

SURFACE INTERACTIONS OF BENZALKONIUM CHLORIDE WITH MEIBOMIAN AND CORNEAL LIPIDS AND WITH WHOLE TEARS.
Georgi As. Georgiev,[1] Norihiko Yokoi,[2] Krasimir Koev,[3] Slavyana Ivanova,[1] Elena Kutsarova,[1] Alexander Kyumurkov,[1] Rumen Krastev,[4] Zdravko Lalchev.[1] University of Sofia[1] and University hospital,[3] Sofia, Bulgaria, Kyoto Prefectural University of Medicine,[2] Japan, Max Planck Institute of Colloids and Interfaces,[4] Potsdam, Germany

Purpose: Surface chemistry study of the interactions between benzalkonium chloride (BAC), a common preservative used in ophthalmic formulations, and tear film (TF) constituents. **Methods:** The interactions between BAC and human tears, meibum, and rabbit corneal epithelium lipid extracts at the air/water interface were examined *in vitro* during an artificial blink (compression/expansion of film area) by Langmuir surface balance, surface potential measurements, and axisymmetric drop shape analysis (PD-ADSA). Surface pressure-area isotherms and isocycles were used to assess the sample's lateral elasticity and capability to compress and spread during dynamic area changes. The lipid films morphology was monitored by Brewster Angle Microscopy. The viability of BAC-treated rabbit corneal cell (SIRC) cultures was also examined. **Results:** In the Langmuir-monolayer and PD-ADSA experiments, the interactions between BAC and lipids or tears result in: a) impaired lipid spread and formation of discontinuous non-uniform surface layers, b) increased surface pressure-area hysteresis during compression/expansion and c) displacement of the lipids by BAC from the surface. A decrease (>50%) in SIRC cell viability was observed. The effects occurred within seconds after BAC exposure and their magnitude increased with BAC concentration (kept within the clinical range of 0.005-0.02%). **Conclusions:** A surface chemistry approach provides molecular scale insights of the detrimental effect of BAC on TF, which well-explain TF instability and corneal epithelial barrier dysfunction after exposure to BAC in the *in vivo* human eye (Ishibashi et al. J Glaucoma. 2002). [This research was supported by a grant from Contract DO 02-280/2008 by the Bulgarian ministry of Education and Science]

CLINICAL SAFETY STUDY OF A NOVEL EYELID WARMING DEVICE USING MOIST HEAT TECHNOLOGY.
Felicity Gill, Paul Murphy, Christine Purslow School of Optometry & Vision Sciences, Cardiff University, UK

Objective: Heat therapy is the standard regimen for obstructive meibomian gland dysfunction (MGD). Research has shown the melting point of the meibum is raised to 35°C in MGD compared to 32°C in normal subjects, and sustained heat delivery is essential in effective treatments. This study was designed to evaluate the changes in ocular surface signs and temperature efficacy of a novel device which delivers latent moist heat to the eye lids and surrounding area for 10 minutes. **Method:** A prospective, controlled, single intervention trial recruited 25 normal subjects (M8, F17; age 29.2 ± 5.7yrs). Ocular temperature, tear film stability, ocular hyperaemia and surface staining were measured, using non-invasive ocular thermography and Efron grading respectively, ,before and after device application. Results were checked for normality and compared using paired t-testing. **Result:** Temperature significantly increased to effective levels in both the upper (37.7±0.58°C; p<0.0001) and lower (37.7±0.57°C; p<0.0001) eyelids. Bulbar conjunctival hyperaemia significantly decreased after treatment (p<0.005), but limbal and palpebral hyperaemia were not significantly affected by treatment (0.11 <0.33). No significant change in corneal or conjunctival staining was observed (0.75 <1.00). Non-invasive tear break-up time (NIBUT) was not significantly changed in this normal cohort (p=0.12). **Conclusions:** The Blephasteam® device provides safe and effective warmth to the ocular area without any adverse effects on the ocular surface in this study. Interestingly, even normal subjects had significantly less ocular redness after treatment. Commercial Relationships: Laboratories Théa, France

REGULATION OF GOBLET CELL DIFFERENTIATION IN THE CONJUNCTIVA: THE ROLE OF THE TRANSCRIPTION FACTOR, SPDEF.
Ilene K Gipson[1], Albert Alhatem[1] Gang Chen[2]. Jeffrey Whitsett[2], and Hans Clevers[3]
[1]Schepens. Eye Research Institute, Harvard Medical School, Boston MA, [2]Cincinnati Children's Hospital Medical Center, University of Cincinnati School of Medicine, Cincinnati, OH and [3] Hubrecht Institute, Utrecht, The Netherlands

Purpose: A key transcription factor involved in inducing goblet cell differentiation from precursor cells in the mouse tracheobronchial epithelium and gut is the SAM (sterile alpha motif) pointed domain epithelia specific transcription factor (SPDEF). The purpose of this study was to determine if SPDEF regulates goblet cell differentiation in the conjunctiva. **Methods:** Conjunctival tissue from mice null for SPDEF were examined by light microscopy for presence of goblet cells, and SPDEF was localized by immunohistochemistry in human and mouse conjunctivae. The amount of SPDEF mRNA in conjunctival biopsies from patients with Sjögren's Dry Eye with documented downregulation of the goblet cell mucin MUC5AC was compared to that of normal conjunctiva using real-time PCR. **Results:** Conjunctival epithelia of SPDEF -/- mice completely lack goblet cells. A few inflammatory cells are present in SPDEF -/- epithelium, and the epithelium appears thicker. There is no grossly obvious corneal or eyelid phenotype in the null mice. SPDEF protein is localized in conjunctival goblet cell nuclei by immunohistochemistry in both human and mouse conjunctival epithelium. A significant decrease in SPDEF mRNA was found in conjunctival epithelia samples from patients with dry eye resulting from Sjögren's Syndrome as compared to that from normal subjects. These data were obtained using cDNA from previous studies of mucin gene expression in Sjögren's dry eye, which showed significant decreases in goblet cell MUC5AC expression and protein. **Conclusion:** Taken together these data indicate that SPDEF is involved in regulation of goblet cell differentiation in the conjunctiva as in other wet surface mucosae in which goblet cells differentiate from precursor cells. Supported by R01 EY03306 to IKG.

DEWS WORKSHOP UPDATE: CLINICAL AND BASIC RESEARCH IN DRY EYE
Ilene K. Gipson. Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School, Boston MA, USA

Objective: To summarize new clinical and basic research findings on Dry Eye since publication of the DEWS report in 2007. **Methods**: Assessment of new research findings relevant to dry eye was done by literature review and polling of the DEWS Research Subcommittee. **Results**: A series of clinical studies on human dry eye published since the DEWS 2007 report, have added new information regarding tear fluid composition differences in dry eye patients compared to normal subjects. Reports describing changes in specific cytokine, chemokine, mucin and other protein levels have been published as well as several proteome comparisons have been done seeking dry eye biomarkers. Comparisons of tear meniscus levels and meibomian/lipid physical character have been done. Studies since 2007 on mice exposed to environmental stress alone or in combination with pharmacologic desiccation have yielded information on immune components of response to desiccation. Use of in vitro systems of human ocular surface epithelia have provided new information on surface barrier composition as related to rose bengal dye penetrance and the components of the membrane mucin rich glycocalyx that give rise to barrier function. The effects of NGF and inflammatory cytokines on surface mucin expression and release have been reported. **Conclusion**: Literature review of research in dry eye since the 2007 DEWS reports suggests an active and energized research effort to understand the pathologic mechanism of the disease. Consensuses are beginning to be built regarding tear/epithelial surface changes in dry eye that will facilitate the development of testable hypotheses of the disease mechanism.

RESOLVINS FOR THE TREATMENT OF FRONT OF THE EYE DISEASES.
Per Gjorstrup, Resolvyx Pharmaceuticals, Inc., Bedford, MA, US.

Purpose: Present data obtained in different translational models on the potential clinical use of resolvins in the treatment of anterior ocular disease. **Methods**: The endogenous resolvin E1 (RvE1) a metabolite of eicosapentaenoic acid was investigated in the following models: human corneal epithelial cell (HCEC) epithelial transmigration in vitro, as a model of corneal wound healing; in models of murine dry eye (low humidity and increased airflow combined with scopolamine treatment, in prophylactic (C57Bl/6) and therapeutic (balb/c) regimens; a murine (balb/c) model of herpes simplex virus (HSV) induced keratitis (both prophylactic and therapeutic regimens). In vivo treatments were topical (100-300 ug/mL, 1-3 uL drop, BID-QID). **Results**: After scratch wounding a HCEC monolayer, addition of RvE1 dose dependently stimulated wound closure at 24hrs (EC50 ~1 nM); the maximal effect was similar to that of EGF (10 ng/mL).. Wound closure was achieved by activating PI3K/Akt and MAPK pathways and phosphorylation of GSK3beta causing paxillin to relocalize to the wound edge. Activity was dependent on EGF receptor phosphorylation following shedding of HB-EGF. In C57Bl/6 mice RvE1 completely prevented goblet cell loss (20% loss in untreated controls) and reduced the increase in corneal staining by 70%.In balb/c mice desiccation was introduced for 7 days before a 7 day treatment. RvE1 improved tear flow (Schirmer's test) and corneal integrity (measured as cell counts in the superficial corneal epithelial cell layer), downregulated COX-2, and prevented leukocyte migration into the cornea. In mice inoculated

with HSV, severe keratitis and angiogenesis developed at 14days, but were prevented by up to 70% in RvE1 treated mice. This was accompanied by reduced number of PMN, Th1 and Th17 cells, together with reduced corneal levels of IL-6, IL-1b, FGFb, and VEGF-A. **Conclusions**: The current results suggest that RvE1 and other resolvins may be useful in the treatment of front of the eye conditions that require control of either innate or adaptive immunity, or promotion of tissue integrity including stimulation of wound healing. Support: Resolvyx Pharmaceuticals, Inc.

COMPARATIVE TEAR PROTEIN PROFILING OF DRY EYE, BLEPHARITIS AND CONTROL PATIENTS BY MALDI-TOF MASS SPECTROMETRY AS A NEW DIAGNOSIS TOOL Nerea González[1], Ibón Iloro[2], Felix Elortza[2] and Tatiana Suárez[1]. [1]Bioftalmik Applied Research S.L. Viacaya Technology Park, Building 800, 48160, Derio, Spain. [2]Proteomics Platform, CIC bioGUNE, CIBERehd, ProteoRed. Vizcaya Technology Park, Building 800, 48160, Derio.

Objective: To demonstrate the use of combined MALDI-TOF mass spectrometry analysis and solid phase extraction methods as a new and powerful tool for tear peptide/protein profiling and diagnosis of ocular surface diseases.**Method:** Tear samples were collected from healthy controls, blepharitis and dry eye affected patients, with a glass micro-capillar. Samples were enriched in peptides and low molecular weight proteins by *Magnetic Bead Purification Kit 100 MB-HIC 18* (Bruker Daltonics), following the protocol provided by the manufacturer. Protein profiles were obtained in a mass spectrometer *AutoFlex III TOF/TOF Smartbeam* (Bruker Daltonics). *R*esults were statistically analyzed with the software *Clinprotools 2.2* (Bruker Daltonics) and the free open-source system Multi Experiment Viewer (MeV, TM4 Microarray software suite).**Results:** Statistical analyses of tear peptide/protein profiles revealed three major clusters corresponding to the studied groups: control individuals, dry eye and blepharitis patients. Additionally, it was also possible to identify several subgroups in both pathologies according to their protein profile and bioinformatics approaches such as Support vector Machine and discriminant analyses. The predictive power of these analyses enables the classification of new unknown samples into a specific group, leading to correct phenotyping of patients. **Conclusions:** Magnetic Beads coupled with MALDI-TOF Mass spectrometryis especially suitable for tear peptide/protein profiling. The three studied populations have been statistically separated by this technique, showing its potential use as a tool for tear diagnosis of different human eye diseases. Different stadia of these pathologies could also be discerned, allowing promising strategies in early diagnosis and prognosis field.The authors have no commercial relationship.

A METALLOPROTEINASE ZmpC SECRETED BY *STREPTOCOCCUS PNEUMONIAE* INDUCES MUC16 SHEDDING FROM OCULR SURFACE EPITHELIAL CELLS B. Govindarajan, B. B Menon, S. Spurr-Michaud, M. Gilmore, P. Argüeso, and I. K. Gipson. Schepens Eye Research Institute, Harvard Medical School, Boston, MA

Objective: The Membrane-tethered mucin MUC16 on ocular surface epithelia acts as a barrier to bacterial adherence. Infections at the ocular surface occur by opportunistic pathogens that bypass the mucin barrier through wounds, or by non-opportunistic pathogens that cross the mucin barrier by unknown mechanisms. We found that a non-opportunistic, nontypeable strain of *Streptococcus pneumoniae* that causes epidemic conjunctivitis secretes a protein that induces shedding of the membrane-tethered mucin MUC16 from corneal and conjunctival epithelial cells. The purpose of this study was to isolate and determine the identity of the protein. **Method:** Human corneal and conjunctival epithelial cells cultured for optimal mucin production were treated with exoproducts of non-encapsulated *Streptococcus pneumoniae* (SPN) strain 168 for 1 hour. The SPN exoproduct sheddase was purified using a 50 kDa cut off filter followed by sequential anionic exchange (DEAE) and size exclusion chromatography (CL-4B). Coomassie blue stained bands on SDS Page were analyzed by mass spectroscopy to identify the sheddase. A mutant of *Streptococcus pneumoniae* lacking the *zmpC* gene was constructed using overlap extension PCR with an erythromycin resistance cassette. **Result:** Extraneous proteins from the SPN culture supernatants (99.9 % of total protein) were removed by the 50 kDa cut off filter. Anionic exchange followed by size exclusion chromatography provided a sequential 10-fold enrichment of the active component. Mass spectroscopic analysis of Coomassie blue stained bands on SDS PAGE gels identified the protein of interest to be ZmpC. A mutant of *Streptococcus pneumoniae* lacking the *zmpC* gene did not induce MUC16 shedding in epithelial cells. **Conclusion:** The metalloproteinase, ZmpC, secreted by pathogenic non-encapsulated *Streptococcus pneumoniae* strain 168, induces shedding of MUC16 from epithelial cells, abrogating the MUC16 pathogen barrier. These data are the first demonstration that bacteria can manipulate the membrane mucin barrier on wet surfaced mucosae. Supported by NIH grant EY018850 to IKG

ALLEVIATION OF DRY EYE DISEASE SYMPTOMS WITH BROMFENAC OPHTHALMIC SOLUTION James A. Gow,[1] Shari L. Rowen,[2] Neal A. Sher,[3] Simon P. Chandler,[1] Timothy R. McNamara.[1] ISTA Pharmaceuticals®, Inc., Irvine, CA, USA[1]; Eye and Cosmetic Surgery Center, Lutherville, MD, USA[2]; Eye Care Associates, PA, Minneapolis, MN, USA.[3]

Purpose. To evaluate the efficacy of bromfenac ophthalmic solution (bromfenac) on symptoms using Ocular Surface Disease Index© (OSDI©) in subjects with Dry Eye Disease (DED). **Methods**: On Day –14 and Day 0, subjects were diagnosed with mild or moderate DED with a mean OSDI grade of ≥12 in the same eye. Baseline grading of the most bothersome ocular symptom was also determined. From Day -14 to Day 0, eligible subjects dosed Refresh Plus® eye drops OU qid. From Days 0 to 42, subjects dosed bromfenac OU bid and Refresh Plus prn (≤qid). Subjects returned to the office on Days 14±2, 28±2, and 42±2 for safety and efficacy evaluation. During a 10-day follow-up period, Refresh Plus was dosed prn (≤ qid), followed by a Day 52±4 visit. **Results**:. A total of 38 subjects were enrolled and analyzed for safety, 38 were analyzed for efficacy in ITT population, and 31 subjects were analyzed for efficacy in PP population. Significant reductions in DED symptoms were observed during the treatment period for the OSDI final score; 8/9 individual OSDI symptoms; 7/8 ocular symptoms, including most bothersome symptom; and artificial tear use. No deaths, SAEs, or discontinuations due to AEs were reported and 9 subjects (23.7%) experienced a total of 18 AEs. Most AEs were either mild (6/9 subjects, 66.7%) or moderate (2/9 subjects, 22.2%); 1/9 subjects (11.1%) had a severe AE (sinusitis) considered not treatment related. Two subjects had AEs (eye discharge and eye pain) considered possibly related to treatment. One subject had a mild AE (foreign body sensation in eyes) during the 10-day follow-up period. Visual acuity and IOP had no significant changes from baseline. **Conclusion**: The data for the subjects treated with bromfenac ophthalmic solution for DED were robust and consistent in showing decreased symptoms for 42 days and at 10 days follow-up period when treatment was discontinued. Sponsored by ISTA Pharmaceuticals®, Inc.

EFFICACY OF TOPICAL PLASMA RICH IN GROWTH FACTOR IN THE TREATMENT OF DRY EYE. Arturo E. Grau MD, Silvia López-Plandolit MD, María C. Morales PhD,Vanesa Freire PhD-Student and Juan A. Durán MD, PhD. Instituto Clínico-Quirúrgico de Oftalmología, Bilbao, Vizcaya, Spain

Objective: Efficacy evaluation of plasma rich in Growth Factors (PRGF) in the treatment of moderate-severe dry eye. **Methods**: PRGF treatment was applied in 16 patients diagnosed with dry eye who had not responded to other treatments. Growth factors were quantified in the PRGF, and symptoms before and after treatment were obtained from a modified Dry Eye Questionnaire (SDEQ) as well impression cytology samples to determine the degree of squamous metaplasia. **Results**: We observed a statistically significant improvement of patients symptoms (87.5%). A change in the squamous metaplasia grade by impression cytology was detected, however was not significant. PRGF treatment led to a reduction of associated treatments and 75% of patients were free of them. **Conclusions**: The PRGF is effective as a therapeutic alternative in the treatment of dry eye, as evidenced by improving patient comfort and reducing the squamous metaplasia grade. However, sometimes there is no correlation between symptoms expressed in the questionnaire for dry eye, and the grade of squamous metaplasia observed in the impression cytology. No conflicts of interest or financial support to be declared.

EVALUATION OF TEAR OSMOLARITY IN PATIENTS UNDERGOING PHACOEMULSIFICATION CATARACT SURGERY Arturo E. Grau (MD), Maria C. Morales (PhD), Juan A. Durán (MD, PhD). Instituto Clínico-Quirúrgico de Oftalmología, Bilbao, Vizcaya, Spain

Objectives: To compare the preoperative and postoperative tear osmolarity in cataract surgery patients using a measurement system of tear osmolarity. **Methods**: Preoperative and postoperative tear osmolarity was evaluated in 52 eyes diagnosed with cataract. Phacoemulsification surgery was performed with no complications. In assessing tear osmolarity was used a portable system with a microchip which measure the tear osmolarity (Tearlab TM). **Results**:When comparing the osmolarity before and after a month of surgery, the 20% of patients had a significant osmolarity increase. However, the 40% of them were keeping the post surgical treatment and 60% stopped the treatment. Thus, the change was more evident in patients without treatment. **Conclusions**:Cataract surgery could cause an alteration in the tear film. The dry-eye symptoms that patients complain could be explained by a change in the osmolarity of the tear. No conflicts of interest or financial support to be declared.

MANAGEMENT OF CORNEAL MELTING IN BOSTON KERATOPROSTHESIS WITH BIOCOMPATIBLE MATERIALS Arturo E. Grau (MD)[1], Jaime Etxebarria (MD) [1, 2] INSTITUTIONS:[1]Instituto Clínico-Quirúrgico de Oftalmología, [2] Hospital de Cruces, Bilbao, Vizcaya, Spain.

Objective: Demonstrate the safety and useful application of biocompatible materials in the management of corneal thinning in Boston keratoprosthesis patients and ocular surface alterations. **Methods**: Depending on the degree of corneal impairment different methods were used to rehabilitate the donor cornea. We describe the utilization of cyanoacrylate and artificial dura mate, the intra and postoperative management and possible complications in four patients. **Results**: The use of cynoacrylate glue in mild corneal melting is safe and simple, with excellent results. The use of non absorbable artificial dura mater in extreme cases allows a good sealing of the cornea and provides additional time for a new surgery if necessary. **Conclusions**: In those situations where there are no available donor corneas, special maneuvers to save the keratoprosthesis are necessary. The use of biocompatible materials provides a good alternative in these cases and often a permanent solution. No conflicts of interest or financial support to be declared.

METHODS FOR ITRAQ ANALYSES FOR QUANTITIAVE ANALYSIS OF PROTEIN EXPRESSION LEVELS IN TEAR FILM. Kari B. Green-Church,[1] Liwen Zhang,[1] Sruthi Srinivasan,[2] Mirunalni Thangavelu,[2] Christopher Paulette,[2] Kelly K. Nichols.[2] Mass Spectrometry and Proteomics Facility[1] College of Optometry,[2] The Ohio State University, Columbus, OH, USA.

Objective: To demonstrate the use of iTRAQ technology to analyze (1) the changes in protein expression levels in (DE) and non dry eye (NDE) patients; and (2) tear film proteins from Sjögren's patients compared with NDE. **Methods**: In iTRAQ experiment1, 24 participants were categorized into NDE (OSDI score ≤ 12; Schirmer scores (SS) ≥ 16mm), mild DE (OSDI 13-22; SS: 6-15mm), moderate to severe DE (OSDI ≥24; SS: ≤5mm) and mixed DE (OSDI score- variable; SS: 6-15mm). Tear samples (n=6/group) were collected using Schirmer strips. In iTRAQ experiment 2, 24 participants were categorized into NN (Normal), NB (mostly normal), NMild (slight dry eye, however patient believes they are normal), SMild (Sjogrens patients with normal tear production), SMod (Sjogrens patients with borderline/normal tear production), and SS (Sjogrens patients with decreased tear production). Tear samples (n=4/group) were collected using Schirmer strips. Proteins were extracted from Schirmer strips using 100mM triethylammonium bicarbonate with 400µl 0.05% ProteaseMAX as extraction buffer. Extracted proteins were quantified using Bradford assay, trypsin digested, labeled with TMT Isobaric Mass Tag labeling reagent (Thermo Scientific). LC-MS/MS analysis was obtained on the LTQ Orbitrap mass spectrometer (Thermo Scientific). Data search was performed with MASCOT search engine against Swiss-prot human database. **Results**: On average ~400 unique proteins are identified. Notable differences in protein expression levels are observed between the different patient groups and disease states with statistical relevance using iTRAQ. Proteins are detected as up- or down regulated and potential biomarkers that are unique within the patient categories are demonstrated. For example, 22 proteins were identified as showing significant changes in protein expression in the moderate to severe dry eye group compared to normal patients. Of the 22 proteins, 4 were up-regulated and 18 were down-regulated. Proteins of interest include lysozyme, lipocalin and mammoglobin B. These proteins of interest in all the iTRAQ results have numerous functions and protective roles including front line defense, tear film stability, lipid scavengers and products of inflammation. Data from the other iTRAQ comparisons are also presented. **Conclusions**: iTRAQ is one of the newest tools for quantitative mass spectrometry in tear proteome research, and yields promising results in for potential biomarker discovery. Differences in the protein ratios can be detected between normal and dry eye patients and continued work to characterize involved pathways is needed. Future work to validate findings using Western blot and ELISA assays are planned.

THE OPHTHALMOLOGIC EVALUATION AND MANAGEMENT OF ACUTE STEVENS-JOHNSON SYNDROME: A COMPREHENSIVE APPROACH. Darren G. Gregory. University of Colorado, Denver, USA

Purpose: To present a new grading system for the ocular manifestations of acute Stevens-Johnson Syndrome (SJS) and to describe the management and outcomes of the SJS patients whose cases were used in the development of this system. **Methods:**

Photographic and clinical records of 34 consecutive patients treated for acute SJS were reviewed and each case was graded based on the extent of epithelial sloughing of the lid margins, conjunctiva and ocular surface as defined by fluorescein staining. The acute phase ocular involvement was graded as mild, moderate, severe, or extremely severe. This severity grade was then compared to each patient's 6 month best correctable visual acuity (BCVA), scarring sequelae and dry eye symptoms. Any acute phase ophthalmologic treatments were also noted. **Results:** Eighteen patients were graded as mild (n=12) or moderate (n=6). All were managed medically and had 20/20 BCVA with no dry eye or scarring. Six were severe. All 6 received acute phase amniotic membrane transplantation (AMT) to the ocular surfaces. All had 20/20 BCVA with minimal dry eye or scarring. Ten were extremely severe. The 2 managed medically both had 20/25 BCVA with severe dry eye, severe photophobia and moderate scarring of the conjunctiva and lid margins. The 8 who received acute phase AMT all had >20/30 BCVA (6 were 20/20). Two had severe photophobia, but none had more than moderate dry eye or mild scarring. **Conclusion:** Mild or moderate ocular involvement in acute SJS carries a low risk of visual or ocular surface sequelae and may be managed medically. Severe or extremely severe involvement carries a high risk of significant dry eye problems and ocular surface scarring. Acute phase AMT can greatly decrease the ocular surface damage and is recommended for such patients. [The author has no financial interest in any products used in this study.]

RELEVANCE OF TEAR FILM PROTEOMICS IN THE DIAGNOSIS OF DISEASE. F. Grus. Experimental Ophthalmology, Dept. of Ophthalmology, University Medical Center, Johannes-Gutenberg-University Mainz, Germany

Purpose: The composition of proteins and peptides in tears plays an important role in ocular surface diseases. Proteomic analyses could reveal biomarkers for the health and integrity of the ocular surface. Several studies could demonstrate changes in the tear protein patterns of dry-eye patients compared to controls. The presentation will give a critical state-of-the-art analysis of the relevance of tear film proteomics in the diagnosis of disease and the problems of translating it into clinical routine. **Methods:** Different proteomic analyses in tear film will be compared such as conventional gel-electrophoresis, micro-array based approaches, and e.g. mass spectrometric profiling (LC-MSMS, Maldi-TOFTOF, Seldi-TOF etc.). Furthermore, different methods for quantification and validation of the biomarkers will be demonstrated. **Results:** Many studies could reveal protein and peptides biomarkers which are consistently up - or downregulated in dry-eye disease and could differentiate between different disease subgroups. Some of these biomarkers are even consistent between different analytical methods. The presentation will compare the results of several of those studies and discuss the clinical implications of these biomarkers and possible implications for new treatment options. **Conclusions:** Proteomic technologies might be a very promising approach in dry-eye disease. In comparison to genetic testing, proteomic biomarkers can describe the actual state of the ocular surface and the ongoing disease processes. However, the application of these technologies in clinical routine is still challenging. Beside of new treatment options, these biomarkers could serve in future to optimize treatments in individual patients in personalized medicine.

A REVIEW OF THE PATIENT-REPORTED OUTCOME INSTRUMENTS TO MEASURE THE IMPACT OF DRY EYE ON HEALTH-RELATED QUALITY OF LIFE. Isabelle Guillemin & Benoit Arnould Mapi Values, Lyon, France

Objective: To review patient-reported outcome (PRO) instruments evaluating symptoms and health-related quality of life (HRQL) in dry eye (DE) patients. **Method**: Literature searches in Medline and Embase databases, using the MeSH terms "dry eye syndromes" AND "questionnaires", identified new instruments in DE. Further searches documented the development methodology, clinical validity, reliability, reproducibility and responsiveness of the instruments identified. **Result**: In addition to the instruments previously reported in the Dry Eye Workshop Report (published in 2007), the Ocular Comfort Index was the only new and relevant instrument found. Most are symptom questionnaires, used to screen for DE, assess prevalence or ascertain DE symptom profile in clinical trials. Only the National Eye Institute Visual Function Questionnaire-25 (NEI-VFQ25), Ocular Surface Disease Index (OSDI), Ocular Surface Disease (OSD) and Impact of Dry Eye on Everyday Life (IDEEL) assess DE symptoms and the impact of DE on quality of life and vision. The NEI-VFQ25 is the best at measuring visual functioning, but was not designed specifically for DE. The OSDI is a reliable, valid and responsive instrument however, it does not fully address issues reported by patients. IDEEL and OSD were developed with standard-recommended methodology, ensuring their content validity. They comprehensively cover HRQL, visual functioning and DE symptoms, and have good psychometric properties. **Conclusion**: Only the IDEEL and OSD comprehensively and specifically document HRQL, visual functioning and symptom impact of DE in patients. IDEEL was developed and validated in line with FDA PRO guidance and is the best candidate to overcome the limitations of OSDI and NEI-VFQ25.

DRY EYE SYMPTOMATOLOGY OF CONTACT LENS WEARERS WITH THE OSDI QUESTIONNAIRE. Michel Guillon, Cecile Maissa, Elizabeth Bolton, Caroline Flomet. OTG Research & Consultancy LondonUK

Objective: Using the OSDI validated questionnaire, this investigation aimed to determine the frequency and nature of dry eye symptoms in contact lens wearers presenting in an optometric practice and to identify the individual questions that best predict the dry eye symptomatology status produced by the overall questionnaire. **Method:** The OSDI questionnaire (the questionnaire made up of twelve questions grouped under the headings of ocular and vision related symptoms and environmental triggers) was administered to 226 soft contact lens wearers (Age 30.0±10.6) who attended OTG Research & Consultancy clinic. The data set was analysed with a Chi Square Automated Interaction Detector (CHAID) test to identify the factors that best predict the dry eye status of individual patients, as determined by their overall OSDI score. **Result:** The average OSDI score for the test sample was 11.3 ± 13.7 and the incidence of dry eye (DE) of varying severity 32% (Mild=14%; Moderate=9%; Severe=9%). Each individual questions produced statistical differences ($p<0.001$) between the normal and dry eye groups; however the capacity of each question to predict individual patient status was highly different. The predictive model was multifactorial: problem during computer use being the primary predictive factor (DE incidence: No symptom = 13%; At least some time =86%). For the asymptomatic computer users no symptomatology under low humidity (DE incidence=2%) and for the symptomatic computer users symptomatology in air conditioned areas at least some time (DE incidence=97%) were the main secondary factors. **Conclusion:** The incidence of contact lens wearers complaining of dry eye in this investigation (33%) was slightly lower than previously reported in the same clinic with the McMonnies questionnaire (43%). The best predictor of individual patients' dry eye status was symptomatology during computer use the additional factors were symptomatology in dry environment and in areas with air conditioning.

MANAGEMENT OF LID MARGIN DISEASES WITH BLEPHACLEAN. Michel Guillon, Cecile Maissa, Stéphanie Wong OTG Research and Consultancy, London UK.

Objective. The principal objective of this investigation was to assess the efficacy of Blephaclean eye pads (Spectrum Théa) in managing anterior blepharitis or MGD associated with dry eye complaints. **Method.** The investigation was a bilateral, prospective, interventional open label investigation of three month duration. The test population was made up of dry eye sufferers with at least mild symptoms (OSDI≥13) who presented with mild to moderate anterior blepharitis or MGD. The subjects used Blephaclean eye pads initially intensively (twice a day) for three weeks and in a maintenance regimen (once a day) for the remainder of the study. At each visit, lid margin and meibomian glands status of the subjects was assessed and their symptoms evaluated. **Result.** 40 subjects aged 22 to 74 years (54±15 years) were enrolled of whom 39 completed the investigation. The product usage revealed good overall compliance throughout the study. The results revealed significant improvement in associated signs and symptoms. Eyelashes visible contamination by flakes, scales and crusts was significantly lower ($p<0.001$) at Day 21 and 90 than at baseline with a significant increase in cases with no visible contamination ("Clear lashes": Baseline 19% Day21 40% Day90 50%). Meibomian gland blockage was also significantly lower ($p<0.001$) at Day 21 and 90 than at baseline (No blocked glands: Baseline 8% Day21 20% Day90 41%) and associated with an improvement in the quality of the meibomian secretion. A significant decrease in symptomatology was also recorded (Mean OSDI: Baseline = 30 Day 21 = 18 Day 90 = 19; Symptomatic: Baseline 100% Day 21 55% Day 90 54%) and end of day comfort increased significantly (Mean score: Baseline =56 Day 21 = 67 Day 90 = 67). **Conclusion.** The results showed significant improvement in signs and symptoms. The data confirms the efficacy and safety of the clinical methodology put forward of 21 days of intensive use (twice a day) of eye pads followed by maintenance use (once a day). The findings also contribute to identify the key parameters to record to monitor the condition in an efficient manner in routine clinical practice. *The study was sponsored by Laboratoires Théa.*

CELL SURFACE MUCIN O-GLYCANS IMPAIR NANOPARTICLE DELIVERY TO CORNEAL EPITHELIAL CELLS. A. Guzman-Aranguez,[1] J. Pintor,[1] P. Argüeso.[2] [1]Department of Biochemistry, School of Optics, Complutense University, Madrid, Spain; [2]Schepens Eye Research Institute and Department of Ophthalmology, Harvard Medical School, Boston, MA, USA.

Purpose: Recent data have shown that mucin O-glycans participate in barrier function at the ocular surface by interacting with -galactoside-binding lectins on the epithelial glycocalyx. The purpose of this study was to determine whether cell surface mucin O-glycans impair the delivery of nanoparticles to human corneal epithelial (HCLE) cells. **Methods:** Downregulation of mucin O-glycosylation in HCLE cells was carried out using a stable tetracycline-inducible RNA interfering system to knockdown c1galt1 (T-synthase), a critical galactosyltransferase required for the synthesis of core 1 O-glycans. HCLE cells were incubated with 0.1 µm carboxylate-modified fluorescent nanospheres for 3 hours at both 37ºC and 4ºC. Nanoparticle uptake was analyzed by confocal microscopy and quantified using a fluorometric assay. Immunofluorescence staining of ZO-1 was used to evaluate tight junction integrity. **Results**: By confocal microscopy, abrogation of mucin Oglycosylation in HCLE cells stably transfected with c1galt1 shRNA resulted in increased nanoparticle uptake at 37ºC as compared to scramble shRNA control. As determined by fluorimetry, cells transfected with c1galt1 shRNA had a 1.63-fold increase in nanoparticle uptake as compared to scramble control. The internalization of the particles was dramatically reduced at 4ºC, when active transport processes are blocked. No differences were observed in ZO-1 immunostaining under the different conditions, indicating that increased nanoparticle uptake occurs through the transcellular pathway. **Conclusions:** These results indicate that cell surface mucin O-glycans impair the penetrance of nanoparticles into corneal epithelial cells. Transient manipulation of the glycocalyx barrier is an alternative approach to delivering therapeutic nanoparticles to the cornea. Supported by NEI R01EY014847 (PA) and BSCH-UCM GR58/08 (JP).

RELAXIN 2 AND INSL3 PROMOTE WOUND HEALING AT THE OCULAR SURFACE. Ulrike Hampel,[1] Thomas Klonisch,[2] Saadettin Sel,[3] Friedrich Paulsen.[1] Departments of Anatomy and Cell Biology,1 Ophthalmology,[3] Martin Luther University of Halle-Wittenberg, Halle/Saale, Germany; Department of Human Anatomy and Cell Science and Department of Medical Microbiology and Infectious Diseases[2], University of Manitoba, Winnipeg, Manitoba, Canada

Purpose: The relaxin-like peptides relaxin 2 (RLN2) and INSL3 are predominantly known for their effects in reproductive systems. Both hormones induce remodelling of the extracellular matrix (ECM) by modulating the expression of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs). Wound healing at the ocular surface is associated with ECM remodelling and accompanied by activation of MMPs. **Methods:** In the present investigation we analyzed transcript levels of RLN2, INSL3, and their cognate relaxin-like receptors RXFP1 and RXFP2 as well as the presence of immunoreactive human RXFP1 in tissues of the ocular surface, the lacrimal apparatus, and human corneal (HCE), conjunctival (HCjE) and sebaceous (SC) cell lines. We analyzed effects of human RLN2 and INSL3 on cell proliferation and migration and quantified MMP and TIMP expression in HCE, HCjE, and SC. **Results:** The presence of RXFP1 and RXFP2 transcripts was detected in the lacrimal gland, meibomian gland, conjunctiva, cornea, primary corneal fibroblast, nasolacrimal ducts, and in all three HCE, HCjE and SC cell lines. Immunoreactive RXFP1 protein localized to Meibomian glands. RLN2 and INSL3 transcripts were rarely detectable in tissues of the ocular surface and lacrimal apparatus, but were always present in HCE, HCjE and SC. Stimulation of HCE, HCjE and SC with RLN2 and INSL3 significantly increased cell proliferation and enhanced cell migration. Relative mRNA expression levels of MMP2, MMP9, TIMP1 and TIMP2 were significantly influenced by RLN2 or INSL3 in all three cell lines at different time points studied. **Conclusions:** Our data support a paracrine role of RLN2 and INSL3 at the ocular surface and in the lacrimal apparatus and a novel role during wound healing at the ocular surface. [This research was supported by DFG grants PA738/6-1 and PA738/1-5.]

TEAR CYTOKINE PROFILES IN SJÖGREN SYNDROME AND IN NON-SJÖGREN DRY EYE. Sang Beom Han, MD, [1]Joon Young Hyon, MD,[1,2] Ji-Won Kwon, MD,[3] Won Ryang Lee, [2,4] MD,[1] Jin Hak Lee, MD.[1,2] Seoul National University Bundang Hospital[1] Seoul National University College of Medicine,[2] Seoul National University Hospital Healthcare System Gangnam Center,[3] Seoul National University Hospital, [4] Seoul, Korea

Purpose. To compare tear cytokine concentrations in patients with Sjögren syndrome (SS) and those with non- Sjögren dry eye (NSDE). **Methods**. Eleven patients with primary SS (SS group) and 13 patients with NSDE (NSDE group) were included. In each patient, one eye with more severe dry eye signs was selected. Dry eye symptoms were evaluated using visual analogue scale and ocular

surface disease index scores. Dry eye signs were measured using Schirmer-I test, tear film breakup time, and the rose bengal stain score. Tear samples were collected and analyzed. Concentrations of Interferon-, interleukin (IL)-1, IL-4, IL-6, lL-8, IL-10, Il-12, IL-17, monocyte chemoattractant protein (MCP)-1, interferon-inducible protein (IP)-10, macrophage inflammatory protein (MIP)-1 and -1 was measured and compared between the two groups. **Results**. No statistically significant difference in dry eye symptoms or signs between the two groups. Tear concentrations of IL-12 (80.39±104.46 vs. 22.00±12.36 pg/mL, *P*=0.023) and IL-17 (86.31±148.27 vs. 12.30±7.48 pg/mL, *P*=0.030) were significantly higher in SS group, and that of MCP-1 (1962.84±2255.99 vs. 9676.14±11189.96 pg/mL, *P*=0.018) was significantly higher in NSDE group. **Conclusions**. Difference in tear cytokine profiles between SS and NSDE was observed, even though there was no significant difference in severity of dry eye signs and symptoms. These findings suggest that difference in pathogenesis may exist between the two entities. Financial disclosure: None

ROLE OF INFLAMMATION IN HSV-1-INDUCED STROMAL KERATITIS. Robert L. Hendricks**.** Department of Ophthalmology, University of Pittsburgh, Pittsburgh, PA USA

Purpose: Recurrent herpes stromal keratitis (HSK) represents an immunoinflammatory process that results in progressive corneal scarring and visual impairment. Our studies are aimed at developing immune-based intervention in HSV-1 recurrence and corneal scarring. **Methods:** We employ a mouse corneal infection model and use whole mounts of infected tissue and flow cytometry on dispersed cells as well as quantitative real time PCR and Luminex technology to evaluate the inflammatory process in the corneas and trigeminal ganglia of infected mice. **Results:** We establish a role for dendritic cells in regulating the initial innate immune response that clears replicating virus from the cornea as well as the $CD4^+$ T cell orchestration of the neovascularization and leukocytic infiltration that characterize HSK. We demonstrate an essential role for $CD8^+$ T cells in preventing HSV-1 reactivation from latency in sensory ganglia. **Conclusions:** Strategies aimed at augmenting $CD8^+$ T cell function in the trigeminal ganglion and inhibiting $CD4^+$ T cell function in the cornea will likely reduce periodic shedding of HSV-1 at the cornea and the subsequent inflammation and scarring of the cornea, respectively.

THE EFFECT OF MEIBOMIAN LIPID FILMS ON EVAPORATION OF WHOLE TEARS *IN VITRO*. George H. Herok,[1,2] Shiwani R. Raju,[1] Thomas J. Millar[1.] School of Natural Sciences, University of Western Sydney[1], Department of Medical and Molecular Biosciences, University of Technology, Sydney[2].

Purpose: Our *in vitro* studies using a controlled environment with minimal air flow have been unable to show that meibomian lipids reduce evaporation using an artificial subphase (ATB). Of concern was that the conditions did not represent those incurred by the lipid layer *in vivo*. Therefore, we have used whole tears and higher air flows to examine the ability of meibomian lipids to prevent evaporation *in vitro*. **Method**: Meibomian lipids in hexane were spread on either ATB or whole tears (40µL, surface area 0.28cm^2). Evaporation was measured gravimetrically. Experiments were repeated using a pendant drop where evaporation was calculated using drop size analysis. Oscillation of the drop ensured spreading of lipids across the surface. **Results**: Gravimetrically, there was minimal difference in evaporation between having tears or ATB as the subphase. Increasing air flow increased evaporation. However, a decrease in evaporation due to a meibomian lipid film was only noticed with higher flow rates (>80mL/min). At 160mL/min flow rate with excessive amounts of lipids (25µg), evaporation was reduced to the equivalent of losing 0.17µL of depth of a tear film per minute. The pendant drop showed similar results in that a meibomian lipid layer with proteins adsorbed from tears gave minimal protection from evaporation. **Conclusion**: Despite mimicking tear film structure by spreading meibomian lipids over whole tears, the ability for the meibomian lipids to prevent evaporation was minimal. Protection occurred only when air flow was high. [This research was supported by the Australian Government linkage project scheme #LP0776482, and Alcon]

NEW FORMULATION BASED ON LIPOSOMES LOADED WITH MEDROXYPROGESTERONE FOR DRY EYE TREATMENT. Rocío Herrero-Vanrell[1] ,Marta Vicario[1] ·José Manuel Benitez del Castillo[2], Beatriz de las Heras[3], Manuel Guzmán[4], Irene T. Molina-Martinez.[1.] [1]Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, Complutense University, Madrid, Spain. [2] Unidad Superficie e Inflamacion Ocular (USIO), Hospital Clínico San Carlos, Madrid, Spain. [3]Department of Pharmacology, School of Pharmacy, Complutense University, Madrid, Spain. [4]Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, Alcalá University, Alcalá de Henares, Madrid, Spain.

Purpose: Stabilisation of preocular tear film can be performed through the administration of formulations which constituents are similar to the ones present in the tear film. Treatments directed to replace a disturbed lipid layer through pharmaceutical nanosystems (liposomes) result of therapeutic benefits for dry eye patients. The formulation can be improved if an anti-inflammatory agent such as medroxyprogesterone is included. The aim of this study was the development of liposomes loaded with medroxyprogesterone. Once prepared the liposomes were dispersed in a hypotonic solution of a bioadhesive polymer. **Materials and Methods**: Phospholipon 90G containing > 95% of phosphatidilcholine (PC) purified from soy lecithin was purchased from Phospholipid GmbH (Cologne, Germany). Cholesterol, Vit E (a-tocopherol), Medroxyprogesterone Acetate (MPA) and Trehalose were purchased from Sigma Chemical Co. (St. Louis, Missouri), Hyaluronic acid Ophthalmic grade (Mw 400.000-800.000Da) was acquired from (Abarán Materias Primas, Barcelona, Spain). Liposomes were prepared according to the technique described by Benitez and col.(ARVO 2006) by employing PC (200mg), Cholesterol(25mg), vitamin E (2mg) and MPA (0,9mg). After extrusion vesicles were dispersed in a hypotonic solution of hyaluronic acid (190mOsm/L). Tonicity of formulation was adjusted with trehalose.The mean particle size and size distribution of liposomes were performed by photon correlation spectroscopy (PCS) in liposomes loaded with MPA. Tolerance of the formulations was evaluated "*in vitro*" (Human Inmortalized-Limbal Epithelial Cells; HCLE). Cytotoxicity studies, based on MTT method, were carried out at short (15minutes) and long term (1 hour) exposures. Viability was set as 100% in untreated cells. **Results:** The size of liposomes loaded with MPA was 188,90±18,21nm.The formulation based on liposomes loaded with MPA (45µg/ml) resulted in cell viability values higher than 80% for short and long exposures. **Conclusions:**The new formulation based on liposomes loaded with MPA could be useful for the treatment of dry eye. Acknowledgements: This work has been supported by FIS PI07/0043 and PI070012, Research Group UCM 920415 (GR58/09) and Spanish Ministry of Health (RETICS RD07/0062/2002).Dr. Gipson is gratefully acknowledged for providing a generous gift of the HCLE cell line

SELENOPROTEIN P CONTROLS OXIDATIVE STRESS IN CORNEA. Akihiro Higuchi[1], Kazuhiko Takahashi[2], Kazuo Tsubota [1,3]. [1]Center for Integrated Medical Research, School of Medicine, Keio

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The ocular surface is always attacked by oxidative stress, and cornea epithelial cells are supposed to have their own recovery system against oxidative stress. Therefore we hypothesized that tears supply key molecules for preventing oxidative stress in cornea. The study on dry eye treatment using autologous serum eye drops revealed selenoprotein P (SeP) was potential target key molecule. SeP is a carrier of selenium, which is an essential trace element for many animals, for oxidative stress metabolism in the organism. An experiment was performed with SeP eye drops in a rat dry eye model, prepared by removing the lacrimal glands. The anticipated improvement in corneal dry eye index and the suppression of oxidative stress markers were observed in SeP eye drops group. When corneal epithelial cells were cultivated in selenium-deficient medium for 2 weeks, glutathione peroxidase (GPx) activity was diminished. GPx activity, which is a sign of selenium uptake, recovered following the addition of SeP to the culture medium. SeP was taken up by the CEPI cells; the selenium in SeP was then used to synthesize GPx. Although SeP had been found in plasma, it was not known that SeP was present in human tears. We found that SeP was extremely expressed in rat lacrimal glands and secreted in human tears. Furthermore, the concentration of SeP was significantly higher in dry eye patients compared with non dry eye patients. We concluded that tear SeP is a key molecule to protect the ocular surface cells against environmental oxidative stress.

COLD-SENSITIVE CORNEAL AFFERENTS IMPLICATED IN BASAL TEAR PRODUCTION, TRPM8 NERVE MEMBRANE RECEPTORS, AND DRY EYE DISEASE. Harumitsu Hirata and Michael L. Oshinsky. Department of Neurology, Thomas Jefferson University, Philadelphia, PA, USA

Purpose. We previously reported that a specific class of corneal afferents responded to the types of ocular stimuli that will trigger tearing such as drying of the cornea and hyperosmolar tears (*Investigative Ophthalmology and Visual Science*, 2010). This study pharmacologically investigates the involvement of the nerve membrane receptors in the responses of these corneal afferents to ocular stimuli important to tear production in an attempt to assess the relationship between the corneal afferent receptors and tearing. **Methods**. In isoflurane-anesthetized adult male rats, the activity of the corneal afferents was recorded *in vivo* from the trigeminal ganglia with tungsten microelectrodes in response to 1) drying of the cornea, 2) hyperosmolar tears, and 3) menthol before and after the ocular application of TRPM8 antagonist, N-(4-t-Butylphenyl)-4-(3-Chloropyridin-2-yl)tetrahydropyrazine-1(2H)-carboxamide (BCTC; 20-50 μM; 20 μl). **Results**. The activity of cold-sensitive corneal neurons to drying of the cornea and hyperosmolar tears applied to ocular surface was significantly inhibited by 20 μM BCTC. The activity of other types of corneal afferents such as low threshold mechanoreceptors was not affected by BCTC (up to 100 μM). However, BCTC did not block the responses to menthol in all cold-sensitive corneal afferents: little or no effect on most but a total blockade in few cells. **Conclusions**. Our findings indicate that a specific type of cold-sensitive corneal afferents previously reported to be the afferent limb of the basal tearing reflex (innocuous cold thermoreceptors) contain the nerve membrane receptors, TRPM8, and their activation may produce basal tears. Although these neurons have been characterized as "menthol" receptors, our observation that not all menthol responses of the corneal afferents were inhibited by TRPM8 antagonist suggest that a heterogeneous pool of receptors (e.g., TRPA1) exists in these afferents. We hypothesize that damage to these receptors, which may occur after ocular desiccation, leads to some forms of the dry eye.

P2X$_7$ RECEPTORS INTERACT WITH a$_{1D}$-ADRENERGIC AND MUSCARINIC RECEPTORS IN RAT LACRIMAL GLAND ACINI. Robin R. Hodges and Darlene A. Dartt, Schepens Eye Research Institute; Department of Ophthalmology, Harvard Medical School, Boston, MA.

Purpose: We previously showed that a1D-adrenergic, cholinergic, and P2X7 receptor agonists increased intracellular calcium ([Ca2+]i) in rat lacrimal gland acini. In this study, we determined if a1D-adrenergic and cholinergic agonists interact with P2X7 receptors and the effects of the interactions on [Ca2+]i and protein secretion. **Methods:** Rat lacrimal glands were removed from male Sprague Dawley rats and acini isolated by collagenase digestion. [Ca2+]i was measured using InCyte Im2TM Ratio Imaging System in acini incubated with the calcium indicator dye fura2. Peroxidase secretion was measured spectrophotometrically. Acini were stimulated with a P2X7 receptor agonist (BzATP, 10-4M), the a1D -adrenergic agonist phenylephrine (Ph, 10-5 M) or the cholinergic agonist carbachol (Cch, 10-4 M). Inhibitors were added prior to agonist addition. **Results:** A43871 (P2X7 receptor inhibitor, 10-4 M) inhibited Ph-stimulated increase in [Ca2+]i by 52 ± 14% but did not inhibit Ph-stimulated secretion. BMY 7378 (a1D-adrenergic receptor inhibitor, 10-4 M) inhibited BzATPstimulated increase in [Ca2+]i by 52±19% but did not inhibit BzATP-stimulated secretion. Simultaneous addition of Ph and BzATP resulted in an increase in [Ca2+]i that was less than additive. However, secretion was additive in the presence of Ph and BzATP. In contrast to Ph, A43871 did not alter Cch-stimulated increase in [Ca2+]i or secretion. However, atropine (muscarinic receptor inhibitor, 10-4 M) blocked BzATP-stimulated increase in [Ca2+]i 68 ± 14% and secretion by 67±19%. Simultaneous addition of Cch and BzATP resulted in an increase in [Ca2+]i that was less than additive. Secretion was additive in the presence of Cch and BzATP. **Conclusions:** We conclude that a1D-adrenergic and muscarinic receptors use the same pathways to increase [Ca2+]i as P2X7 receptors but use separate pathways to stimulate protein secretion. Supported by NIH EY06177.

REPROGRAMMING OF CELLULAR TRANSCRIPTION BY SIGNALING THROUGH MUC1. Michael A. Hollingsworth, Michelle E. Behrens, Samuel J. Erb. Eppley Institute for Research in Cancer, Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE, USA 68198-5950

The MUC1 cytoplasmic tail (MUC1.CT) conducts signals from spatial and extracellular cues (growth factor and cytokine stimulation) to evoke a reprogramming of the cellular transcriptional profile. Specific phosphorylated forms of the MUC1.CT achieve this function by differentially associating with transcription factors and redirecting their transcriptional regulatory capabilities at specific gene regulatory elements. The specificity of interaction between MUC1.CT and several transcription factors is dictated by the phosphorylation pattern of the 18 potential phosphorylation motifs within the MUC1.CT. Microarray gene expression analysis and ChIP-chip promoter analysis identified genome-wide transcriptional targets of MUC1.CT signaling. We examined in detail the molecular mechanisms of MUC1.CT signaling that induced expression of connective tissue growth factor (CTGF/CCN2), a potent mediator of ECM remodeling and angiogenesis. There was a robust induction of CTGF synthesis and secretion in response to serum factors that was enabled only when MUC1 was highly expressed. There was a requirement of phosphorylation at distinct tyrosine motifs within the MUC1.CT for MUC1-induced CTGF expression and a

phosphorylation-specific localization of MUC1.CT to the CTGF promoter. MUC1 reorganizes transcription factor occupancy of genomic regions upstream of the CTGF gene, directing b-catenin and mutant p53 to CTGF gene regulatory elements to promote CTGF expression and destabilizing the interaction at these regions of the transcriptional repressor, c-Jun. Phosphorylation of specific residues on the MUC1CT enabled binding to the tetramerization/regulatory domain of p53. Unphosphorylated MUC1CT or MUC1 phosphorylated on other residues did not bind p53 demonstrating that binding is phosphorylation-specific. Bound MUC1 stabilized the disordered p53 C-terminal regulatory domain and induced oligomerization of p53. These findings are consistent with MUC1CT acting as a co-regulator of p53 activity. With this example we illustrate the capacity of MUC1.CT to modulate transcription factor activity in a context-dependent manner to achieve widespread and robust changes in gene expression.

TOPICAL JAK INHIBITOR, TASOCITINIB (CP-690,550), MODULATES OCULAR SURFACE INFLAMMATION IN DRY EYE. Jing-Feng Huang, Rolla Yafawi, Min Zhang, Michael McDowell, Kay D Rittenhouse, Frederick Sace, Melissa Liew, Scott R Cooper, Eve H Pickering. Pfizer Inc., San Diego, CA, USA

Purpose: To evaluate the pharmacological activity and mechanism of action of ophthalmic tasocitinib (CP-690,550), a selective small molecule inhibitor of the Janus family of kinases (JAK), in dry eye. **Methods**: In a randomized and double-masked clinical study with tasocitinib in moderate/severe dry eye patients, tear fluid and conjunctival impression cytology (IC) specimens were collected from a subset of subjects (82) at baseline and the end of 8-week treatment period. Conjunctival cells from IC were analyzed with flow cytometry for HLA-DR, and tear concentrations of 29 immune mediators and protein biomarkers were measured by multiplex bead analysis. **Results:** Subjects treated with tasocitinib for 8 weeks had significantly reduced level of HLA-DR from baseline: in tasocitinib 0.005% QD and 0.003% BID groups, HLA-DR levels at week8 were 73% (P=0.091) and 67% (P=0.007) of Day 0, respectively, while in the vehicle control, it was 133% (P=0.145) of Day 0. Compared with changes from baseline in the vehicle group, changes in HLA-DR in tasocitinib 0.005% QD and 0.003% BID groups were significantly different: 55% (p=0.0296) and 51% (p=0.0062) of vehicle control, respectively. In tears, several inflammatory mediators were decreased significantly after tasocitinib treatment. In particular, tasocitinib (0.005% QD) group had reduced MMP-3 (44% of Day0, P=0.063), MMP-9 (42%, P=0.093) and IL-17 (47%, P=0.16) at week 8, while the vehicle group was unchanged or increased. Among the 29 analytes measured in tears at baseline, MMP-9 and IL-17 were highly correlated with each other (correlation coefficient: 0.91). Changes at week 8 from baseline in MMP-9 were correlated with changes of IL-17 in tears, as well as with changes of HLA-DR in IC. (For this study, <0.2, 2-sided) **Conclusions**: Topical ophthalmic tasocitinib shows pharmacological activity in dry eye and appears to inhibit the Th17 pathway, MMP-9, MMP-3 and MHC II expression. Inhibition of ocular surface inflammation provides mechanistic bases for further developing tasocitinib for dry eye.

MEASUREMENT OF TEAR MENISCUS IN DRY EYE PATIENTS WITH FOURIER-DOMAIN OPTICAL COHERENCE TOMOGRAPHY. David Huang, Pho Nguyen, Matthew C. Bujak, Ethan Tittler, Xinbo Zhang, Yan Li, Samuel Yiu.Doheny Eye Institute, University of Southern California, Los Angeles, CA, USA

Purpose. To assess the utility of tear meniscus measurement in dry eye patients. **Methods.** Dry eye patients were recruited in a prospective observational study. The lower tear meniscus was imaged at the inferior cornea-lid junction with a 6-mm vertical line scan using a Fourier domain optical coherence tomography (OCT) system (RTVue by Optovue, Inc.) with 5 micron resolution. Two scans were performed before (baseline) and 1, 2, 5, 10, and 15 minutes after the instillation of 0.5% carboxymethylcelluose artificial tear (Optive by Allergan, Inc.). Scanning was performed 2 seconds after a blink. The tear meniscus was measured using computer calipers by 3 human graders. Symptom scores were evaluated with the Indiana Dry Eye Questionnaire 2002. **Results.** Fourteen subjects (27 eyes) were measured. The meniscus height averaged 0.257 (range 0-0.605) mm, depth averaged 0.143 (0-0.343) mm, and cross-sectional area averaged 0.021 (0-0.083) mm2. Linear regression showed that the baseline meniscus parameters were all positively correlated ($p < 0.05$) with the post-anesthetic Schirmer's test, and negatively correlated ($p < 0.05$) with the tear breakup time and symptom score. The inter-grader coefficients of variation were 0.11, 0.13, and 0.19 for height, depth, area, respectively. The meniscus parameters were significantly elevated ($p < 0.05$, Wicoxon signed rank test) compared to the baseline at 1, 2, and 5 minutes after artificial tear instillation, but not after 10 minutes. **Conclusions.** Tear meniscus measurement with OCT is an objective noncontact test that correlated well with dry eye severity. It may be useful for the evaluation of tear dynamics after artificial tear therapy. The reproducibility of Fourier-domain OCT meniscus measurement is better than previous results with lower resolution time-domain OCT. This research is supported by NIH grant EY018184 and a grant (Huang, Li, Zhang) from Optovue, Inc. David Huang also received stock options, speaker fee, travel support, and patent royalty from Optovue, Inc.

ANTIMICROBIAL SUSCEPTIBILITY OF OCULAR BACTERIAL PATHOGEN TO LEVOFLOXACIN, MOXIFLOXACIN, GATIFLOXACIN, AND CIPROFLOXACIN. Joon Young Hyon, Hung Won Tchah. Korean Corneal Disease Study Group

Objective: To compare the antimicrobial susceptibility of ocular pathogens to levofloxacin, moxifloxacin, gatifloxacin, and ciprofloxacin. **Method**: Total 60 ocular pathogens from bacterial keratitis were collected. Antimicrobial susceptibility was determined either disk diffusion (in 37 strains) or MIC method (in 23 strains). Suceptibility profiles by disk diffusion method was compared among levofloxacin, moxifloxacin, and gatifloxacin. MICs were compared among levofloxacin, moxifloxacin and ciprofloxacin. **Result**: Out of 60 strains, 55 strains (91.7%) including three methicillin-resistant staphylococcus strains were susceptible to the all fluoroquinolones tested. One Enterococcus faecalis strain was resistant to gatifloxacin, but showed intermediate susceptibility to levofloxacin. One Staphyloccoccus epidermidis strain was resistant to ciprofloxacin, but susceptible to moxifloxacin and had intermediate susceptibility to levofloxacin. Ciprofloxacin had lower MIC against pseudomonas strains, and levofloxacin showed tendency to have lower MIC against staphylococcus strains. **Conclusion**: Most ocular pathogens were susceptible to fluoroquinolones widely used in the practice, but 5% of pathogens showed resistance to ciprofloxacin and the fourth generation fluoroquinolones.

COMMUNITY BASED STUDY IN ELDERLY POPULATION FOR THE ASSOCIATION BETWEEN DEPRESSIVE SCORE / DEMENTIA SCORE AND DRY EYE. Joon Young Hyon[1], Sang Beom Han[1], Ji Won Kwon[2], Se Joon Woo[1], Jung Jae Lee[3], Tae Hui Kim[4], Ki Woong Kim[4] 1 Department of Ophthalmology, Seoul National University Bundang Hospital, Seongnam, Korea 2 Seoul National University Healthcare System Gangnam Center, Seoul,

Korea 3 Department of Psychiatry, Kyungbook National University Hospital, Daegu, Korea 4 Department of Neuropsychiatry, Seoul National University Bundang Hospital, Seongnam, Korea

Objective: To determine the association between depression score / dementia score and dry eye in elderly Korean population. **Method:** This community based study included 657 participants of more than 65 years old from mixed rural/urban population of Korea. Dry eye symptoms were assessed using a 6-item questionnaire. Dry eye signs, including Schirmer test and fluorescein stain score, tear -film breakup time, meibomian gland dysfunction were evaluated. Mini-Mental Status Examination in the Korean Version of the CERAD Assessment Packet (MMSE-KC) for dementia, and a Korean version of the Short Geriatric Depression Scale (SGDS-K) for depression were also evaluated. Association between dry eye signs and symptoms and MMSE-KC or SGDS-K were evaluated. **Result:** The depression scale (SGDS-K) score was significantly higher in dry eye group compared to non-dry eye group (5.53 ± 4.48 vs. 3.73 ± 3.77, P<0.001). Prevalence of dry eye was significantly higher in subjects with major depression, defined as SGDS-K score of ≥ 8. The number of positive responses in dry questionnaires was significantly highter in depression group. However, no association was found between dry eye signs and depressive mood. There was no significant difference in MMSE-KC score between dry eye and non-dry eye group. **Conclusion:** Depression scale score has strong association with dry eye symptoms, but not with dry eye signs.

CHANGES IN TEAR FUNCTIONS AFTER CHOLINERGIC TREATMENT IN DRY EYE PATIENTS. Osama M.A. Ibrahim, [1, 4] Murat Dogru, [1, 2] Yoji Takano, [3] Yoshiyuki Satake, [2] Tais Hitomi Wakamatsu, [1, 4] Kazumi Fukagawa, [4] Kazuo Tsubota, [4] Hiroshi Fujishima [5] 1-Keio University School of Medicine, Johnson & Johnson Ocular Surface and Visual Optics Department, Tokyo, Japan 2-Tokyo Dental College, Department of Ophthalmology, Chiba, Japan 3- Kitasato University School of Medicine, Department of Ophthalmology, Tokyo, Japan 4-Keio University School of Medicine, Department of Ophthalmology, Tokyo, Japan 5-Saiseikai Central Hospital, Tokyo, Japan

Objective: To investigate the efficacy of cholinergic parasympathomimetic agonist pilocarpine (Salagen) treatment for dry eye disease and changes in tear meniscus in a prospective controlled study. **Methods:** Ten dry eye female patients received 5 mg Pilocarpine tablets twice a day for 3 months. Visual analog scale assessment for dry eye and dry mouth symptoms was also carried out. Patients underwent tear meniscus height (TMH) measurement with slit-microscopy graticule scale and Visante optical coherence tomography, strip meniscometry testing, tear film break-up time (BUT) measurement, ocular surface vital staining with fluorescein (F) and Rose Bengal (RB) dyes and the Schirmer-1 test. The data was analyzed one week, 1 month and 3 months after treatment. Mann-Whitney Test was performed. The study was conducted in compliance with the Tennets of the Declaration of Helsinki. **Results:** Visual analog scale of dry eye and mouth symptoms showed a significant time wise improvement. The graticule scale and Visante OCT TMH measurements significantly improved after one week and one month of treatment (p<0.001). Strip meniscometry values, mean tear stability, and vital staining scores remained improved in dry eye patients 3 months after treatment (p<0.001), while Schirmer-1 test values tended to improve without statistical significance. **Conclusion:** Systemic pilocarpine treatment appeared to be effective in the improvement of signs and symptoms in patients with dry eye disease. Visante OCT was effective in monitoring the tear meniscus changes during the course of treatment. The authors have no proprietary or commercial interest in any of the products mentioned in this work. No grant support.

IgE and ECP AS MARKERS OF SEVERITY IN DIAGNOSIS OF ATOPIC KERATOCONJUNCTIVITIS. Ayako Igarashi, Tais Hitomi Wakamatsu,[2]Yoshiyuki Satake, Yoji Takano, Osama Ibrahim, Naoko Okada, Kazumi Fukagawa, Murat Dogru, Jun Shimazaki, Kazuo Tsubota, Hiroshi Fujishima[5]. Tokyo Dental College Ichikawa Hospital, Chiba, Japan [2] Keio University School of Medicine,Tokyo,Japan [3] Kitasato University School of Medicine,Tokyo, Japan [4] Ryogoku Eye Clinic,Tokyo,Japan [5] Saiseikai Central Hospital, Tokyo, Japan

Objective:To evaluate IgE and ECP as severitymarkersforAtopic Keratoconjunctivitis (AKC). **Methods**: Thirty eyes of 30 patients (23 males,7 females) with AKC(mean age: 23.4±13.2 years, range 6-64 years) and 10 healthy control subjects (3 males,7 females; mean age 23.3±5.5 years) were examined in this prospective study. All subjects underwent fluorescein staining, conjunctival injection, proliferation,edema grading and tear collection. Peripheral blood was also collected from 10 patients and 10 control subjects.Tear and serum ECP and IgE levels from the same patientswere measured ,the correlation of which were investigated with theocular surface clinical parameters. **Results**:The mean conjunctival proliferation, injection and edemascoreswere significantly higher in AKC patients compared with the controls. The mean fluorescein score (2.9±3.84) was significantly higher than the control group (0.14±0.38). Higher total IgE levels were detected in AKC tears (967.83±1231.75ng/ml)when compared with the control group (1.5±0.87ng/ml). The ECP levelsin AKC patients and controls were1406.0 ± 2719.7ng/ml and 9.8 ± 19.47ng/ml, respectively. Tear ECP levels showed a significant correlation with staining andpapillary proliferationscores(r=0.67, 0.38 respectively). Tear IgE hadno correlation with keratoconjunctivalsigns. Serum IgE and ECP concentrations were elevated in AKC patients but did not show any correlation with clinical parameters. **Conclusion**:This study suggests the presence of an overalleosinophilic response in AKC disease independent of IgE-sensitization. Tear ECP was a useful marker delineatingthe severity of the ocular surface diseasein AKC. Commercial interest:none

CHRONICALLY DISTURBED IP_3 RECEPTOR-MEDIATED CA^{2+} SIGNALING IN EXOCRINE GLANDS CAUSES SJÖGREN'S SYNDROME-LIKE AUTOIMMUNE DISEASE. Takaaki Inaba[1, 2], Chihiro Hisatsune[2], Yasumasa Sasaki[1], Yoko Ogawa[1], Taishin Akiyama[3], Etsuko Ebisui[2], Naoko Ogawa[2], Minoru Matsui[4], Tsutomu Takeuchi[5], Katsuhiko Mikoshiba[2] & Kazuo Tsubota[1] [1]Department of Ophthalmology, Keio University School of Medicine [2]Laboratory for Developmental Neurobiology, Brain Science Institute, RIKEN [3]Division of Cellular and Molecular Biology, Department of Cancer Biology, Institute of Medical Science, University of Tokyo [4]Department of Pharmacy, Chiba Institute of Science [5]Department of Rheumatology, Keio University School of Medicine

Objective: Sjögren's syndrome (SS) is a cryptogenic chronical autoimmune disease characterized by severe lacrimal gland and salivary grand dysfunction. It was reported that lacrimal gland acinar cells from some SS patients showed reduced Ca^{2+} sensitivity to cholinergic stimulation and that the autoantibody against IP_3 receptors (IP_3Rs) was frequently detected in SS patients; however, the causal relationship between the aberrant Ca^{2+} signaling and the onset of SS disease was entirely unknown. **Method and Result:**We used IP_3RKO and wild-type mice (WT) in all experiments.IP_3Rs play a central role in tear secretion, and that the mice lacking both IP_3 receptor type2 (IP_3R2) and type3 (IP_3R3) cause SS-like autoimmune disease. IP_3R2/3 knockout mice showed severe defect of tear secretion due to impaired Ca^{2+} signaling of lacrimal acinar cells, resulting in abnormal phenotype at cornea and conjunctiva. Importantly, degeneration of exocrine gland with infiltrating

lymphocytes occurred as the $IP_3R2/3$ knockout mice got elder, even though their lymphocytes normally developed. Moreover, autoantibody against SS-A antigen, an SS marker, was detected in the mice serum. **Conclusion:** Our results suggested that tear fluid secretion was fully dependent on the IP_3R-mediated lacrimal gland Ca^{2+} signaling and that chronic acinar cell hypofunction caused by disturbed IP_3R-mediated Ca^{2+} signaling may be a cause of the onset of SS disease.

MOUSE LACRIMAL GLAND IS A REGENERATABLE ORGAN. Masataka ITO Department of Developmental Anatomy and Regenerative Biology, National Defense Medical College, Saitama, Japan.

Objective: The main aim of this study was to observe how mouse lacrimal gland regenerate after severe ischemic atrophy. We have generated an atrophy model of exorbital lacrimal gland (EOL) by cauterizing main arteries nourishing EOL in the adult mice (ARVO 2009, 2010), in which wet weight of the gland and tear volume decreased by 60% and 80%, respectively, in a couple of weeks after cauterization. In the present study, we performed immunohistochemical observations on this atrophy model to study how regeneration of the gland occurs. **Methods:** The arteries to right EOL in 8-9 week-old C57BL/6 mice were cauterized. Then immunohistochemical staining for cytokeratin 14 (CK14), Ki67, Caspase 3 and aquaporin 5 (Aqp5), which are markers for basal cell layer of the secretion duct, cell proliferation and apoptosis, respectively, were performed on the paraffin sections of the gland. **Results:** In the first three days after cauterization, massive necrosis with apoptosis occurred in EOL. On day 3 after cauterization, epithelial proliferation began in the survived ductal epithelium, followed by the expansion of the ducts with CK14-positive epithelial cells. By 6 weeks after cauterization, apparently normal terminal acini with Aqp5 positively stained on the apical side of the cells appeared in the atrophied tissue, and by 8 weeks, those regenerated acini got predominant in the tissue. Ki67 was only transiently positive on the ductal epithelium between 1 and 2 weeks after cauterization, thereafter Ki67-positive cells were rare in the regenerating EOL. **Conclusions:** Complete regeneration from severe atrophy of the gland suggests mouse lacrimal gland have a strong regenerative potency. Temporal emergence of Ki67-positive cells indicates that regenerative process of this ischemic model begins with the proliferation of the duct and that regenerated acinar cells were differenciated from the proliferated ductal epithelium. Progenitor cells for lacrimal gland tissues were considered to be located in the ductal epithelium and were activated by the ischemic condition.

LIPID PENETRATION INTO CONTACT LENSES: A CONFOCAL MICROSCOPY VIEW. J. Jacob, J. Guinn, T. Edwards Louisiana State University Health Sciences Center, Dept of Ophthalmology, New Orleans, LA 70112

Objective*:* To quantitiate and visualize the degree of tear film lipid penetration into silicone hydrogel contact lenses over wear times. **Methods***:* Four contact lens types (Lotrafilcon B, Balafilcon A, Senofilcon A, and Comfilcon A) were exposed to artificial tear solution containing fluorescently-labeled cholesterol and phosphocholine (PC) under wear conditions for 1, 12 and 24 hours. The lenses were then analyzed cross-sectionally with confocal microscopy to image the penetration of cholesterol and PC through the lens. Additionally, lenses were analyzed for total protein and total individual lipid (cholesterol and PC) content. **Results***:* Confocal microscopy provided specific visualization of the lipids as they entered and penetrated the lenses. Adsorption/penetration profiles for each lens and lipid type were able to be generated. A significant difference in the penetration characteristics was seen between cholesterol and PC as well as the different lens types. In general, cholesterol penetration was imaged to be more diffuse than the PC penetration which was more punctuate in nature. The rate limiting step for adsorption of both lipids was initial penetration into the lens surface which was dependent upon material type. Once the surface was penetrated, further adsorption of the lipids followed more simple diffusion characteristics. Cholesterol and PC adsorbed to the different lens materials to significantly different degrees. Lotrifilcon B which showed the lowest cholesterol adsorption did not show the lowest PC adsorption. The lens materials ranked as Balafilcon A > Senofilcon A > Comifilcon A > Lotrafilcon B for total cholesterol adsorption. However, for total PC adsorption the lens materials ranked Senofilcon A > Lotrfilcon B > BalafilconA > Comifilcon A. **Conclusions***:* Confocal microscopy allows for the spatial imaging of lipid adsorption into contact lens material. The specific lipid penetration profile of a lens material may provide surface and bulk characteristic information important not only for fouling and comfort screening but also for contact lens induced dry eye.

TEAR FILM OSMOLARITY IN DRY EYE DISEASE. Christina Jacobi, Friedrich E Kruse, Claus Cursiefen

Purpose: Dry eye is defined as multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface. Tear hyperosmolarity causes damage to the surface epithelium by activating a cascade of inflammatory events at the ocular surface and a release of inflammatory mediators into the tears (MAP kinases, NFkB signalling pathways) which leads to the generation of inflammatory cytokines (IL-1, IL-1, TNF-) and Matrix Metalloproteinases (MMP 9). Because of these pathogenetic mechanisms, tear film osmolarity could be an important diagnostic tool as indicator of ocular surface health in keratoconjunctivitis sicca and other ocular surface diseases. The aim of this prospective, non-randomized, clinical, single-centre study was to assess the changes in the osmolarity in tear samples of patients with keratoconjunctivitis sicca compared to healthy controls. **Methods:** 98 patients (age 48±10, 32 males and 66 females) with severe keratoconjunctivitis sicca and 84 controls (age 52±22, 36 males and 48 females) were enrolled in the trial. Tear samples were collected from the inferior-temporal conjunctival sac. Inclusion criteria were a break up time (BUT) < 5sec and a Jones- Test < 5mm. Tear film osmolarity was analyzed by the OcuSense TearLab. Measurements were performed twice at the right eye, using the mean value for statistical analysis. Statistical analyses were performed using StatisticaTM software (Mann-Whitney-U-Test), p-values < 0.05 * were considered significant. **Results:** There was a significantly higher tear film osmolarity in patients with keratoconjunctivitis sicca (321±19.5 mOsmol/l) compared to the control group (298±12.6 mOsmol/l). The results of this study showed a sensitivity of 71% and a specificity of 83%. **Conclusions:** Tear film osmolarity can be determined using the OcuSense TearLab osmometer. Our results approved the cutoff value for dry eye of approximately 316 mOsmol/l as described in the literature. Testing tear film osmolarity, applied singly or in combination, can be a very effective objective diagnostic tool in the diagnosis of dry eye disease.

IS BLINKING ALTERED IN DRY EYE? Meredith E. Jansen, Carolyn G. Begley, Minhua Chen, Haixia Liu. Indiana University School of Optometry; Bloomington, IN USA

Purpose: Previously, we have shown that most blinks are incomplete and the blink amplitude (BA) is highly correlated with habitual

ocular symptoms as measured by the Dry Eye Questionnaire (DEQ). The purpose(s) of this study were (1) to investigate blink parameters while manipulating cognitive demand and ocular surface stimulation to determine their input into blinking parameters, and (2) to determine whether these parameters correlate with dry eye symptoms. **Methods**: Ten subjects completed the DEQ and 2µl of 2% fluorescein was instilled into the right eye. Tear film break-up (TBU) and blink parameters were recorded by 2 digital cameras while listening to music (low cognitive demand) and playing a game (high cognitive demand), both with (decreased surface sensation) and without anesthetic (normal surface sensation). Custom MATLAB® (The Mathworks™) programs were used to analyze blink parameters and TBU. Repeated measures ANOVA with Bonferroni post hoc testing was used to compare conditions. **Results**: Blink rate (BR) significantly decreased with both game and anesthetic conditions ($p \leq 0.02$) and there was a significant decrease in the BA of the game with anesthetic ($p \leq 0.01$). The duration of the blink significantly decreased and the up and down phase velocity increased with the game ($p \leq 0.03$). There was significantly more TBU during the game with anesthetic, but TBU occurred in all tasks, mostly in the inferior area, and often remained following incomplete blinks. The DEQ score and many symptoms were highly correlated ($0.594 \leq r \leq 0.792$) with increased blink duration and decreased blink velocities. **Conclusion**: Our data suggests that tasks involving increased cognitive demand tend to decrease the rate, fullness and duration of the blink, presumably to minimize interference by the eyelids. Anesthetic increased this effect, in theory by removing ocular surface input. The opposite effect occurred in association with habitual dry eye symptoms, representing a likely input for blinking from irritation at the ocular surface.

CONTACT LENS SOLUTIONS: WHERE NEXT? Lyndon Jones PhD FCOptom DipCLP DipOrth FAAO (DipCL) Professor, School of Optometry Associate Director, Centre for Contact Lens Research University of Waterloo Ontario, Canada

Over the past 30 years the development of contact lens solutions has shown tremendous changes, with products becoming increasingly complex and diverse in their composition and performance. For much of this time, companies concentrated on formulating lens care regimens that provided maximum disinfection strength, with an aim of providing safe, effective regimens that. Recently, more emphasis has been placed on producing regimens that assist with improving lens comfort, with new regimens incorporating a variety of surface active agents to aid wettability and, hopefully, improve end of day comfort. Traditional lens materials based on combinations of polyHEMA with other more hydrophilic monomers have a propensity to deposit proteins. Most care regimens to-date have been formulated to remove proteins, primarily lysozyme, often through a passive soaking mechanism. However, silicone hydrogels offer a new challenge as they are far more hydrophobic, primarily deposit lipid and have an increased potential to denature any lysozyme deposited on the lens surface. In addition, proteins deposited on conventional hydrogels often become absorbed into the lens matrix, as opposed to being adsorbed onto the surface with lower water content, siloxane-based materials. These deposit patterns necessitate a different emphasis for cleaning and may require considerable efforts to formulate care regimens specifically targeted for silicone hydrogels. Finally, studies over the past 5 years have increasingly shown that certain lens materials and care system combinations have a high probability of producing abnormally high levels of corneal staining, often with no signs of discomfort being exhibited by the patient to warn practitioners that such an unacceptable response is occurring. These developments often occur in patients who have previously used a care system for many years with no problems being seen. Understanding and unravelling these interactions is of considerable importance for practitioners. This presentation will review the major components of care regimens, describe their interaction with the ocular surface and discuss what changes are likely to occur over the next 5-10 years as "next generation" care regimens become available.

EFFECTS OF AMNIOTIC MEMBRANE SUSPENSION IN HUMAN CORNEAL WOUND HEALING IN VITRO. Choun-Ki Joo. Department of Ophthalmology and Visual Science, Seoul St. Mary\'s Hospital, College of Medicine, the Catholic University of Korea.

Purpose: To investigate the biochemical mechanism of amniotic membrane (AM) suspension on corneal wound healing, particularly on epithelial proliferation and migration. **Methods**: Human corneal epithelial cells (HCECs) were cultured in media with different concentrations of AM suspension. In an effort to evaluate the migratory potential of AM, migration assays were conducted via the manual scraping of HCECs and immunocytochemical staining of cell adhesion molecules (E-cadherin). The relative expression of MMP9 and adhesion molecules was determined via RTPCR and western blot analysis. The proliferative potential of AM was evaluated via a proliferation assay using BrdU incorporation and western blot analysis for proliferating cell nuclear antigen (PCNA). In addition, ELISA was used to measure the protein concentrations of mitogenic growth factors. **Results**: Migration assay rates were enhanced as AM concentrations increased. RT-PCRs revealed that the expression of the MMP9 gene was upregulated by AM, and the expressions of E-cadherin and fibronectin genes were downregulated by AM. Western blot analysis demonstrated significantly higher MMP9 expression in AM-treated groups, versus significantly lower levels of E-cadherin and fibronectin expression in AM-treated groups. Immunocytochemistry showed large quantities of E-cadherin near the wound edges after 24 h of injury in the AM-treated groups. The proliferation assay showed that the BrdU positive cell counts/total cell counts were augmented by AM to a statistically significant degree. Western blot analysis showed that the expression cell cycle-associated protein, PCNA, increased gradually as a result of AM treatment. ELISA showed that our AM suspension contained 4 growth factors (HGF, EGF, KGF, and FGF). The amount of HGF was especially large, followed by that of EGF. **Conclusions**: These results demonstrate that the suspension form of AM maintains its beneficial effect on corneal epithelial wound healing in vitro, and that AM suspension leads to significant increases in corneal epithelial migration and proliferation with increasing AM concentrations.

MORPHOMETRIC DESCRIPTION OF CORNEAL EPITHELIAL CELLS WITH LOW DENSITY OF MICROVILLI IN DIFFERENTS DRYING TIMES Gemma Julio[1], Mª Dolores Merindano[1], Sara Lluch[1], Carme Caum[21]Department of Optics and Optometry, Universitat Politècnica de Catalunya (UPC), Spain. [2]Faculty of Mathematics and Statistics, Universitat Politècnica de Catalunya (UPC), Spain.

Objective:To establish the differences between corneal cells with low density of microvilli in several drying times in order to improve the description of desiccation process in a model of dry eye. **Method**:The eyes of sixteen rabbits were kept open for different drying times (DT) (DT1<1h, DT2=1-2h, DT3=2-3h and DT4=3h) in groups of three rabbits (4 in control group). Epithelial cells (988) of the central cornea were examined by scanning electron microscopy (mean of 30.9cells per cornea) and quantitative data of microvilli density (percentage of *cell surface covered by microvilli* (*SCM*)), cell size (*Area*), shape (*Shape*), electron reflex (*Shade*) and cell to cell adhesion (*CCA*) (the only categorical variable) were obtained, using image processing techniques. Low microvilli density threshold (*SCM*<41%)

was chosen because 80% of epithelial cells in a healthy cornea show SCM values >41%. Morphometric changes of cells with SCM< 41% (390 cells) in different DT were evaluated by applying Kruskal-Wallis and Mann Whitney test. **Result**:Percentage of cells with low *SCM* was increased as DT enlarged (13.30% (DT0); 18.34% (DT1); 58.64% (DT2); 66.83% (DT3) and 41.36 (DT4)). *Area* did not show significant differences between different DT. *Shade* was similar, except in all the comparison with DT1 ($p<0.05$), and *Shape* is only significantly different in DT2/DT4 and DT3/DT4 comparisons ($p<0.05$). *CCA* clearly showed more heterogeneity as DT increased with a greater number of altered junctions (2.6% in DT0; 5.4% in DT1; 19.9% in DT2, 14.63% in DT3 and 37.7% in DT4). **Conclusion**:This study quantitatively confirms that cells with low density of microvilli increase as DT enlarged. These cells are morphologically similar in all DT. Only cell to cell adhesion is clearly different.

SOURCES OF VARIABILITY IN MORPHOMETRIC CLASSIFICATION OF CORNEAL EPITHELIAL CELLS.
Gemma Julio[1], Mª Dolores Merindano[1], Sara Lluch[1], Carme Caum[2]
[1]Department of Optics and Optometry, Universitat Politècnica de Catalunya (UPC), Spain. [2]Faculty of Mathematics and Statistics, Universitat Politècnica de Catalunya (UPC), Spain.

Objective:The aim of this study is to establish the main variability sources of cell morphometric characteristics to improve the assessment of corneal epithelium. **Method**:226 epithelial cells of the central cornea of four New Zealand white rabbits were examined by scanning electron microscopy (SEM). Quantitative data of microvilli density (*cell surface covered by microvilli* (*SCM*)), cell size (*Area*), shape (*Shape*) and electron reflex (*Shade*) were obtained, using image processing techniques. Possible sources of variability (rabbit, cornea (right or left), zone of the central cornea (5-6 per cornea) and cell (with a mean of 5.08 ±1.1 cells per zone)) were evaluated in each variable by applying a variance component analysis with a hierarchically nested random-effect model. **Result**:The factor that contributed most to the variance was cell, in all the variables studied (88.52 % for *SCM*, 78.15 % for *Area*, 96.66 % for *Shape* and 63.44 % for *Shade*). The other sources of variability showed minimal influence (mean of 2.39% ±1.92) except in the case of rabbit factor (15.20%) for *Area* and cornea (24.08%) and zone (12.48%) factors for *Shade*. **Conclusion**:This study quantitatively confirms that some cell features, as microvilli density, are almost independent from factors outside the cell itself whereas others, like cell size or cell electron reflex, are seriously influenced by them. This influence could threat the repeatability of the data.

POLYMER NANOMATERIALS FOR THERAPEUTIC DRUG DELIVERY.
Alexander V. Kabanov. Center for Drug Delivery and Nanomedicine, College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska, USA

A new generation of polymer therapeutics has emerged which uses self-assembling nanomaterials for delivery of drugs, genes, and imaging molecules. Examples of such materials include *polymer micelles*, *cross-linked nanogels*, and *block ionomer complexes* that entrap small drugs, DNA or siRNA (*"polyplexes"*), as well as therapeutic enzymes (*"nanozymes"*) or antibodies (*"nanobodies"*). These materials are designed to improve therapeutic index of their biologically active payloads by 1) protecting the payload against degradation in the body, 2) improving its pharmacokinetics and targeted bioavailability, 3) increasing its transport across cellular membranes (e.g. in cancer cells) or other biological barriers (such as the blood brain barrier (BBB)), and 4) ultimately, releasing the active payload at the site of disease. These materials are also used in combination with cell-based therapies. For example, they are loaded into immunocytes, which then carry and release the payload to the inflamed sites. Vaccination approaches using polymeric materials are also explored, such as DNA vaccination, which exploits capabilities for targeting specific immune cell populations for antigen presentation, and activation of cell-signaling that favors the immune response. The studies in this field have provided observations regarding interactions of such nanomaterials with living cells and sub-cellular statures, including their cellular uptake and transport itineraries, which are sometimes remarkably specific and precise for a man-made material. Furthermore, *polymer genomics* has emerged as a field that explores ability of synthetic polymers to affect various signal transduction mechanisms involving inflammation, differentiation, proliferation, and apoptosis. The ability of the cells and organisms to respond to the effects of these polymers can be dependent on phenotype or genotype. Overall, these effects are relatively weak as they do not result in cytotoxicity or major toxicities in the body. However, when combined with biologically active agents, such as cytotoxic agents, bacterial DNA or antigens, either by mixing or covalent conjugation, the polymers can drastically alter specific genetically controlled responses to these agents. Some examples, of such materials that reached clinical stage will be discussed such as SP1049C, a polymeric micelle doxorubicin for highly resistant tumors. ACKNOWLEDGEMENT: The United States National Institutes of Health (1P20RR021937, CA89225, CA116591, NS051334) and the Department of Defense (USAMRMC 06108004, W81XWH-09-1-0386) are acknowledged for the support of research. The presenter is a co-founder and has interest in Supratek Pharma Inc. (Montreal, Canada), a developer of SP1049C.

VASOACTIVE INTESTINAL PEPTIDE ACTIVATES THE ADENYLYL CYCLASE PATHWAY IN HUMAN MEIBOMIAN GLAND EPITHELIAL CELLS.
Wendy Kam and David A. Sullivan, Schepens Eye Research Institute and Harvard Medical School, Boston, MA, USA

Purpose: One of the most striking characteristics of the human meibomian gland is its rich sensory, sympathetic and parasympathetic innervation. No other human sebaceous gland shares this characteristic, yet the functional relevance of these nerve fibers remains unknown. We hypothesize that neurotransmitters are released in the vicinity of the gland, act upon glandular receptors, and influence the production, secretion and/or delivery of meibomian gland secretions to the ocular surface. If correct, this would indicate an important role for the nervous system in maintaining the tear film lipid layer, and thus the health of the ocular surface. Our goal in this study was to begin to determine whether neurotransmitters do influence the meibomian gland. Towards that end we examined whether: (1) the adenylyl cyclase pathway, which mediates several neurotransmitter activities and generates cAMP, is operative in meibomian gland epithelial cells; and (2) vasoactive intestinal peptide (VIP), which is present in nerves contacting the gland, activates this pathway. **Methods**: Immortalized human meibomian gland epithelial cells were treated with vasoactive intestinal peptide and/or forskolin, in the presence or absence of phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX). Intracellular cAMP levels were determined by colorimetric enzyme-linked immunoassay. **Results**. Our results demonstrate that forskolin, a secretagogue known to activate adenylyl cylase, increases cAMP levels in meibomian gland epithelial cells. This forskolin effect is amplified in the presence of IBMX. Similarly, our findings show that VIP activates the adenylyl cyclase pathway, and that this effect is increased dramatically in combination with IBMX. **Conclusions**. Our study demonstrates that VIP does influence the function of human meibomian gland epithelial cells. This research improves our understanding of signaling pathway activation in the human

meibomian gland and provides a basis for further investigation of the possible activators of lipid synthesis and/or secretion in these cells. (Supported by NIH grant EY05612)

MESENCHYME/EPITHELIUM INTERACTION IN EYELID AND MEIBOMIAN GLAND MOPHORGENESIS. Winston W-Y Kao, Yujin Zhang, Chia-Yang Liu and Mindy K. Call. Edith J. Crawley Vision Research Center, Department of Ophthalmology, University of Cincinnati

Purpose: Tissue morphogenesis during development is regulated by mesenchyme/epithelium interaction via growth factors and cytokines. In this study, we examined the possible role of mesenchyme/epithelium interaction on eyelid and Meibomian gland morphogenesis. **Methods:** Bi-transgenic *Kera-Cre/ZEG* and tri-transgenic *Kera-rtTA/tet-O-Cre/mTmG* mice were used to track the cell lineages of periocular mesenchymal cells of neural crest origin in the formation of eyelids. Mouse lines in which eyelid stromal cell-specific genetic perturbation was achieved by keratocan promoter driven tet-O-biglycan transgene expression and -catenin gain of function mutant (*Ctnnb1 gof*) triple transgenic *Kera-rtTA/tet-O-Cre/Ctnnb1*^{tE3} micewere used to determine the role of eyelid levator muscle on Meibomian gland morphogenesis. **Results:** Cell lineage analysis indicated that the periocular mesenchymal cells of neural crest origin contributed to dermal papilla of hair follicles and the mesenchymal cells surrounding the bulge of hair follicles where epidermal stem cells are located. We also showed that transgenic mice over expressing biglycan under the keratocan promoter exhibited exposure keratitis and premature eye opening from non-infectious eyelid ulceration due to perturbation of eyelid muscle formation and the failure of Meibomian gland formation. Our data revealed that biglycan binds to TGF-, thus interrupting EGFR signaling pathways essential for mesenchymal cell migration induced by eyelid epithelium. The *Ctnnb1gof* mutant embryos and neonates exhibited an anomaly of bilateral concaved eyelids at birth and severe eyelid margin malformation at P21. Histological and immunofluorescence examinations showed that the eyelid epithelial sheet from both -catenin wild-type and *gof* mutant mice began to migrate and expressed phospho-JNK at E14.5. However, the subsequent eyelid stromal elongation and eyelid closure which normally occurred at E16.5 was disrupted in the mutants. It was noteworthy that the constitutive activation of -catenin by *gof* mutant caused the formation of a highly proliferative cell mass accumulated in the eyelid stroma that was characterized by the malformation of the tarsal plate and Meibomian gland. **Conclusions**: Our results indicated that TGF-signaling and coordinated activation of Wnt/-catenin signaling are essential for normal eyelid and Meibomian gland morphogenesis.

ELEVATED EXPRESSION OF TLRS, DECTIN-1, AND IL-1B IN HUMAN CORNEAS INFECTED WITH THE FILAMENTOUS FUNGI *ASPERGILLUS* AND *FUSARIUM*. R. Siva Ganesa Karthikeyan[1], Sixto M. Leal[2], Lalitha Prajna[1] and Eric Pearlman[2]. [1]Aravind Eye Hospital, Madurai, Tamil Nadu, India, [2]Department of Ophthalmology and Visual Sciences, Case Western Reserve University, Cleveland, Ohio

Objective: Although Fusarium species were the cause of a recent contact lens related outbreak of keratitis in the USA, the major risk factor for fungal keratitis worldwide is ocular trauma. In South India 65% of total corneal ulcers (including bacterial) are caused by *Fusarium* and *Aspergillus* species. The purpose of this study was to examine the host response in corneal tissues from patients with fungal corneal ulcers. **Methods**: Corneal scrapings were collected for diagnosis from patients between February and July 2009, and categorized as *Fusarium* or *Aspergillus* species. Total RNA from the corneal scrapings was extracted by the TRIzol method, and quantitative PCR was performed for expression of TLR2, TLR4, Dectin - 1, Dectin - 2, IL - 1, IL - 8, TNF - , and IFN – g. Cadaver corneas were used as controls. Histological sections of paraffin embedded infected corneas obtained after transplant during this time period were examined for infiltrating cells. **Results:** The total number of patients with corneal ulcers presenting during this period was 519, of which 332 were fungal ulcers (64%). In the current study, 40 patients (21 *Fusarium sp* and 19 *Aspergillus sp*) were analyzed by quantitative PCR. We found that expression of TLR2, TLR4, Dectin-1, IL-1, IL-8, and TNF-, was higher in corneal tissues from patients infected with control, donor corneas, whereas there was no difference between control and infected corneas in expression of IFN-g or Dectin-2. The histopathological analysis of the infected corneal tissue showed consistent neutrophil and mononuclear infiltration into the corneal stroma. There was no difference in expression of these mediators between patients infected with *Fusarium sp* and those infected with *Aspergillus sp.* **Conclusions:** TLRs and Dectin-1 are involved in the fungal recognition, and the release of chemotactic cytokines which recruits the inflammatory cells to the infected cornea. Findings from corneal ulcers from patients will be related to studies using mouse models of *Aspergillus* and *Fusarium* keratitis. Studies were supported by a grant from Alcon Laboratories, Inc. for basic research (LP), and by NIH EY01862 (EP). There are no commercial interests.

MENISCOMETRY USING ANTERIOR SEGMENT OPTICAL COHERENCE TOMOGRAPHY Hiroaki Kato,[1,2] Norihiko Yokoi,[2] Anthony J Bron,[3] John M Tiffany[3] and Shigeru Kinoshita[2]. National Center for Geriatrics and Gerontology,[1] Aichi, Japan; Kyoto Prefectural University of Medcine,[2] Kyoto, Japan; University of Oxford,[3] Oxford, UK.

Objective: This study investigates the correlation between video-meniscometry (VM) (R_{VM}; Yokoi N, et al. Cornea 2000; 19S) and anterior segment optical coherence tomography (ASOCT) (R_{ASOCT}; RTVue-100^{TM}, Optovue, Inc) for the measurement of the radius (R) of curvature of the central lower tear meniscus (TM). **Methods**: Forty eyes of 21 subjects [7 males and 14 females; mean age: 59.2±17.0(SD)] were studied. For the measurement of R_{ASOCT}, a cross-section of the TM was imaged, and R was measured by fitting a circle to the image, passing through points A (where the TM contacts the corneal surface), B (where the TM contacts the lower lid margin), and C (where a line perpendicular to and passing through the midpoint of AB crosses the TM curve). The relationship between the values of R_{ASOCT} and R_{VM} was then investigated. The precision of each technique was compared by measuring R by reflection from the inner surface of glass capillary tubes of a specific radius, halved lengthwise to provide access. **Results**: No significant differences were found between the values of R_{VM} (0.29±0.26 mm) and R_{ASOCT} (0.29±0.29 mm) with significant correlation (R_{ASOCT}=1.07×R_{VM} -0.02, R=0.97, p<0.0001), which was also confirmed in the glass capillary model. **Conclusions**: This study shows that ASOCT can also measure R and that R_{ASOCT}, like R_{VM}, is a useful indicator of tear volume.

ISOLATION, CULTURE OF MOUSE LACRIMAL GLAND EPITHELIAL CELLS. Tetsuya Kawakita,[1] Shinya Kobayashi,[1] Motoko Kawashima,[1] Naoko Okada,[1] Kenji Mishima,[2] Masataka Ito,[3] Ichiro Saito,[2] Shigeto Shimmura,[1] Kazuo Tsubota[2]. [1] Department of Ophthalmology, Keio University School of Medicine,[2] Department of Pathology, Tsurumi University,[3] Department of Anatomy, National Defense University,[3] Japan.

Purpose: Lacrimal gland epithelial cells of newborn mice was reported to culture, but not in adult mouse. In this study, we have

establish the culture of adult murine lacrimal gland epithelail cells. **Methods:** Mouse lacrimal glands were removed from postnatal 1 day mouse and 7 weeks mouse. After surgery, enzymatically digested and then dissociated. Isolated epithelial cells were cultured in Cnt 07 media with or without cholera toxin and/or 3T3 feeder layers to demonstrate the colony forming efficiency. Characterization of cultivated cells was perfomed by immunostaining (Cytokeratin14, Aquaporin8, and alpha-SMA). **Results:** Our studies demonstrate that it is possible to isolate viable human lacrimal gland epithelial cells and to culture them in serum-free media, which phenotype was K14+ and alpha-SMA-, and generate colony fomation on 3T3, and sphere formation on Matrigel. **Conclusions:** Our results show that mouse adult lacrimal gland epithelial cells could be isolated and cultured. In addition, our data indicate that these cells were duct-like phenotype. [This research was not supported by any grants]

AUGMENTATION OF TEAR FILM LIPID LAYER BY AN NEW ARTIFICIAL TEAR EMULSION. Howard Ketelson[1], Robert Baier[2], Anne Meyer[2], Jonathan Prindle[2], Michael Christensen[1], and Michelle Senchyna[1].[1]Alcon Research Ltd;[2]SUNY Buffalo.

Purpose: Surface analytical methods were applied to rationalize how an new artificial tear emulsion (ATE) containing mineral oil, surfactants and an anionic phospholipid in a HPGuar (HPG)-supplemented borate buffer, increases tear film lipid layer thickness by more than 50 nm, as demonstrated in dry eye clinical studies. **Methods:** Droplet-applied aliquots of ATE ramp-spread at the air/saline interface in a Langmuir trough spontaneously formed surface films at their intrinsic spreading pressure of approximately 12 mN/m as first determined by contact angle measurements on reference-grade PTFE film. Pre-positioned underfilm, glow-discharge-cleaned, hydrophilic and optically-flat infrared-transparent trapezoidal germanium prisms collected geometrically equal surface areas of these films by Langmuir-Blodgett lifts. The films were subsequently analyzed by 1) Multiple Attenuated Internal Reflection (MAIR) InfraRed spectroscopy for composition; 2) Thin Film Ellipsometry for thickness; and 3) Contact Potential Determination for electrical dipole orientation. Multi-dip/lift film transfers did not increase transferred film thickness. The initially spread films, reduced in surface area with a moving Teflon barrier, thickened to show grey-white interference colors. These were transferred for similar analyses to a separate prism. **Results**: ATE droplets spontaneously formed surface films at the air/aqueous interface, comprising mineral oil, surfactants, lipid and HPG based on MAIR-IR spectra with thicknesses not exceeding 2 nm and having a negative surface potential of 300mV. Cyclic compression/expansion to/from smaller areas consistent with interference colors quantified by interferometry in clinical studies revealed significant thickening of the oil phase with retention of surfactant/lipid and tissue-ontissue friction-reducing HPG ingredients. **Conclusions**. A new ATE containing HPG is capable of delivering and releasing surface-active anionic phospholipid and mineral oil components to the anterior tear interface in a superficial film dynamically compressible to significantly augment lipid layer thickness.

MEASURING OSMOLARITY WITH THE TEARLAB™. Santosh Khanal, Thomas J Millar. School of Natural Sciences, University of Western Sydney, Australia

Purpose: The TearLab™ has enabled a possibility of measuring tear osmolarity in clinical settings for the diagnosis of dry eye. Previous reports indicate that the TearLab™ gives statistically equivalent readings to other osmometers. More recently, it has been shown that TearLab™ measurements provide a reliable reading if averaged over a number of consecutive tests. Clinically, a single rather than multiple measurements for diagnosis would be ideal. In this study, we aimed to identify the extent of the variance between osmolarity readings. **Methods**: Seven non-contact lens wearers without any signs and symptoms of dry eye were recruited. Three osmolarity measurements were taken at 1 minute intervals on different occasions: about 9am; 12 noon to 1pm; and about 4pm for 2 consecutive days. For two of the subjects, osmolarity measurements at midday were continued over 7 days. One of these subjects had consistently high osmolarity values and the other with consistently low values. Osmolarity of a standard solution, 305mOsm/L, was measured 5 times consecutively with two different TearLab™ stations by 3 examiners. **Results**: Consistent with other reports, the range of consecutive tear osmolarity readings made with the TearLab™ varied up to 20mOsm/L, but an average over 3 readings can be a reliable indicator of tear osmolarity at a confidence level of 95%. This range of variation would be acceptable for definitive dry eye and normals but could significantly distort the diagnosis in borderline patients. For population studies, a power analysis based on the variability of the data showed that 3 repeat measurements would be required to obtain reliable data for a study with less than 50 subjects whereas 1 measurement would suffice for 500 or more subjects. Tests on a standard solution showed similar variability to those found with tears. This variability was the same on two different stations. However, occasionally aberrant results (up to 390mOsm/L) were obtained. **Conclusion**: Readings obtained with the TearLab™ can vary randomly in consecutive measurements and this could limit its discriminatory ability between borderline dry eye and normals. [This research was supported by a UWS seed grant]

A CASE OF GRANULOMA FORMATION AFTER CORNEAL REFRACTIVE SURGERY IN STEVENS-JOHNSON SYNDROME Eung Kweon Kim, MD, PhD,[1-3]Kyung Eun Han, MD,[1] Jae Hoon Kim, MD,[1] Sang Min Nam, MD,[1] Tae-im Kim, MD, PhD,[1] Kyung Ryul Seo, MD, PhD[1]. [1]Corneal Dystrophy Research Institute, Department of Ophthalmology, Yonsei University College of Medicine, Seoul, Korea, [2]Severance Medical Research Institute, Yonsei University College of Medicine, [3]Brain Korea 21 Project for Medical Science, Yonsei University, Seoul, Korea

Objective: To report a case after operating LASEK in one eye and LASIK in the other eye in Stevens-Johnson syndrome (SJS). **Method**: A 39-year-old female visited to our hospital due to ocular pain of her right eye. She had SJS for 30 years. At the acute attack of SJS, she experienced multiple bullae on whole body and ocular pain in both eyes. Ten years ago, she received LASEK in the right eye and LASIK in the left eye at local clinic. She had suffered from herpetic keratitis on her right eye for last 10 years. Before presentation, she was treated at local clinic because of corneal ulcer on her right eye. **Result**: Slit-lamp examination revealed a geographic corneal ulcer and some corneal erosions in her right eye and a large amorphous granuloma with new vessels and numerous small dot lesions on the nasal part of her left cornea. Fourier-domain optical coherence tomography of left cornea showed that the granuloma was located from subepithelial layer to anterior stroma and dot lesions were located around the LASIK flap interface. Meibomian gland plugging and trichiasis were noted in both eyes but severe conjucntival cicatrization or symblepharon was not observed. With acyclovir ointment and oral acyclovir, the epithelial lesion on her right eye was controlled. **Conclusion**: LASEK or LASIK should not be done in SJS patients, even though the ocular surface seemed to be normal or not having severe sequelae. Financial disclosure:The authors have no financial interest.

THE THERAPEUTIC EFFECT OF EYELID BOTULINUM TOXIN A INJECTION FOR THE DYSFUNCTIONAL TEAR SYNDROME. Jee Taek Kim, Hyun Koo, Jae Chan Kim. Yong-san Hospital, Chung-Ang University

Purpose: To evaluate the effect of an injection of Botulinum Toxin A into the medial side of lower eyelid for the treatment of dysfunctional tear syndrome(DTS). **Methods:** Prospectively, dry eye patients (Delphi grade 2 or severe) with and without excessive lid tension were selected. Botulinum toxin A (2.5 IU/0.1cc) was injected between the medial canthus and punctumof lower eyelids. Ocular examinations including Shirmer test without local anesthesia(Shirmer I test), tear break up time (TBUT), and the fluorescein clearance test (FCT) and vital staining were performed before, 1 month and 3 month after the injection.**Results:** A total 40 eyes of 20 patients were enrolled, and the mean age was 58.0±14.9 years. Patients were classified into three subgroups. Group A had only DTS patients (10 patients); Group B had DTS patients with lid tension abnormality (essential blepharospasm, hemifacialspasm and superior limbic keratitis: SLK : 7 patients); Group C had DTS patients with recurrent filamentary keratitis (3 patients). Shirmer I test, TBUT, FCT and vital staining were significantly improved after Botulinum toxin Ainjection. Improvement for DTS was definite in Group B patients, especially SLK patients. Filamentary keratitis was also resolved without recurrence. **Conclusions:** The eyelid botulinum toxin A injection in the DTS patients with aqueous deficiency is useful and effective treatment especially for patients with excessive eyelid blinking or severe DTS with filamentary keratitis.

PHARMARCODYNAMICS OF DA-6034 OPHTHALMIC SOLUTION IN NORMAL RABBIT. Ju Mi Kim, Moon Jung Goo, Kyung Koo Kang, Byoung Ok Ahn. DONG-A PHARM.CO.,LTD.

Objective: DA-6034, a secretogogue, is now under being developed as a treatment agent for dry eye symptom. This study was performed to evaluate the pharmacodynamics of DA-6034 ophthalmic solution on tear fluid volume (quantity) and mucin-like glycoprotein contents in tear (quality) using normal rabbits. **Method**: Tear fluid volumeand mucin-like glycoprotein contents were evaluated at 0, 10, 30, 60, 90, 120, 180, 240 and 360 min after topical application of DA-6034 ophthalmic solution of 0, 1, 3, or 5%. Tear fluid volume and glycoprotein contents were measured using Schirmer's strips and Enzyme-linked lectin assay, respectively. **Result**: DA-6034 ophthalmic solution significantly increased the tear fluid volume ($p<0.05$) with dose-dependency and its increase reached to the highest level at 10 min after instillation. At that time point, the increase of tear fluid was found to be 1.44-(1%), 1.68(3%)-, 1.72-fold (5% DA-6034) higher than that of the vehicle treated group, respectively. In addition, increased tear volume was maintained for 240 min after instillation of 5% DA-6034 ophthalmic solution. In mucin-like glycoprotein contents, DA-6034 ophthalmic solution showed significant and dose-dependent increase, and continued the increase until 90, 120, 240 min after instillation of 1, 3, 5% DA-6034 ophthalmic solution, respectively. **Conclusion**: These results suggest that DA-6034 ophthalmic solution stimulates not only tear fluid secretion but also mucin-like glycoprotein contents in tear and maintains its pharmacodynamic effect for 4 hours. Therefore, we concluded that DA-6034 ophthalmic solution would be a good candidate for dry eye treatment by improving the quantity and quality of tears. Keywords : DA-6034, dry eye, tear fluid volume, mucin-like glycoprotein

EFFICACY OF SODIUM HYALURONATE AND CARBOXYMETHYLCELLULOSE IN TREATING MILD TO MODERATE DRY EYE DISEASE. Tae-im Kim,1 Ji Hwan Lee,1 Ji-Won Kwon,2 Hyun Suk Ahn,1 Eung Kweon Kim,1 1The Institute of Vision Research, Department of Ophthalmology, Yonsei University College of Medicine, Seoul, Korea 2 Department of Ophthalmology, Seoul National University Hospital, Seoul, Korea

Purpose : We compared the efficacy and safety of sodium hyaluronate (SH) and carboxymethylcellulose (CMC) in treating mild to moderate dry eye. **Methods :** Sixty-seven patients with mild to moderate dry eye were enrolled in this prospective, randomized, blinded study. They were treated six times a day with preservative-free unit-dose formula eyedrops containing 0.1% SH or 0.5% CMC for 8 weeks. Corneal and conjunctival staining with fluorescein, tear film break up time (TFBUT), subjective symptoms, and adverse reactions were assessed at baseline, 4 weeks, and 8 weeks after treatment initiation. **Results :** Thirty-two patients were randomly assigned to the SH group and 33 were randomly assigned to the CMC group. Both the SH and CMC groups showed statistically significant improvements in corneal and conjunctival staining sum score, TFBUT, and dry eye symptom score at 4 and 8 weeks following treatment initiation. However, there were no statistically significant differences in any of the indices between the two treatment groups. There were no significant adverse reactions observed during follow-up. **Conclusion :** The efficacies of SH and CMC were equivalent in treating mild to moderate dry eye. Both preservative free artificial tear formulations appropriately manage dry eye sign and symptoms and show safety and efficacy when frequently administered in a unit-dose formula.

DRY EYE AND *DEMODEX* BLEPHARITIS. Jae Chan Kim, Jee Taek Kim, Seok Hyun Lee, Yeoun Sook Chun. Department of Ophthalmology, College of Medicine, Chung-Ang University, Seoul, Korea

Objective : To determine the correlative relationship between the *Demodex* in eyelashes and dysfunctional tear syndrome by demographic epidemiology and analyzing proinflammatory cytokine levels in tear fluid.**Methods :**Three hundred thirty five patients with ocular surface discomfort underwent epilation of eyelashes to count the number of *Demodex*, and they answered questionnaires about ocular surface discomfort and underwent ophthalmic examinations. IL-1, 5, 7, 12, 13, 17, Granulocyte colony-stimulating factor (G-CSF), and macrophage inflammatory protein -1 beta (MIP-1) of tear fluid in patients with ocular demodicosis group and control group were analyzed using a Luminex® 200™ Total System. After treatment of *Demodex* blepharitis using tea tree oil, patients rechecked questionnaires, ophthalmic examinations, and cytokine of tear. **Results :***Demodex* was found in 282 of the 335 tested patients (84%). The number of *Demodex* showed significant positive correlations with increased age, ocular discomfort, and 1/BUT ($p<0.05$). Tear concentrations of IL-7, IL-12, IL-17, MIP-1 were significantly higher in patients with ocular *Demodex* than in control groups. And IL-5, IL-13 were not significantly higher in patients with ocular *Demodex*. After treatment, the number of *Demodex* and ocular discomfort were significantly decreased, and IL-1 and IL-17 were significantly reduced. **Conclusions :**Elevated levels of IL-7, IL-12, and IL-17 in the tears of patients with *Demodex* blepharitis, and decreased levels of IL-1 and IL-17 after treatment suggest that *Demodex* trigger the innate immune response and acts important role in dysfunctional tear syndrome. The authors indicate no potential conflicts of interest.

LONG TERM CLINICAL RESULTS OF LIMBAL CONJUNCTIVAL AUTOGRAFT VERSUS AMNIOTIC MEMBRANE TRANSPLANTATION IN PTERYGIUM SURGERY. Hyung Joon Kim, Sin Hoo Kim. Department of Ophthalmology, Catholic University of Daegu, Daegu, Korea

Purpose. To compare the long term clinical outcome of limbal

conjunctival autograft versus amniotic membrane transplantation in surgically treated pterygium patients. **Methods.** 86 cases of pterygium in patients older than 50 years were surgically excised and received either limbal conjunctival autograft or amniotic membrane transplantation. Mean follow-up period was 36 months. All patients were examined for recurrence, which was graded from G0 to G3, and complications such as delayed epithelial wound healing and granuloma formation. **Results**. In limbal conjunctival autograft group, 35 out of 40 cases showed no recurrence(grade 0), and 2 cases of grade 1 recurrence and 3 cases of grade 2 were observed. On the contrary, 33 out of 46 cases in amniotic membrane transplantation group showed no recurrence, and 7 cases of grade 2 recurrence and 6 cases of grade 3 recurrence were observed. Conjunctival granuloma formation was occurred in 4 cases in amniotic membrane transplantation group and none in limbal conjunctival autograft group. Epithelial wound healing is not delayed in both group. **Conclusions**. Both limbal conjunctival autograft and amniotic membrane transplantation can be safe and effective adjunctive treatments for primary pterygium. However, limbal conjunctival autograft has a lower recurrence rate than amniotic membrane transplantation for long term follow up period. Limbal conjunctival autograft appears to be a more effective technique to reduce the recurrence after pterygium excision. No commercial relationship

IF TEAR EVAPORATION IS SO HIGH, WHY IS TEAR OSMOLARITY SO LOW? P. Ewen King-Smith[1], P. Ramamoorthy[1], K.K. Nichols[1], R.J. Braun[2], J.J. Nichols[1]. College of Optometry, The Ohio State University[1], Department of Mathematical Sciences, University of Delaware[2]

Objectives: 1. To test whether the thinning of the tear film between blinks is mainly due to evaporation. 2. To evaluate why osmolarity is increased much less than expected by the high evaporation rates we deduce. **Methods:** Fluorescent video imaging, over cornea and conjunctiva, was performed on 20 subjects (7 female, mean age 35 years) who were asked to keep their eyes open for as long as possible (up to 60 seconds) after a blink,. The recordings were performed using one microliter of 0.1% fluorescein and repeated ten minutes later using one microliter of 5% fluorescein. If thinning is due to evaporation rather than "tangential flow", fluorescence decay from increasing fluorescein concentration and quenching should be much greater for the high concentration. **Results:** 1. Analysis started 2 seconds after the blink, avoiding the upward tangential flow immediately after a blink. In the period between 2 and 6.9 seconds (the shortest inter-blink interval for all subjects), fluorescent decay rate for the high concentration, 3.16 ± 2.88%/second (mean ± SD) was much greater than for the low concentration, 0.23 ± 0.62%/second (binomial test, $P<0.001$). 2. For the 14 subjects who kept their eyes open for all 60 seconds for the high concentration, fluorescence decay between 2 and 57 seconds after the blink was much less over the conjunctiva, 8.8 ± 6.5%, than over the cornea, 53.9 ± 6.7% ($P<0.001$). **Conclusions:** 1. Tear thinning is largely due to evaporation rather than tangential flow. 2. There is little tear thinning over exposed conjunctiva. Because tear thinning rate equals evaporation rate minus osmotic flow rate (ignoring tangential flow), and evaporation rate is expected to be similar over conjunctiva and cornea, this indicates that osmotic flow out of all the conjunctiva is relatively large and limits the osmolarity increase from evaporation. Commercial relationships: none Supported by R01 EY017951 (King-Smith), R01 EY015519 (K. Nichols) and NSF 0616483 (Braun).

HEALTH AND DISEASE OF THE OCULAR SURFACE. Shigeru Kinoshita, M.D., Ph.D. Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan

The ocular surface serves a critical function as the defensive front line of the cornea, and it is key to maintaining corneal transparency. Whether the ocular surface remains healthy or succumbs to disease heavily depends upon such factors as tear-fluid function, ocular surface epithelial integrity, ocular surface innate immunity, and commensal and/or pathogenic bacteria. Of those factors, this presentation will specifically focus on the basic understanding of ocular-surface mucosal innate immunity on interaction with commensal and/or pathogenic bacteria, as this is one of the enlightening scientific fields of ocular-surface research. The ability of cells to recognize pathogen-associated molecular patterns depends on the expression of a family of TLRs, RIG-1, MDA5, etc. In fact, immunocompetent cells such as macrophages can recognize various microbial components and induce inflammation that then excludes the microbes. Ocular surface epithelial cells are, however, selectively responsive to microbial components and induce limited inflammation, probably because of the unique innate immune response to the coexistence with commensal bacteria on the ocular surface. Based upon the evidence mentioned above, it is reasonable to consider that there is an association between ocular surface inflammatory diseases and a disordered innate immune response. In fact, we previously documented the association with TLR3 and IL4R gene polymorphisms in Japanese SJS/TEN patients with ocular surface complications, thus suggesting a genetic-background involvement in this syndrome. A similar event may be considered in patients with meibomitis-related keratoconjunctivitis, a similar form of acne rosacea. Therefore, there exists the possibility that sustained, inflamed ocular surface diseases may be closely related to a disordered mucosal innate immunity, as its incidence may be higher than we had previously suspected.

DO MARINE MAMMALS HAVE A UNIQUE TYPE OF MEIBOMIAN GLAND? Nadja Knop,[1] Erich Knop, [1] Robin Kelleher Davis.[2,3] Research Laboratory, Dept of Ophthalmology CVK, Charité - Universitätsmedizin Berlin, Germany; [1] Schepens Eye Research Inst, [2] Harvard Medical School, [2,3] Boston, MA, USA.

Objective: In a previous study, we determined that the tears of pinnipeds and cetaceans contain proteins, but that in the pinniped, the tear film appears to lack the lipid layer found in human tear film. Hence, it was of interest to determine whether marine mammals have meibomian glands. **Methods:** Eyes and lid tissues were obtained from a sea lion that had been euthanized for reasons unrelated to this study. Tissues were fixed in formalin, investigated by a stereo magnifier, photodocumented, and embedded in paraffin. Sections of 10-20 µm thickness were performed with a rotary microtome and stained with haematoxilin and eosin. **Results:** In stereo magnifier analysis, a distinct tarsus was missing and the lid margin was seen to form a tip. The lid consisted mainly of muscular tissue and to the outside, underneath the epidermis, there was a whitish granular layer. In light microscopy, it was detected that this whitish layer consisted of hair follicles and sebaceous glands. Bundles of holocrine sebaceous acini were arranged between hair follicles. Towards the lid margin, the number of hair follicles decreased and the relative volume of sebaceous glands increased. At the lid margin, the sebaceous glands formed several solitary gland bodies without association to hairs or with only rudimentary hair shafts. The acini drained via short ductules into a straight duct with an about four-layered stratified squamous epithelium. The straight ducts opened via a terminal part that represented an ingrowth of the epidermis onto the outer lid skin. **Conclusions:** Our results indicate that pinnipeds have solitary sebaceous glands at the eyelid margin that resemble human meibomian glands in structure but are different in orientation and size. It remains to be determined whether the sea lion eyelid sebaceous glands represent an equivalent to human meibomian glands. Support: NIH EY05612, DFG KN317/11, Arey's Pond Boat Yard, Inc. We thank the Marine Mammal Center (Sausalito, CA) for contribution of tissues, and David A. Sullivan for advice.

TEAR MENISCUS AREA SOFTWARE (TMAS) FOR THE ASSESSMENT OF TEAR MENISCUS CHARACTERISTICS IN THE DIAGNOSIS OF DRY EYE DISEASE. Takashi Kojima[1,2], Osama M.A. Ibrahim[1,2], Tais Hitomi Wakamatsu[1,2], Koji Tonomura[3], Yukihiro Matsumoto[1,2], Murat Dogru[1], Kazuo Tsubota2.1. Johnson & Johnson Ocular Surface and Visual Optics Department, Keio University School of Medicine 2. Department of Ophthalmology, Keio University School of Medicine [3]. Konan Medical

Purpose: To evaluate the tear meniscus area using a newly developed software in patients with dry eyes and compare the findings with healthy control subjects. **Methods**: Seven eyes of 7 control subjects (2 male, 5female; mean age:47.4±9.7) and 7 eyes of 7 dry eye patients (2male, 5 female; mean age: 53.7±16.0) were enrolled in this prospective study. Dry eye was diagnosed according to the criteria described by Japanese dry eye research society. After instilling 2µl of 0.5% fluorescein dye, slit lamp digital photographs with a cobalt filter were taken to cover the whole area of tear meniscus in each eye. **Results**: The tear meniscus area in each eye could successfully be analyzed in all cases. The mean total meniscus areas were 6162±1376 and 7427±1030 pixels in dry eye and control subjects, respectively. The mean total meniscus area in dry eye patients was significantly smaller than the control subjects (p = 0.0291). **Conclusion**: The tear meniscus area software was found to be a useful tool in evaluating the ocular surface tear volume changes, and seems to be a promising method for the diagnosis and follow-up of dry eye disease.

CORNEAL ANTIMICROBIAL PEPTIDE EXPRESSION IN RESPONSE TO CANDIDA ALBICANS AND FUSARIUM SOLANI. Satya Sree Kolar, Hasna Baidouri, Wanyu Zhang, Alison M McDermott. University of Houston, College of Optometry, Houston, TX, 77204

Purpose: Antimicrobial peptides such as defensins and cathelicidin are an important component of innate immunity but little is known of their role in protecting the ocular surface from fungal infection. Here antimicrobial peptide expression in response to fungal exposure was evaluated in vitro and in vivo. **Methods**: Cultured human corneal epithelial cells were exposed to extracts of killed C. albicans or F. solani for 18 hours. RNA was harvested and RT-PCR performed to investigate expression of antimicrobial peptides human beta-defensin (hBD)-1, -2, cathelicidin and histatin. For in vivo studies the corneas of C57BL/6 or 129/SVJ mice were scratched then infected with C. albicans or F. solani. 24 hours later corneas were harvested and expression of murine beta defensins (mBD) and cathelicidin determined by RT-PCR. Expression of antimicrobial peptides was also investigated in murine neutrophils and macrophages. **Results**: Expression of hBD-2 was increased up to 3 fold in C. albicans and F.solani exposed human corneal epithelial cells whereas cathelicidin was increased up to 2.5 fold in F.solani exposed cells. There was no change in hBD-1 expression. Additionally, C. albicans but not F.solani treated human corneal epithelial cells expressed histatin-3. Murine (129/SVJ) corneas infected with F. solani demonstrated up to 3 fold enhanced expression of mBD-4, 6, 14 and cathelicidin compared to uninfected corneas. Corneas of C57BL/6 mice infected with F. solani demonstrated up to 3 fold enhanced expression of mBD-3, 4, 6, 14 and cathelicidin. Murine neutrophils (peripheral blood and extravasated) and macrophages were found to express mBD-1 and cathelicidin but not mBD-3, 4, 5 or 14, thus observed changes in mBD-3, 4 and 14 were likely due to modulated antimicrobial peptide expression in epithelial cells. **Conclusions**: Fungal exposure induces expression of antimicrobial peptides at the ocular surface which in turn are likely to participate in promoting elimination of these pathogens.

EFFECTIVENESS OF SINGLE ORAL PILOCARPINE ADMINISTRATION IN PATIENTS WITH SJÖGREN SYNDROME. Aoi Komuro[1, 2], Norihiko Yokoi[2], andShigeru Kinoshita. [1]Department of Ophthalmology, Nishijin Hospital, Kyoto, Japan, [2]Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan

Objective: To evaluate the effectiveness of single oral pilocarpine on tear film and dry eye and dry mouth symptoms in patients with Sjögren syndrome. **Methods:** Fifty-three eyes of 27 Sjögren syndrome patientsexperiencing dry mouth (26 female, 1 male; mean age: 52.9 yrs)were enrolled in this study. To evaluate changes in tear volume, a video meniscometer was used to measure the radius of the lower central tear meniscus curvature (R, mm) of each eye before administration of 5mg oral pilocarpine, and at 15 [R:(15)], 30 [R:(30)] and 60 [R:(60)] minutes after. The fluorescein breakup time (F-BUT, sec) and dry eye and dry mouth symptoms were evaluated before and 60 minutes after administration, using visual analogue scale (VAS, mm). **Results:** The respective R values (mean±SD)were [before: 0.16±0.07; R(15); 0.18±0.08; R(30): 0.18±0.07; R(60): 0.17±0.08]. There were significant differences between R beforeand at 15 (p=0.0313) and 30 (p=0.0025) minutes.F-BUT showed significant improvement before (0.7±0.5)and at 60 minutes(1.2±0.8) (p<0.0001). VAS score for dry eye and dry mouth symptoms decreased significantly (p<0.0001) before (respectively 63.9±26.7; 67.5±29.9) and at 60 minutes (37.9±30.3; 41.8±32.7). **Conclusions:** The oral pilocarpine administration seems to have a beneficial effect on tear film and dry eye symptoms as well as dry mouth symptoms in patients with Sjögren syndrome.

A NOVEL THERMAL PULSATION AND INNER EYELID HEAT APPLICATION FOR THE TREATMENT OF OBSTRUCTIVE MEIBOMIAN GLAND DYSFUNCTION. D.R. Korb[1,2], LipiFlow Study Group[2]. [1]Korb Associates, Boston, MA; [2]TearScience, Morrisville, NC.

Objective: To evaluate the safety and effectiveness of the LipiFlow® Thermal Pulsation System (LTPS) compared to standardized iHeatTM Warm Compress (WC) System for adult patients with chronic meibomian gland dysfunction (MGD). **Methods:** The study was a non-significant risk, prospective, open-label, randomized, crossover multicenter clinical trial. The 139 enrolled subjects were randomized between LipiFlow® (n=69) and WC control (n=70). LipiFlow® subjects received a single 12-minute LipiFlow® treatment and were reexamined at 1-day, 2-weeks and 4-weeks. Control subjects received a 5-minute iHeatTM treatment with instructions to perform the same 5-minute treatment daily for 2 weeks. They were examined at 2-weeks before receiving the LipiFlow® crossover treatment with follow-up at 1-day and 2-weeks. Effectiveness parameters: MG assessment, tear break-up time (TBUT) and dry eye symptoms. Safety parameters: adverse events, slit lamp and retinal evaluation, ocular surface staining, intraocular pressure, visual acuity, discomfort during and after treatment. **Results:** Only the LipiFlow® treatment resulted in significant improvement in the objective signs of increased MG secretion and TBUT at 2 and 4 weeks. MG secretion at baseline = 6.3±3.5; 2 wks = 14.3±8.7; 4 wks = 16.7±8.7 (p<0.05); no significant change in the control group. TBUT at baseline = 5.5±2.9; 2 wks = 6.9±5.0; 4 wks = 7.4±5.5, (p<0.05); no significant change in the control group. The single LipiFlow® treatment resulted in a greater significant reduction in dry eye symptoms than the 14-day, once-a-day iHeatTM warm compress treatment. There were no unanticipated or serious adverse events with either treatment. **Conclusion:** The data support the safety and effectiveness of the LTPS, applying heat to the inner eyelid surface, in treating MGD. The LipiFlow® was significantly more effective than the iHeatTM WC.

EVALUATION OF EXTENDED TEAR STABILITY BY TWO EMULSION BASED ARTIFICIAL TEARS. Donald Korb1 Caroline Blackie[1], David Meadows[2], Mike Christensen[2], Marion Tudor [2] : [1] Korb Associates, Boston, MA; [2] Alcon Research LTD. Fort Worth, TX;.

Purpose: Clinical studies were conducted evaluating the performance of Systane® BALANCE Lubricant Eye Drops (Alcon) vs Soothe™ XP Lubricant Eye Drops (B&L). Study 1 measured lipid layer thickness (LLT) and study 2 evaluated tear film breakup time (TFBUT), drop haze, comfort and acceptability. **Methods**: Systane® BALANCE is a unique oil-in-water emulsion containing propylene glycol as the active demulcent and POLYQUAD® as the preservative. Soothe™ XP contains light mineral oil and mineral oil as actives with PHMB as the preservative. Study 1: 40 patients were enrolled in a randomized, double masked, contralateral eye study. A baseline LLT of < 75nm in both eyes not varying by more than ± 15nm over the course of a 10-min observation period was required to qualify. One drop of the assigned test article was administered per eye per randomization. LLT was assessed at BL, then 1, 5, 15, 60 and 120 min post drop instillation. Study 2: 38 MGD patients with TFBUT ≤ 5 sec were enrolled in a double masked, randomized two-period cross over study. After drop instillation, a 3-min haze profile (30 sec intervals) was measured followed by comfort and acceptability scales. TFBUT was evaluated at 15, 30, 60 and 120 min post drop instillation. **Results:** Study 1: Significant improvements in LLT favoring Systane® BALANCE were seen at 5 (p=0.0015), 15 (p=0.0011), 60 (p=0.0001), and 120 (p<0.0001) min vs the control. Study 2: Systane® BALANCE showed less haze at time 0 (p<0.0001), 30 (p<0.0001) and 60 sec (p=0.0044). Drop comfort was significantly better for Systane® BALANCE (p=0.041). Drop acceptability was numerically but not significantly better for Systane® BALANCE vs Soothe XP. TFBUT was significantly longer for Systane® BALANCE at 2 hr (p< 0.0001). Longer time points are being evaluated. **Conclusion:** Systane® BALANCE demonstrated significant improvements in LLT and TFBUT up to 2 hr post drop instillation as compared to a control and demonstrated less haze (blur) and better comfort as compared to Soothe™ XP.

QUANTIFICATION OF FORCES OF MEIBOMIAN GLAND EXPRESSION RELATED TO TYPE OF EXPRESSION AND PAIN. D.R. Korb[1,2], C.A. Blackie [2,1].[1]TearScience, Morrisville, NC; [2]Korb Associates, Boston,MA.

Objective: (1) Determine the force required to assess whether individual meibomian glands (MG), which test non-functional, have the potential to secrete, (2) determine the force required for therapeutic expression for obstructive MGD and (3) document the reported pain. **Methods:** Twenty-eight subjects were recruited. Custom instrumentation designed to quantify the force applied during MG expression was used. The expression instrument was applied to the inner surface of the lower lid, which was compressed between the thumb and the expression instrument contact surface. The applied force was recorded. The first procedure evaluated the force required to obtain the first visible material from non-functional glands. The second evaluated the force required for effective therapeutic expression. The pain response was monitored throughout. **Results:** The force to obtain the first visible material (the potential to secrete) ranged from 1-60 PSI (mean = 16.0±8.2 PSI), and for effective therapeutic expression from 1-80 PSI (mean = 34.6±11.4 PSI). The maximum tolerable force, despite topical anesthesia, was limited by pain, varying from 5-80 PSI (mean = 23.9±9.3 PSI). Only 21% of patients could tolerate the forces necessary for effective therapeutic expression to all lower eyelid MGs. **Conclusions:** Forces of significant magnitude are required for both determination of whether non-functional glands have the potential to secrete and for therapeutic expression. Pain is the limiting factor for the conduct of these procedures.

ROLE OF SPDEF IN PULMONARY GOBLET CELL DIFFERENTIATION. Tom Korfhagen, Perinatal Institute, Section of Neonatology, Perinatal and Pulmonary Biology, Cincinnati Children's Hospital, Cincinnati, Ohio

Goblet cell hyperplasia is a significant pathological finding in the lungs of patients with chronic inflammation including chronic asthma, chronic obstructive pulmonary disease, and cystic fibrosis (CF). Using a transgenic mouse model of conditional SAM-pointed domain-containing Ets-like factor (SPDEF) expression in the conducting airway epithelium, we detected significant increases in goblet cells without detectable increases in airway inflammation. Exposure of the lung to pulmonary allergens induced differentiation of nonciliated secretory epithelial cells, Clara cells, into goblet cells. In vivo expression of SPDEF in Clara cells was sufficient to cause reversible goblet cell differentiation in the absence of cell proliferation. Increased RNA for genes regulating goblet cell differentiation and protein glycosylation, including forkhead box A3 (Foxa3) and anterior gradient 2 (Agr2), were detected in isolated bronchiolar cells by microarray. SPDEF, FOXA3, and AGR2 were also detected in goblet cells lining the airways of patients with chronic lung diseases including CF and smokers. *Spdef* null mice did not have goblet cells in upper airway submucosal glands and OVA sensitized *Spdef* null mice did not produce increased pulmonary goblet cells. Considered together, these data show that SPDEF plays an important role in a transcriptional network leading to goblet cell differentiation associated with chronic pulmonary disorders. References: Chen G, Korfhagen, TR, Xu Y, Kitzmiller J, Wert SE, Maeda Y, Gregorieff A, Clevers H, Whitsett JA. SPDEF is required for mouse pulmonary goblet cell differentiation and regulates a network of genes associated with mucus production. J Clin Invest. 119: 2914-2924, 2009. Park KS, Korfhagen TR, Bruno MD, Kitzmiller JA, Wan H, Wert SE, Khurana Hershey GK, Chen G, Whitsett. SPDEF regulates goblet cell hyperplasia in the airway epithelium. J Clin Invest. 117: 978-988, 2007.

MEASURING OF THE LOWER TEAR MENISCUS HEIGHT WITH TEARSCOPE® Krisztina Kosina-Hagyó, Amarilla Veres, Eszter Fodor, Béla Csákány, János Németh. SemmelweisUniversity, Department of Ophthalmology

Objective: To exam the reliability of measuring of lower tear meniscus height (LTMH) with Tearscope® and compare it with the optical coherence tomographic (OCT) method. **Method**: The LTMH was examined in thirty-six subjects. The meniscus was pictured five times after voluntary complete blinks with Tearscope® attached to a digital slit-lamp. According to basic guidelines, three independent observers evaluated the photos. The photos were re-evaluated after a meeting where the measurement was discussed in detail. The agreement between the observers, interobserver and interblink coefficient of variation (CV) were analysed. Additionally, LTMH detected with Tearscope® and RTvue® OCT were compared in fourteen participants. Friedman, Wilcoxon signed ranks test and Spearman correlation test was applied for statistical analysis. **Result**: The mean LTMH of the observers were 0.228±0.05, 0.186±0.04, 0.204±0.05 mm before and 0.215±0.05, 0.196±0.05, 0.203±0.05 mm after the meeting. There were significant differences between the observers before and after the meeting as well. The results of observers showed significant correlations with each other (r=0.884-0.970, p=0.000) at both time. The interblink CV were 9.88% and 10.30%, respectively. The interobserver CV decreased from 11.14% to 6.84% after the meeting, and the post meeting interobserver CV

decreased under the interblink CV. In the comparative analysis, the mean LTMH was 0.366±0.19mm with OCT and 0.257±0.11 mm with Tearscope®. The results of the two methods differed significantly, but correlated well (r=0.692, p=0.006).**Conclusion**: The Tearscope® is useful to measure non-invasively the LTMH beside other tear film parameters. This method of evaluate of LTMH is subjective, but the interobserver variability decreases below the natural variability of LTMH after discussion of the way of analysis. Supported by a research grant from Semmelweis University Doctoral School, Budapest, Hungary. None of the authors has a proprietary or financial interest in any materials or methods mentioned.

CYCLOSPORINE A PREVENTS ENHANCED NEURAL ACTIVITY OF CORNEAL COLD SENSORY NERVE TERMINALS IN CHRONIC DRY EYE. Illés Kovács[1,2], Susana Quirce[1], Carolina Luna[1], M. Carmen Acosta[1], Carlos Belmonte[1], Xavier Gasull[3], Juana Gallar1.1Instituto de Neurociencias, Universidad Miguel Hernandez-CSIC, San Juan de Alicante, Spain [2]Dept. of Ophthalmology, Semmelweis University, Budapest, Hungary [3]Dept. of Physiology-IDIBAPS, University of Barcelona, Barcelona, Spain

Objective: Topical cyclosporine A (CSA) ameliorates ocular dryness sensations and these effects are assigned to antiinflammatory properties of the drug. Our aim was to evaluate the influence of CSA on activity of corneal cold nerve terminals (CNT) in experimental dry eye. **Method:** In 9 guinea-pigs, the main lacrimal gland was removed. Four weeks later, nerve terminal impulse activity of corneal CNTs was recorded using microelectrodes in a recording chamber perfused at 34ºC or cooling down to 22ºC. Spontaneous activity at 34ºC (SA, impulses/s), cold threshold temperature (ºC) and maximum response during cool ramps (MRC, impulses/s) were analyzed from control and dry eye (DE) corneas before and during perfusion with 50µM CSA. **Result:**In dry eye, significantly higher SA (DE: 9.7±1.0, control: 6.4±0.9; p<0.05) MRC (DE: 32.7±4.5, control: 28.4±5.4; p<0.05) and lower cold threshold (DE: 2.2±0.1, control: 3.01±0.1) was observed. CSA had no effect on SA (p>0.05) but significantly decreased MRC and increased cold threshold in dry eye (p<0.05) corneas. **Conclusion:** CSA decreased the enhanced responsiveness of corneal cold receptors in dry eye, suggesting its direct effect on membrane Na^+ and/or TRPM8 channels involved in cold sensation. Support: Human-MB08A-80372 (IK), SAF2008-00529 (JG), CSD2007-00023 (CB).

PROTECTIVE EFFECT OF AMINO ACIDS AND COMMERCIAL OPHTHALMIC INGREDIENTS ON 4-HYDROXYNONENAL-INDUCED CYTOTOXICITY IN HUMAN CORNEAL EPITHELIAL CELLS. Takahiro Kurose[1, 2], Kazuhiro Tsuji[2], Takayuki Miyano[1, 2], Yoichi Honma[2], Norihiko Yokoi [1] and Shigeru Kinoshita[1] Kyoto Prefectural University of Medicine, [1] Kyoto, Japan; Rohto pharmaceutical co., Ltd, [2] Kyoto, Japan

Objective: 4-Hydroxynonenal (4HNE), a major lipid peroxidation product, is reportedly increased in cornea of rat dry eye models. We evaluate the cytotoxicity of 4HNE in human corneal epithelial cells (HCE-T) and protective effect of amino acids and commercial ophthalmic ingredients. Also, we analyze whether c-Jun N-terminal kinase (JNK)-mediated intrinsic pathway signaling contributes to the cytotoxicity. **Method**: HCE-T were grown to confluent, and then exposed to 4HNE (1-100 µM) for 24 hrs. Amino acids or commercial ophthalmic ingredients were added to the cells treated with 75 µM 4HNE. Cell viability was examined by ATP quantification method. Phosphorylation of JNK was examined by western blot and immunohistochemistry. **Result**: 4HNE induced the cytotoxicity in a dose dependent manner, accompanied by the induction of JNK phosphorylation. Cells treated with reduced glutathione (GSH), cysteine, taurine, and -cyclodextrin was significantly greater than viability of cells treated with 4HNE alone. 4HNE-induced JNK phosphorylation was attenuated in the cells treated with taurine. **Conclusion**: In this study, we first showed that 4HNE induces the cytotoxicity on corneal epithelial cells. Moreover, GSH, cysteine, taurine and -cyclodextrin showed greater protective effect on 4HNE-induced cytotoxicity. The present results suggest that these cell-protective agents may become a treatment for dry eye.

DO CONTACT LENSES ELEVATE TEAR OSMOLARITY IN THE TYPICAL LENS WEARER? Alan Landers, Mary Mowrey-McKee, Walter Nash, Robert Scott, CIBA VISION Corporation, Atlanta, GA.

Purpose: It has been suggested that elevated tear osmolarity may play a role in contact lens-induced dryness/discomfort. Therefore, we investigated the impact of contact lenses (CLs) of differing materials on tear osmolarity in what we assumed to be the typical lens wearer (i.e. asymptomatic). **Methods:** 30 subjects were exposed to five different wearing cycles in random sequence: 1) enhanced-lotrafilcon A CLs 2) No lens wear 3) nelfilcon A CLs 4) No Lens wear 5) comfilcon A CLs. The TearLab™ Osmolarity System was used to measure tear osmolarity of both eyes and was the first clinical measure performed at each follow-up visit to avoid any potential carryover effects. The data were inspected for differences between the two 'no lens' wearing cycles and for the difference between each contact lens type and the average of the 'no lens' wearing cycles. **Results:** The length of time in lenses at the follow-up visits was equal for all lens types, with a mean wear time of 7.1 hours. There were no significant differences between the two 'no-lens' wearing cycles (296.6±11.2 vs. 298.6±13.5 mOsm/L). Therefore, these two values were averaged for comparison to the lens-wearing cycles. The tear osmolarity for the contact lens wearing cycles were 304.2±19.2 mOsm/L for enhanced-lotrafilcon A, 302.7±17.0 mOsm/L for nelfilcon A, and 297.8±11.6 mOsm/L for comfilcon A. There were no significant differences found between nelfilcon A and comfilcon A when compared to no lens wear. A statistical difference was reached for enhanced-lotrafilcon A when compared to no lens wear (p=0.008). However, this would not be considered a clinically significant result based on reported values in the literature for normal tear osmolarity. **Conclusions:** These results indicate that, in this population of contact lens wearers, the presence of a contact lens (regardless of material) in the eye did not significantly elevate the tear osmolarity. This data seems to suggest that either contact lenses do not disturb the tear film as much as previously thought or the healthy eye has the ability to compensate for the tear film de-stabilization and regulate the tear osmolarity.

CELL TARGETING BY TEAR PROSECRETORY MITOGEN – LACRITIN. Gordon W. Laurie-, Yinghui Zhang Cell Biology, University of Virginia

Objective: Lacritin is an eye-specific mitogen in human tears that when added to the rabbit ocular surface promotes basal tearing. Tearing is sustained for 240 min after a single dose, or for at least one week after doses three times daily for 2 wks have ended (Samudre et al, unpublished). Lacritin targets the cell surface via co-receptor syndecan-1 (SDC1) in a tear heparanase-dependent manner. Here we explore the lacritin-SDC1 binding site. **Method**: Human SDC1 or newly generated SDC1 cDNA's lacking 20 or 30 amino acids from the N-terminus; or in which serines 15, 23 or 25 were mutated to alanine; or in which the SDC1 sequence GAGAL (amino acids 26 - 31) was switched out for the equivalent sequences in SDC2 (GDLDD) or SDC4 (GADED) were expressed in 293-6E

suspension culture and assayed in lacritin pulldown experiments. Also assayed were lysates from 4-methylumbelliferyl--D-xylopyranoside-treated 293-6E cells expressing wild type SDC1, a series of lacritin N-deletion and point mutants, and newly generated synthetic peptides corresponding to SDC1 amino acids 1 - 20, 10 - 30, 20 - 30 and 30 - 50. **Result**: A) SDC1 truncation of 30, but not 20, amino acids, and synthetic peptides corresponding to amino acids 20 - 30 and 10 -30, but not 1 - 20 and 30 - 50 abrogated binding to lacritin. B) Binding affinity was significantly diminished by switching out GAGAL for equivalent SDC2 or SDC4 sequences. C) SDC1 point mutants S15A, S15/23A and S15/25A bound lacritin, but not S23/25A. No binding was observed to SDC1 lacking all HS chains after xyloside treatment. D) Truncation of up to 75 amino acids from lacritin's N-terminus did not affect binding. Point mutagenesis of lacritin's hydrophobic binding face revealed substantially reduced binding to L108S and L109S. G101S, F104S and F112S mutations did not affect binding.**Conclusion**: These data suggest that the role of tear heparanase is to generate heparan sulfate stubs that serve as negatively charged elements of the hydrophobic GAGAL binding motif. GAGAL likely interacts with hydrophobic leucines 108 and 109 of lacritin's C-terminal amphipathic alpha helix. Supported by EY013143 (to GWL). G.W. Laurie, EyeRx, C; UVa Patent Fdn, P. Y. Zhang, none.

HYPERLIPIDEMIA - A PREDISPOSING FACTOR TO MEIBOMIAN GLAND DYSFUNCTION. Souhad Lawand. MD, Ph.D Zulekha hospital, Ophthalmology department. UAE – Sharjah

Purpose: this clinical research was conducted to determine the correlation of hyperlipidemia, specifically low density lipids (LDL), and meibomian gland dysfunction – one of the major causes of superficial lipid layer deficiency leading to dry eye. **Methods:** During a 3 months clinical research, 232 patients of different nationalities (130 male, 102 female) diagnosed with dry eye of different severity related to meibomian gland dysfunction (MGD) of different intensity submitted blood serum for lipid profile after 12 hours of fasting. **Results:** although almost all patients were systemically asymptomatic, 70% of patients had LDL levels over the normal range of 0-100 mg/dl, out of which 26% of the patients required systemic treatment due to elevated LDL levels over 140mg/dl. Female patients had less elevated levels of LDL in comparison to Male patients. Patients from the Arabian Peninsula had the lowest rates of hyperlipidemia whereas Islanders from such countries such as the Philippines and Sri Lanka had the highest. **Conclusions:** hyperlipidemia, especially elevation of LDL levels, could be one of the major reasons leading to meibomian gland dysfunction. Hyperlipidemia could be suspected upon diagnosing MGD in an ophthalmic patient. Healthy food intake and cholesterol management could be recommended to reduce the severity of meibomian gland dysfunction. No grant support or commercial benefits were related to this research.

CHANGES OF DYNAMIC WAVEFRONT ABERRATION AFTER PUNTAL OCCLUSION IN DRY EYE PATIENTS. Do hyung Lee, MD, PhD., Hyung seok Cho, MD, Jin Hyoung Kim, MD, Suk Kyue Choi, MD. Department of Ophthalmology, Ilsan Paik hospital, Inje University, Korea

Objective :To compare and analyze the effect of two punctual occlusions with dynamic wavefront aberrations in dry eye patients. **Method** : Fourty-two eyes with dry eye syndrome participated in this study. All eyes were randomized to either a collagen or synthetic insert inferior punctal occlusion. Ocular higher-order aberrations with a Hartmann-Shack aberrometer were sequentially measured before and after punctual occlusion (1week and 1 month later) for 10 seconds without blinking. **Result**: Total higher-order aberrations were reduced 1week after punctual occlusion in both plug insertion groups, but statistically not significant($p>0.05$).Fluctuation index was significantly reduced only in group I at post 1 week($p<0.05$). **Conclusion**: Both collagen and synthetic plug may be effective to decrease total higher-order aberrations after punctal occlusion. Collagen plug may have longer effect to improve tear-film stability than absorbable synthetic plug. No author has a financial or proprietary interest in any material or method mentioned.

THE INFLUENCE OF HUMAN MEIBOMIAN LIPIDS ON THE WETTING PROPERTIES OF A DROPLET. Danielle L. Leiske,[1] Cécile Monteux,[2] Michelle Senchyna,[3] Howard A. Ketelson,[3] David Meadows,[3] Gerald G. Fuller.[1]. Stanford University, Stanford, CA USA,[1] ESPCI, Paris, France,[2] Alcon Research, Ltd. Fort Worth, TX USA.[3]

Objective: Rapid dewetting of the tear film from the ocular surface may lead to epithelial cell damage and consequently discomfort associated with dry eye disease. As the influence of tear film components on dynamic movement of the contact line has not been well studied we present a fundamental study on the effects of thin films of human meibomian lipids (ML) and pure lipids on advancing (ACA) and receding (RCA) contact angles of a droplet. **Methods:** Samples of ML were collected via manual expression of the glands in the lower lid of a healthy volunteer. Multiple collections were pooled in chloroform, dried, and weighed. ML and DPPC (dipalmitoylphosphatidylcholine) were dissolved in chloroform and stored at -20°C. The experiment was set up as follows: a drop of water was placed on a hydrophilic or hydrophobic substrate next to a teflon block. Lipids were added to low surface pressures on the drop surface. The glass substrate was pulled or pushed (resulting in ACA or RCA) at a constant velocity so that the droplet remained in contact with the block. The contact angle between the droplet and the substrate, humidity and temperature were monitored. **Results:** We observed similar behavior of ACA for DPPC and ML on both substrates. On the hydrophilic substrate the ACA was constant at high plate velocities (>5mm/s), but for velocities 0.01-mm/s we observed a stick/slip regime of the contact line where the contact angle would gradually increase then suddenly drop at regular intervals (period of 1mm for DPPC and 2mm for ML). RCA on the hydrophobic substrates showed the greatest differences between the lipids: DPPC droplets stretched out to low contact angles while ML droplets resisted deformation resulting higher contact angles. **Conclusions:** ACA at high speeds is relevant to tear film spreading following a blink; our results show minimal differences between lipid types. ML resistance to deformation of RCA compared to DPPC implies that lipid layer properties such as surface tension could affect dewetting rates of the tear film.

VISCOELASTIC AND STRUCTURAL CHANGES OF MEIBOMIAN LIPIDS WITH TEMPERATURE. Danielle L. Leiske,[1] Michelle Senchyna,[2] Howard A. Ketelson,[2] Gerald G. Fuller.[1] Stanford University, Stanford, CA,[1] Alcon Research, Ltd. Fort Worth, TX,[2] USA.

Purpose: As the outermost layer of the tear film, it is believed that the lipid layer is a significant factor in tear film stability. The lipid layer mechanical properties influence the ability of this layer to stabilize the tear film; the mechanical properties, in turn, are a result of the local structure and organization of the lipids. **Methods:** Interfacial mechanical properties of meibomian lipid (ML) monolayers were monitored using an interfacial stress rheometer. Material was spread neat in a Langmuir trough. After compression monolayers were held at a fixed surface area while temperature was increased at 0.2°C/min.

Crystalline structure of bulk meibum was probed by small angle x-ray scattering (SAXS) between 25 and 45°C, grazing incidence x-ray diffraction was used to measure order of ML monolayers at 25°C. **Results:** At 15 mN/m ML monolayers were quite elastic with high surface moduli values, 2-7 mN/m. As the monolayers were heated from 18°C to 35°C, the interfacial moduli values decreased significantly. By 30°C the monolayers were primarily fluid and at 35°C the interfacial viscous modulus was 0.02 mN/m. SAXS showed that bulk ML have a crystalline structure with lamellar packing ~49Å, roughly the length of the molecules. As the samples were heated the peak intensity decreased and was absent at 45°C, meanwhile the centers shifted to larger length scales due to thermal expansion. Preliminary in-plane scattering experiments showed that ML monolayers form a tilted lattice with spacing ~4.85 Å. **Conclusions:** We have shown there is an association between structure and mechanical properties of ML. Moreover, the dramatic changes in interfacial viscoselastic properties could have a number of clinical implications. If the melt temperature of lipids in dry eye individuals is significantly altered (because, for example, of compositional differences) delivery of the lipids from the gland to the tear film and mechanical properties of the tear film itself may be modified in a manner that leads to instability. [This work was supported by Alcon Research, Ltd. and a Stanford Graduate Fellowship.]

UPDATE ON THE DEWS REPORT: THERAPY AND MANAGEMENT. Michael A. Lemp, Georgetown University

Objective: To report on developments in the treatment and management of dry eye disease (DED) since the publication of the DEWS Report **Method**: Literature review **Results**: No new therapeutic drug for the treatment of DED has been cleared in North America or Europe. Immune-modulating and anti-inflammatory drugs dominate those agents in active clinical trials. Additional treatment modalities include: polymer-eluting contact lenses, agents aiding in repair of tissue damage, artificial tears with tear stabilizing and anti-evaporative effects, the controlled release of heat in the treatment of meibomian gland dysfunction (MGD), and the, as yet , off-label use of topical macrolide anti-microbials for blepharitis including MGD. **Conclusion**: Breakthroughs in treatment may well await the development of better endpoints and metrics for assessing improvement in signs and symptoms in clinical trial design.

RECENT ADVANCES TOWARDS UNDERSTANDING THE GENETIC BASIS OF SJÖGREN'S SYNDROME. Christopher Lessard and Kathy L. Moser. Arthritis and Immunology Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA

Sjögren's syndrome (SS) is a clinically heterogeneous autoimmune disease that involves both innate and adaptive immunity. A complex genetic architecture has been hypothesized, however, genetic studies to date have been primarily limited to candidate genes approaches. We have established an international collaborative group, the Sjogrens Genetics Network (SGENE), to assemble the thousands of samples needed for large-scale genetic studies. Using high-density genotyping arrays, we have performed an initial genome-wide association (GWA) scan to identify SS susceptibility loci. We used the Illumina OMNI1-Quad arrays containing >1.1x10E6 variants in a discovery cohort of 272 primary SS cases and 387 healthy controls. For replication, we used a DNA pooling approach in an independent collection of cases and controls, also using the OMNI1-Quad arrays. The most significant region associated with disease risk was the major histocompatibility complex (MHC) with 45 SNPs exceeding a genome-wide significance threshold of 5x10E-8, all of which replicated in our independent samples. Within the MHC, peak significance was observed at HLA-DRA for rs9268832 (meta p=2.56x10E-13). Additional results across the extended MHC support association with multiple loci throughout this region. Evidence for novel genetic associations outside of the MHC were also observed, including a SNP located in a region of high regulatory potential near the musculin (MSC) gene (meta p=1.93x10E-6). MSC codes for a multi-functional transcription factor involved in signaling pathways downstream of B cell receptor activation. Evidence for association of SS with loci previously established in closely related autoimmune diseases was also observed and included IRF5, TNIP1, BANK1, PRDM1, JAZF1, STAT4, and IL12B. This study represents the most comprehensive assessment of the genetic contribution to SS performed to date. Further characterization of these effects is warranted to precisely define causal variants and determine functional consequences that contribute to disease pathogenesis. Grant support: NIH U19 AI082714 and RO1 DE018209

INTERLEUKIN 33, A NOVEL EPITHELIUM-DERIVED CYTOKINE, LINKS INNATE IMMUNITY TO ALLERGIC INFLAMMATION ON OCULAR SURFACE. De-Quan Li, M.D., Ph.D., Lili Zhang, M.D., Xiaofen Zheng, M.D., Ph.D., Guiqiu Zhao, M.D, Ph.D., Matthew A. Cunningham, M.D., Cintia S. De Paiva, M.D., Stephen C. Pflugfelder, M.D. Ocular Surface Center, Cullen Eye Institute, Department of Ophthalmology, Baylor College of Medicine, Houston, Texas, USA

Objective: Toexplore the toll-like receptor (TLR)-mediated induction of interleukin (IL) 33 by human corneal epithelial cells and its potential role in ocular allergic inflammation. **Method**: IL-33 and receptor ST2 were determined in ocular surface of atopic conjunctivitis patients and mice with short ragweed (SRW)-induced experimental allergic conjunctivitis (EAC). Primary human corneal epithelial cells were cultured to evaluate the expression and regulation of IL-33 by reverse transcription and real time PCR, ELISA, immunobead assay, immunostaining and Western blot. **Result**: IL-33 and its receptor ST2 were highly detected in conjunctival impression cytology specimen from atopic conjunctivitis patients. In the SRWinduced EAC mice, IL-33 and ST2 transcripts significantly increased in the corneal/conjunctival epithelia and/or cervical lymph nodes (CLN); and the CD11c+ dendritic cells (DCs) and CD4+ Th2 cells largely infiltrated the conjunctiva with increased transcripts and immunoreactivity of CD11c, CD4, IL- 4, IL-5 and IL-13 detected in the conjunctiva and CLN. In human corneal epithelial cultures, IL-33 was induced by various microbial components(dsRNA, flagellin and FSL-1, the ligands to TLR-3, -5 and -2/6, respectively) and proinflammatory cytokines (IL-1β and TNF-α) through TLR and NFkB pathways. Furthermore, IL-33 stimulated expression of inflammatory mediators (TNF-, IL-6, RANTS, MMP-1, MMP-3 and MMP-9) by human corneal epithelial cells. **Conclusion**: IL-33, an inflammatory cytokine, is induced by human corneal epithelial cells through TLR-mediated innate response, and it promotes ocular allergic inflammation through activating ST2 signaling for Th2 cytokine response. CR: N. Support:DOD CDMRP PRMRP Grant FY06 PR064719 (DQL), NIH Grant EY11915 (SCP), Research to Prevent Blindness, Oshman Foundation, William Stamps Farish Fund.

IN VIVO ASSESSMENT OF THE OCULAR SURFACE EFFECTS OF TRAVOPROST BAK-FREE VERSUS BAK-PRESERVED TRAVOPROST AND LATANOPROST OPHTHALMIC SOLUTIONS. Liang H[1,2,3,4], Brignole-audouin F[1,2,3,4,5], Riancho L[1,2,3,] Baudouin C[1,2,3,4.] [1]INSERM, U968, [2]UPMC Paris 06, [3]CNRS, UMR_7210, [4]CHNO des XV-XX, [5]Université Paris Descartes,Paris, France

Purpose: To evaluate the *in vivo* ocular surface toxicity of a new formulation of travoprost ophthalmic solution, 0.004% (trav BAK-

free), preserved with polyquaternium-1 (PQ), compared with commercially available BAKpreserved travoprost ophthalmic solution, 0.004% (trav BAK) and BAK-preserved latanoprost ophthalmic solution, 0.005% (lat BAK). **Methods**: Adult male New Zealand albino rabbits (N=36) randomly received 15 bilateral drops at 5 minute intervals of phosphate-buffered saline (PBS), PQ 0.001%, BAK 0.015%, trav BAK-free, trav BAK, or lat BAK. At hour 4 and day 1, the ocular surface reactions were evaluated using slit-lamp examination; in vivo confocal microscopy (IVCM) for cornea, limbus, and conjunctiva/conjunctiva-associated lymphoid tissue (CALT); conjunctival impression cytology; and standard immunohistology in cryosections for detecting CD45+ infiltrating cells and MUC- 5AC-labeled cells. **Results**: Trav BAK-free and PQ were similar to PBS, and did not induce clinically-observed irritation, changes in microstructures of the whole ocular surface as measured by IVCM, inflammatory infiltration or cell damage as measured by impression cytology, altered goblet cell counts, or significant corneal infiltration of CD45+ cells. In contrast, trav BAK, lat BAK, BAK 0.015%, and BAK 0.02% induced diffuse conjunctival hyperemia and chemosis, abnormal changes in the ocular surface microstructure, significant total ocular surface toxicity scores, damaged epithelial cells, inflammatory cell infiltration, and decreased goblet cell density. **Conclusion**: Trav BAK-free did not elicit ocular surface toxicity when administered to rabbit eyes, indicating that it may be safer for the ocular surface than products containing BAK. Clinical studies are needed for confirmation in patients with open angle glaucoma or ocular hypertension. [Research supported by an unrestricted grant from Alcon Research Ltd.]

ASSOCIATION OF TEAR FILM WAVEFRONT METRICS WITH GRADE OF PRE-LENS TEAR BREAK-UP AND VISION. Haixia Liu, C.G. Begley, N.L. Himebaugh, L.N. Thibos, Z. Wu, School of Optometry, Indiana University, Bloomington, IN

Purpose: Tear film break-up (TBU) causes local, highly irregular changes in tear film thickness. We hypothesize that this causes deterioration of the optical quality of the eye, impacting vision. The purpose of this study was to determine whether changes in optical metrics correlate with grading of TBU and with contrast sensitivity (CS) loss in contact lens (CL) wearers. **Method**: Soft CL wearers (n=28) kept one eye open for 18 sec during a 3-way concurrent measure of CS, retroillumination (RI), and wavefront aberrometry with a modified COAS™ wavefront aberrometer (AMO Wavefront Sciences). A masked observer graded each RI image of TBU (0 to 4 scale). Higher order (up to 6th) root mean square error (RMS-HO) and root mean square fit error (RMS-FE) were calculated for a 6 mm pupil and analyzed by TBU-RI grade. **Results**: Five subjects had no TBU (Gr 0) with no change in ocular aberrations (mean Δ RMS-HO = 0.05±0.10 μm; mean Δ RMS-FE = 122±241 μrad; paired t-test, p>0.3). For those with TBU, the median Δ in TBU grade was 2. Both RMS-HO and RMS-FE increased significantly over the 18 sec trial (mean Δ RMS-HO = 0.12±0.14 μm; mean Δ RMS-FE = 1197±909 μrad, paired t-test, p<0.001). Compared to RMS-HO, RMS-FE correlated significantly better with TBU grade (rho = 0.85±0.12 and 0.46±0.46, respectively, paired t-test, p<0.001). Mean % change in RMS-HO per grade TBU-RI ranged from 7±21 (Gr 0) to 150±141 (Gr 4) and 12±20 (Gr 0) to 245±58 (Gr 4) for RMS-FE. RMS-FE % change was significant across grades except for Gr 3 and 4 (Mann- Whitney, p<0.0001). CS decreased significantly from 11.76 to 6.41±3.41 (paired t-test, p<0.001). RMS-HO and RMS-FE were both correlated with TBU grade and CS. The average correlations were RMS-HO and TBU (0.46±0.46); RMS-HO and CS (-0.50±0.54); RMS-FE and TBU (0.85±0.12); RMS-FE and CS (-0.74±0.23). **Conclusions**: RMS-FE, which is a measure of the irregular aberrations, yields an accurate metric for changes to the local tear film seen with RI and was highly correlated with TBU-RI grade and CS loss. It can be used to accurately monitor tear film stability across time in s dry eye and contact lenses.

REGULATION OF THE PROLIFERATION AND DIFFERENTIATION OF HUMAN MEIBOMIAN GLAND EPITHELIAL CELLS. Shaohui Liu and David A Sullivan, Schepens Eye Research Institute and Harvard Medical School, Boston, MA, USA

Objective. Our laboratory seeks to identify factors that control meibomian gland function in both health and disease. We also seek to translate this knowledge into the development of novel and unique therapeutic strategies to treat meibomian gland dysfunction. To help achieve these objectives, we have established a culture system for the maintenance of primary human meibomian gland epithelial cells, and have also immortalized these cells. This study's purpose was to explore the regulation of the proliferation and differentiation of human meibomian gland epithelial cells. **Methods.** Cell proliferation and differentiation were evaluated by using 5-bromo-2-deoxyuridine and Oil red O. Gene expression was analyzed by using Illumina HumanHT-12 BeadChips, Illumina BeadStudio and Geospiza bioinformatics. **Results.** Our findings show that growth factors (e.g. epidermal growth factor [EGF] and bovine pituitary extract [BPE]), as well as exogenous calcium and FBS, stimulate the proliferation and/or differentiation of human meibomian gland epithelial cells. The extent of growth factor and media influence on proliferation is dependent upon the cell seeding density and cell passage number. Our results also demonstrate that: [a] differentiation is associated with a significant change in the expression of thousands of genes in both primary and immortalized cells. Differentiation is linked to an upregulation in gene activity related to tissue development, lysosomes and apoptosis, and a reduction in that associated with cell cycle and translation. The precise nature of the molecular response to differentiation-inducing agents appears to depend upon the stimulus (e.g. calcium or FBS); and [b] proliferation is also associated with alterations in the expression of thousands of genes (e.g. cell cycle, keratinization$^-$). These genes are typically different than found with cellular differentiation, and the profile also seems to depend upon the inducing agent (e.g. EGF or BPE). **Conclusions.** Our findings offer new insight into the proliferation and differentiation of human meibomian gland epithelial cells. (Supported by NIH grant EY05612)

DRY EYE SYMPTOMATOLOGY OF NON CONTACT LENS WEARERS WITH THE ODSI QUESTIONNAIRE. Cécile Maissa, Michel Guillon, Caroline Flomet, Elisabeth Bolton. OTG Research & Consultancy London UK

Objective: Using the OSDI validated questionnaire, this investigation aimed to determine the frequency and nature of dry eye symptoms in non contact lens wearers presenting in an optometric practice and to identify the individual questions that best predict the dry eye symptomatology status produced by the overall questionnaire. **Method:**The OSDI questionnaire (the questionnaire made up of twelve questions grouped under the headings of ocular and vision related symptoms and environmental triggers) was administered to 171 non contact lens wearers (Age 34.6±14.1) who attended OTG Research & Consultancy clinic. The data set was analysed with a Chi Square Automated Interaction Detector (CHAID) test to identify the factors that best predict the dry eye status of individual patients, as determined by their overall OSDI score. **Result:**The average OSDI score for the test sample was 15.7 ± 17.2 and the incidence of dry eye (DE) of varying severity 47% (Mild=22%; Moderate=10%; Severe=15%). Each individual questions produced statistical differences (p<0.001) between the

normal and dry eye groups; however the capacity of each question to predict individual patient status was highly different. The predictive model was multifactorial: symptoms in areas with air conditioning was the primary predictive factor (DE incidence: No symptom = 24%; At least some time =74%; More often than some time = 100%). For those asymptomatic in air conditioned areas, no problem while watching TV (DE incidence = 8%) and for those symptomatic some of the time while in air conditioned area, blurred vision at least some time (DE incidence=92%) were the main secondary factors. **Conclusion:** The incidence of non contact lens wearers complaining of dry eye in this investigation was relatively high (47%) but dominated by mild cases (~1 in 2). The best predictor of individual patients' dry eye status was symptomatology in air conditioned areas and the additional differentiating factors were problems while watching TV and blurred vision.

COMPUTATIONAL MODELING OF TEAR FILM DYNAMICS ON AN EYE-SHAPED DOMAIN. K.L. Maki,[1] R.J. Braun,[1] P. Ucciferro,[1] W. D. Henshaw[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 solve mathematical models for tear film dynamics on an eye-shaped domain. **Methods**: A mathematical model is derived for the thin tear film on a fully open eye-shaped domain. Boundary conditions are specified on the thickness and the flux of tear fluid through the boundary. The problem is solved numerically in the Overture framework developed at LLNL. We explore the effects of viscosity, surface tension, gravity and the boundary conditions. **Results**: The simulations recover features seen in one-dimensional simulations and capture some experimental observations of tear film dynamics around the lid margins. In some instances, the influx from the lacrimal gland splits with some fluid going along the upper lid towards the nasal canthus and some traveling around the temporal canthus and then along the lower lid [e.g., Harrison et al, OVS 85:706-714, 2008]. Tear supply can also push through some parts of the black line near the eyelid margins with difficulty. The flow around the menisci is consistent with a larger area of the meniscus providing less resistance to flow than the thin black line. [Maki et al, Journal of Fluid Mechanics, 647:361-390, 2010.] **Conclusions**: The model captures some aspects of tear film dynamics on the eye-shaped domain. Additional effects such as blinking (moving boundaries), evaporation, osmolarity and surfactant transport should be added in the future. [This work is supported by NSF 0616483 (Maki, Braun, Ucciferro, King-Smith). Commercial relationships: none.]-

THE EFFECT OF CONTACT LENS WEAR ON THE DIURNAL PROFILE OF MATRIX METALLOPROTEINASE-9 AND ITS INHIBITOR IN THE TEAR FILM. Maria Markoulli,[1,2] Eric Papas,[1,2] Nerida Cole,[1,2] Brien Holden.[1,2] [1] Brien Holden Vision Institute, Sydney, Australia [2] School of Optometry & Vision Science, University of New South Wales, Australia

Purpose: Matrix Metalloproteinases (MMPs) are a family of degrading enzymes whose function is to maintain and remodel tissue architecture. Increased levels of MMP-9 have been associated with recurrent corneal erosions as well as the epithelial defect which results in corneal ulceration. We have previously determined the diurnal variation of MMP-9 in the healthy tear film and with this study set out to determine how contact lens (CL) wear affects this profile in both the neophyte and after a period of adaptation. **Methods:** Tears were collected using a flush technique from 38 healthy neophytes before CL wear, during the first day of CL wear and after one month. Participants were randomised to wear either Acuvue Oasys® or O2Optix® and placed into either extended (EW) or daily wear (DW). Each time, tears were collected at midday, before sleep and upon waking and analyzed for concentrations of total protein, MMP-9 and its inhibitor, TIMP-1. Statistical analysis was performed using Repeated Measures Analysis of Variance. **Results:** Before CL wear, group mean MMP-9 levels were 11.6 ± 15.2ng/ml (mean ± SD), 8.2 ± 11.3ng/ml and 2,430.7 ± 1,972.7ng/ml for midday, before sleep and upon waking, respectively, the latter being significantly greater than the others (p<0.001). Upon initial CL wear, EW resulted in significantly elevated MMP-9 levels upon waking (5,747.9 ± 4,125.6ng/ml) compared to the same timepoint at baseline (p=0.03) whilst DW remained unchanged (2405.6 ± 2140.7ng/ml, p=0.11). After one month of EW, the levels upon waking were no longer different to baseline (2873.0 ± 1142.8ng/ml p=1.00). DW levels remained unchanged (p=0.11). The MMP:TIMP ratio during EW was greatest after the first night (24.4 ± 20.7) than both no wear (16.4 ± 16.4) and after one month (12.8 ± 7.2) but only the latter was significant (p=0.03). **Conclusions:** In the neophyte, EW appears to initially disturb the tear film homeostasis with a doubling of MMP-9 upon awakening compared to baseline and DW. These levels return to baseline after one month, suggesting adaptation. Commercial Relationships **and Grant Support:** The authors report no commercial relationships. This study was supported by the Australian Federal Government through the Australian Postgraduate Award and by a scholarship from the Brien Holden Vision Institute. This work was conducted while the first author was a recipient of a William C. Ezell Fellowship from the American Optometric Foundation.

TH17 PROMOTING ENVIRONMENT IN THE LACRIMAL GLAND OF THROMBOSPONDIN-1 DEFICIENT MICE WITH OCULAR SURFACE DISEASE. Sharmila Masli, Bruce Turpie. Schepens Eye Research Institute, Harvard Medical School, Boston, MA.

Objective: Deficiency of Thrombospondin-1 (TSP1) in mice leads to chronic lacrimal gland inflammation as seen in autoimmune Sjögren's syndrome and ocular surface disease. We evaluated if TSP-1 deficiency of the lacrimal glands creates environment supportive of Th17 cells implicated in chronic inflammation associated with autoimmune diseases. **Methods:** Lacrimal glands from WT and TSP1null mice (8 wk) were processed to generate frozen sections or lymphoid cells for immunohistochemical analysis to detect TSP-1 or macrophage markers F4/80, CD11b and MHC class II by flow cytometry. Expression of cytokines IL-6, TNF-alpha, IL-1beta was assessed by real-time PCR. Glandular apoptosis was assessed by Annexin-V staining and by detecting apoptotic cleavage product (120 kD) of structural protein a-fodrin by western blot. Expression of IL-23 was assessed in LPS-stimulated WT macrophages in the presence of TSP-1 derived peptide or control peptide. Protein released in culture supernatant detected by ELISA and message expressed by real-time PCR. **Results:** Immunoreactivity to anti-TSP-1 was detected in lacrimal glands of WT mice. Significantly increased infiltration of F4/80 and CD11b positive macrophages in TSP-1null lacrimal glands was detectable as compared to the WT glands. Macrophages from TSP-1null glands expressed increased MHC class II levels compared to WT controls supporting their activated state. Increased Annexin V staining and increased amounts of 120 kD apoptotic cleavage product of a-fodrin in TSP-1null glands compared to controls indicated increased apoptosis in the former than latter. Expression of Th17 promoting cytokine IL-6 was significantly increased in TSP-1null glands while that of TNF-alpha and IL-1beta remained comparable to that detected in WT glands. In *in vitro* experiments, significant inhibition of IL-23, a cytokine involved in

expansion of Th17, was detected at both protein and message levels, in WT macrophages cultured with TSP-1 derived peptides compared to control peptides. **Conclusions**: TSP-1 deficiency results in increased glandular apoptosis accompanied with increased presence of activated macrophages. The presence of IL-6 and absence of TSP-1 together provide Th17 promoting environment that is consistent with the chronic glandular inflammation and subsequent ocular surface disease.

INVESTIGATION OF TEAR FERNING IN NORMAL AND DRY EYES BEFORE AND AFTER USING ARTIFICIAL TEARS. Ali Masmali[1'2], Christine Purslow[1], Paul Murphy[1]. [1]School of Optometry & Vision Sciences, Cardiff University, Cardiff, United Kingdom [2]Optometry Department, School of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia

Objective: Tear ferning (TF) provides useful information about the gross composition and quality of tear fluid; as a clinical test it has previously demonstrated sensitivity and specificity for dry eye diagnosis. This study aimed to investigate TF patterns before and after artificial tears treatment in both normal and dry eye subjects. **Method:** 20 normal subjects (11M, 9F; 20-41 yrs) and 20 dry eye subjects (12M, 8F; 20-53 yrs) were classified according to McMonnies dry eye questionnaire and were monitored in the laboratory during one day. At enrolment (T0) 5µl of tear fluid was collected using a glass capillary. Three drops of artificial tears (TheraTearsâ, Advanced Vision Research) were then applied to both eyes. After 15 minutes (T15), another tear sample was collected. One hour later (T75) three more drops were applied, and after one further hour (T135), tear sampling was repeated. All samples were prepared and examined using a standardised protocol. TF patterns were graded according to an interpolated Rolando's scale where a higher score indicates dry eye. Differences between time intervals were analysed using repeated measures ANOVA and posthoc analysis, and independent t-tests between groups. **Result:** At baseline, TF grades in dry eye subjects were significantly higher (2.75 versus 1.53; $p<0.001$); at T135 grades were similar (2.10 versus 1.70; $p=0.05$). There was no significant difference in the mean TF grade over time from normal subjects ($p=0.245$), whilst a significant difference was observed between samples from dry eye subjects ($p=0.007$). In dry eye subjects the significant change occurred at T135 compared to T0 ($p<0.05$). **Conclusion:** TF patterns are significantly different in subjects identified with dry eyes by McMonnies. Significant improvements in TF pattern are observed after two applications of Theratears® in dry eye subjects. TF appears to be a useful, simple clinical test. No commercial sponsorship

EFFICACY AND SAFETY OF DIQUAFOSOL TETRASODIUM OPHTHALMIC SOLUTION IN DRY EYE PATIENTS: A PHASE 2 , RANDOMIZED, DOUBLE-MASKED, PLACEBO-CONTROLLED CLINICAL TRIA.L Yukihiro Matsumoto[1)], Yuichi Ohashi[2)], Hitoshi Watanabe[3)], Kazuo Tsubota[1)] 1) Department of Ophthalmology, Keio University School of Medicine 2) Department of Ophthalmology, Ehime University School of Medicine 3) Department of Ophthalmology, Kansai Rosai Hospital

Purpose: To investigate the efficacy and safety of diquafosol tetrasodium ophthalmic solution for the treatment of dry eye. **Methods**: Two hundred eighty six dry eye patients who agreed to receive the treatment randomly with topical 1% diquafosol (n=96), 3% diquafosol (n=96), and placebo (n=94) were recruited. Following placebo washout period for 2 weeks, the subjects were treated with the ophthalmic solution 6 times daily for 6 weeks. Fluorescein (FL) staining score, Rose-Bengal (RB) staining score, tear film break-up time (BUT), and subjective symptom were examined.**Results**: Topical 3% diquafosol improved significantly FL staining score compared to placebo at the week 4, primary efficacy time point ($p<0.05$). There was dose-response effect. Both topical 1% and 3% diquafosol improved significantly RB staining score compared to placebo ($p<0.05$). Subjective dry eye symptom was also significantly improved by both diquafosol ophthalmic solutions ($p<0.05$) in spite of no improvement in BUT compared to placebo . Any serious adverse events were not occurred through the period of this study. **Conclusion**: 3% diquafosol tetrasodium ophthalmic solutions as optimal concentration were considered to be effective and safe for the dry eye syndrome.

THE ROLE OF AQUEOUS TEAR EVAPORATION IN NORMALS AND PATIENTS WITH DRY EYE DISEASE. James P. McCulley, M.D., F.A.C.S, F.R.C. Ophth (U.K.) UT Southwestern Medical School

Purpose To assess the role of aqueous tear evaporation in normals and patients with either presumed hyposecretory or hyperevaporative dry eye disease. **Methods** Normal as well as those with aqueous deficient dry eyes (ADDE) with and without associated meibomian gland dysfunction (MGD) were evaluated clinically and had aqueous tear evaporative rates determined between relative humidities (RH) of 20-45% using an evaporameter. Rates were calculated individually between 20 and 25% as well as 40 and 45% RH. Additionally meibography was done on normals and both patient groups. **Results** The contribution of evaporation to aqueous tear loss in normals was 23.47% and 41.66% at RH of 40-45% and 20-25% respectively. The contribution in ADDE without MGD was 30.99% and 57.67% respectively and 25.44% and 50.28% in those with associated MGD. The contribution to aqueous tear loss by evaporation was statistically significantly greater in all groups at a relative humidity of 20-25% versus 40-45% and represented a 99.43% increase in aqueous tear loss to evaporation at the lower RH compared to the higher RH. There was a trend toward a greater contribution to aqueous tear loss in both dry eye patient groups compared to normals. There was a significant increase in meibomian gland drop out by meibomography in both groups of dry eye patients compared to normals but no difference between the groups. **Conclusions** Relative humidity influences significantly the rate of aqueous tears loss through evaporation with a 99% increase in contribution at a relative humidity of 20-25% compared to 40-45%. There was a trend toward aqueous tear evaporation being higher in both dry eye groups compared to normals. Evaporation contributes significantly to the loss of aqueous tears in normals and even more so in patients with aqueous deficient dry eyes. Dr. McCulley is a consultant for Alcon Labs, Inc.

HOW ARTIFICIAL TEAR PRODUCTS CAN MODIFY & RESTORE THE PHYSICAL/CHEMICAL CHARACTERISTICS OF THE HUMAN TEAR FILM. D. Meadows[1], H. Ketelson[1], Robert Baier[2], Gerald G. Fuller[3], Donald Korb[4], Tom Millar[5], Robert Pelton[6], [1]Alcon Research Inc, Ft. Worth, TX, [2]SUNY Buffalo, [3] Stanford University [4]Korb Associates, [5]Univ. of Western Sydney, [6]McMaster University.

Purpose: To characterize critical physical/chemical properties of human tear film constituents and determine how artificial tear products affect dynamics of tear film behavior in normal and dry eye patients. **Methods:** Key physical/chemical properties of the human tear film have been identified that play an important role in overall function: bulk viscosity at low shear, interfacial tension at water/air and water/lipid interfaces, electrostatic interactions, interfacial rheology of the lipid layer, temperature, humidity and pH. Changes in these properties can potentially affect several clinical indicators

commonly used to diagnose dry eye: FlBUT, NIBUT, pre-corneal retention and tear film interferrometry. Interfacial tension measurements were made using Wilhelmy plate, Langmuir trough and interfacial stress rheology methods. Iso-thermal titration, light scatter and electrophoretic mobility were used to study interactions between tear proteins, mucin, polymers, meibum lipids and divalent ions. Additional techniques used included Multiple Attenuated Internal Reflection (MAIR), Dynamic Light Scattering, Thin Film Ellipsometry and Small Angle X-ray Scattering (SAXS). Clinical studies in normal and dry eye patients evaluated the impact of artificial tear products on key indicators of tear film function. **Results:** Dramatic viscosity differences were observed for both the lipid films and the polymer systems. Interfacial measurements indicated that the lipid layer has a crystalline structure with lamellar packing ~49Å, roughly the length of the molecules. This structuring is very temperature and composition dependent. Clinical studies showed that interferrometry patterns, elimination rates, and breakup times can be strongly affected by the type of lipid and polymer system used in artificial tear solutions. **Conclusions:** Physical/chemical characterization of tear film constituents confirmed that interactions between mucin, protein, lipids and ions are important in establishing a stable tear film. Dramatic changes in one or more of these interactions could have a number of clinical implications leading to poor tear film quality.

NONCLINICAL PHARMACOLOGY, OCULAR DISTRIBUTION, AND SAFETY OF MIM-D3, A NOVEL NGF MIMETIC FOR THE TREATMENT OF DRY EYE. Karen Meerovitch, Teresa Lama and Garth Cumberlidge. Mimetogen Pharmaceuticals Inc. Montreal, Quebec, Canada

Purpose: To evaluate the pharmacology, ocular distribution, and safety of MIM-D3. MIM-D3 is a small molecule NGF peptidomimetic, which has potential to increase conjunctival and tear glycoconjugate secretion *in vitro and in vivo*. **Methods**: *(a)* Scopolamine-treated rats were dosed daily with vehicle, 0.4%, 1% or 2.5% MIM-D3 for 17 days. Tear breakup time (tBUT), corneal staining and tear glycoconjugate levels were evaluated during for 28-days. *(b)* Rabbits received topical doses of vehicle, 0.5%, 1% or 2% MIM-D3 four times/day for 7-days. On day 8, the aqueous humor, conjunctiva, cornea, iris, and eyelid were collected for MIM-D3 analysis. *(c)* Male and female rabbits and dogs were dosed topically with vehicle, 1%, 3% or 5% MIM-D3 eight times/day for 28-days. Biomicroscopy (McDonald- Shadduck) and dilated ophthalmoscopy were evaluated predose and at the end of each week. Gross ocular observations for irritation (Draize scoring scale) were monitored twice daily. Ocular tissues were evaluated by histopathology. **Results**: *(a)* Dry eye conditions were established 5 days after subcutaneous implantation of a
scopolamine pump in rats. Daily topical instillation of 1% MIM-D3 (days 5-21) produced a significant decrease in corneal staining, increase in tBUT and tear glycoconjugates on day 28. *(b)* MIM-D3 was detected in relatively high concentrations in the rabbit eyelid and conjunctiva, low concentrations in the cornea and minimal detection in the aqueous humor and iris. *(c)* No signs of ocular toxicity were observed in rabbits and dogs in any MIM-D3 dose groups. There was a dose-dependent increase in discharge noted in dogs, but not rabbits, dosed ^{3}3% MIM-D3 after the 8th daily dose. There was mild congestion and chemosis, without any correlating histopathology. **Conclusions**: MIM-D3 improved clinical signs of dry eye in an experimental rat model. Therapeutic concentrations of MIM-D3 were achieved in the rabbit conjunctiva. MIM-D3 exhibited an excellent ocular safety profile in rabbits and dogs, which supports clinical development as a topical agent for the potential treatment of dry eye.

SEX DIFFERENCE IN INNATE ANTI MICROBIAL FACTORS IN RAT LACRIMAL GLAND. Lilian Eslaine Costa Mendes da Silva, Ana Carolina Dias, Carolina Maria Módulo, Stella Felippe de Freitas, a, Leonardo Tannus Malki, Eduardo Melani Rocha Department of phthalmology, Faculty of Medicine of Ribeirão Preto, USP, Ribeirão Preto, Brazil

Introduction: Several proteins are part of the innate anti microbial defense and are being used as biomarkers of exocrine gland function. However, their sexual differences and the expression of different peroxidases remain unclear . The aims of this study are 1) to compare the expression of the factors involved in innate anti microbial defense and 2) to identify and distinguish the members of peroxidase superfamily in rat lacrimal gland.
Methods: Male and female Wistar rats lacrimal glands (LG) were homogenized and evaluated by Western blot to compare the expression of glutathione peroxidase-3 (GPO3), lactoperoxidase (LPO) and lactoferrin . Total peroxidase activity was also compared among the groups. **Results**: western blot analysis had shown that GPO3, LPO and lactoferrin proteins were expressed in LG. GPO3 expression was significantly reduced in LG of female compared to male ($P<0.05$), however LPO and lactoferrin were significantly higher in LG of female rats ($P<0.05$). Total peroxidase activity was 0.29±0.03 mU/ml in male and 0.98±0.04 mU/ml in female rat lacrimal gland ($P=0.0022$). **Conclusions**: The present work shows that LPO and lactoferrin, proteins related to innate anti microbial defense are higher in LG of female rats. Those data suggest that innate defense against external agents is more intense in female, however the protective capacity against internal damage is higher in male rats. Moreover, it suggests that peroxidase activity, as measured by enzymatic assays express better the secretory function rather than antioxidant capacity. Financial support: CNPq, FAPESP, FAEPA

EVALUATING BIOCHEMICAL PATHWAYS IN THE MEIBOMIAN GLAND Thomas J. Millar[1] and Frank Schirra[2] School of Natural Sciences, University of Western Sydney, Australia,[1] Klinik für Augenheilkunde, Universitätsklinikum des Saarlandes, Homburg/Saar, Deutschland[2]

Purpose:The synthesis of extremely long fatty acids and fatty alcohols by the meibomian gland requires enormous amounts of energy in the form of NAD(P)H, and a source of carbons. Given that the acinar cells are progressively displaced from their scanty blood supply, easy access to carbon sources and oxygen for energy is not apparent. Our studies explore possible biochemical pathways in the meibomian gland that could account for this conundrum. **Methods**:Meibomian glands were stimulated by systemic testosterone administration in orchiectomised male mice. Differential gene expression in the meibomian glands was examined using microarray hybridization. Data mining based on gene ontology data and visualization within pathways of the Kyoto Encyclopedia of Genes and Genomes (KEGG) was used to highlight biochemical pathways most affected. **Results**:Our studies demonstrate concurrent down regulation of aldolase and fructose bisphosphatase 1 which serve opposite roles (a futile cycle) in glycolysis and gluconeogenesis. This indicates driving of carbons from glucose-6-phosphate into the pentose phosphate pathway (NADPH synthesis), and carbons from glycerolphosphate into pyruvate (carbons for fatty acid synthesis). In addition, there were multiple indicators of -oxidation (fat breakdown) occurring which is enigmatic in a lipid synthesizing gland. **Conclusions**.Our results suggest that phospholipids comprising the multiple membranes in the immature acinar cells are the source of both energy and carbons for the synthesis of extremely long chain fatty acids reported in meibomian gland secretions. The lipophilic nature of the enzymes involved suggests that this process could continue after the cells had died. [This research was supported

by NIH grant EY05612, the German Research Society DFG SCHI 562/1-1 and 1-2, and the Australian Government linkage project scheme #LP0776482,]

INCREASING THE BLINKING RATE USING THE "PISC" DEVICE FOR PATIENTS WITH EVAPORATIVE DRY EYE. Danielle L. Miura, Rossen M. Hazarbassanov, Camila K.N. Yamasato, Jose A.P. Gomes. Department in Ophthalmology and Visual Science, Federal University of Sao Paulo, Sao Paulo, SP, Brazil

Purpose: PISC is composed by a micro electronic controller circuit and a microprocessor. It has a frequency controlling crystal and a sensor that emits red and green luminous signs on the same rate as the human blinking frequency (6-7 seconds intervals). Our purpose was to evaluate the effectiveness of PISC as an adjunct treatment for Evaporative Dry Eye (EDE).**Methods**: 20 patients with EDE and 20 controls were enrolled in the study. A webcam was positioned on front of the patient's face, at 1 meter distance. The blinking rate and clinical tests for dry eye were evaluated on two randomized visits, differing by PISC and air conditioning (Ac) presence or absence. The conditions were named: A, without PISC, without Ac; B, without PISC, with Ac; C, with PISC, without Ac; D, with PISC, with Ac. Patients were recorded for 10 minutes per session, with the task of reading a text. Subjects had the following tests performed: Ocular Surface Disease Index (OSDI), patient symptomatology questionnaire, visual acuity (VA), biomicroscopy, Schirmer test I without anesthesia, tear film osmolarity, fluorescein break up time (FBUT) and staining with fluorescein and lissamine green 1% (Oxford grading). **Results**: There was statistical increase of blinking rate (one way ANOVA, p=0.005) when PISC was used with and without Ac for the EDE group only. The following analyses maintained high correlation between their values before and after PISC application: FBUT (A: r = 0.959, P <0.0001; B: r = 0.964, P < 0.0001; C: r = 0.956, P < 0.0001; D: r=0.986, P< 0.0001), fluorescein (A: r = 0.623, P =0.0044; B: r = 0.867, P < 0.0001; C: r = 0.975, P < 0.0001; D: r=0.7196, P= 0.0005) and lissamine green staining (A: r = 0.667, P =0.0018; B: r = 0.824, P < 0.0001; C: r = 0.8408, P < 0.0001; D: r=0.848, p< 0.0001), for the EDE group. **Conclusion**: PISC device increased blinking frequency in patients with EDE and was capable to reduce loss of lubrification, represented by no change in ophthalmological exam values.[Authors have no financial interest]

WHAT STAINS WITH FLUORESCEIN IN PUNCTATE EPITHELIAL EROSIONS? Maryam Mokhtarzadeh[1], Richard Casey[1], Ben J. Glasgow[1]. [1]Jules Stein Eye Institute, University of California Los Angeles

Purpose: The cellular basis of the fluorescein associated superficial punctate pattern in dry eye disease is controversial. Explanations include intercellular trapping of fluorescein, intracellular staining in dead cells, and pooling over surface irregularities. The hypothesis is tested that
punctate "erosions" are individual cells with enhanced fluorescence. **Methods:** Cytologic Correlation of Punctate Spots: Ten impression cytology membrane materials were compared to obtain optimal cellular yield in buccal mucosa and cornea (analyzed by Student's t-test). Removal of fluorescent spots by impression cytology was investigated in four dry eye patients (DEWS criteria) with punctate staining. Individual punctate spots were localized by fiducials in photographs before and after impression cytology and correlated in fluorescence microscopy and cytologic stains. Punctate spots were correlated with cells removed by the membrane using a two way contingency table analysis. Histopathologic Correlation of Punctate Spots: Nine corneas from dry eye patients received corneal transplants for concurrent diseases. Punctate fluorescence, photographed preoperatively, was localized within each cornea specimen aided by fiducials and epifluorescence. After nuclear staining, confocal microscopy precisely localized the fluorescent spots in layers of the cornea. **Results:** Cellular yield was greatest with Teflon (Biopore) impression cytology (p=0.019, buccal mucosa). Punctate fluorescent spots that disappeared after impression cytology (71%) correlated significantly with cells on the impression membranes (p=0.009). Confocal microscopy confirmed that punctate fluorescence correlated with cells in the superficial epithelial layer (47%) and immediately subjacent two wing cell layers (53%) but not in the basal layer (0%). **Conclusions:** Punctate epithelial "erosions" correspond to enhanced fluorescence in cells predominantly in the superficial layers of the cornea and would be more aptly named fluorescent epithelial cells (FLECs). This research was supported by NIH EY11224, The Edith and Lew Wasserman Endowed Professorship in Ophthalmology, and NIH EY007026.

MODIFICATION OF THE TEAR FILM OSMOLARITYWITH THE USE OF CONTACT LENSES IN OMAFILCONA AND METHAFILCONA MATERIALS. Montani Giancarlo Institutions: University Of Salento Formazione Continua In Medicina, Lecce, Italy

Objective: The purpose is to evaluate the effect of contact lenses (CL) in different materials on tear film osmolarity (TFO) in a group of patients with moderate dry eyes. **Method:** We selected 50 patients between 18 and 42 years of age with no disease of anterior segment, no prior use of CL and no contraindication for CL use. The refractive error was similar in both eyes and included between -1,00 dt and -4,00 dt to maintain a similar thickness of contact lenses used. We measured the TFO using the TearLAb™ and the patients that were selected had a TFO between 308,2 mOsm/L and 328,2 mOsm/L, which represents an interval that can be considered associated with marginal dry eyes with no significant difference in both eyes. Afterward we fitted the right eye of all patients with a lens in Omafilcon A material (Hema + PC) hydration 60 % and the left eye with a lens in Methafilcon A (Hema +MMA/EGDMA) hydration 55% . After 7 hours of wear, before removing the lenses, we measured the TFO and the comfort using a NRS divided in ten steps from 0 (not tolerable) to 10 (excellent comfort) in both eyes. **Result:** The average of TFO in RE before CL fitting was 323 mOsm/L (SD±4,3) and 325 mOsm/L (SD±13.3) with the Omafilcon A CL with a not significant difference between the measures for the t test (p=0.17). In the LE the average TFO before CL fitting was 323 mOsm/L (SD±4) and 340 mOsm/L (SD±15.64) after the use of Methafilcon A CL with a significant difference between the measures for the t test (p<0.001). A low correlation was found between TFO and comfort of CL used r^2=0,178 for Omafilcon A lenses and r^2=0,16 for Metahfilcon A lenses. **Conclusion:** The results indicate that lenses in different materials can have a different impact on the TFO. Since a low correlation was found between comfort and TFO, one can think that its increase is not the major factor that determines a CL wearer's discomfort. In conclusion the measurement of TFO can help clinicians to identify materials that increase TFO least.

QUANTITATIVE ANALYSIS OF CORNEAL STAINING. Montani Giancarlo, Romano Francesco Institutions: Università Del Salento Formazione Continua In Medicina, Lecce, Italy

Objective: The aim of the work is to propose a new classification for the staining type and extent and to present a new software developed by Innovative Solution for Eyes (ISE) specifically designed for corneal staining assessment. **Method**: In order to obtain an objective analysis

of the staining, the contribution of different staining was differentiated by means of the following classification: micropunctate area≤ 0.002 mm^2, macropunctates 0.002 ≤ area ≤ 0.01 mm^2, coalescent macropunctates 0.01 ≤ area ≤ 0.3 mm, patch area > 0.3 mm^2. The software employ the five-zone model and determine the exact area of cornea involved by staining (both the absolute value in $mm^2$and the relative value in term of percentage of area) in each zone and for each staining type. To validate the software 20 images of the corneal staining were considered. The images were acquired with direct illumination and 16X magnification after the instillation of sodium fluorescein, by the slit lamp, Topcon™ SLD7 including digital units DV3 and yellow filter Wratten#12. The images were analysed by four trained Eye care practitioner using the CCLRU type and extent scales and the evaluations were compared with the results obtained by the software analysis. **Result**: The use of the analyzing and processing images program allowed obtaining an accurate determination of the area of cornea involved by staining, The analysis allowed to measure the number of the staining and to distinguish the contribute of the single staining type to the total area. Starting from the quantitative analysis a new classification for the type and extent was proposed. The type was defined calculating the average of the staining type weighted by its area percentage and the extent was defined by an exponential function.The quantitative analysis allows exactly evaluating the staining extent also when very limited area is involved, while CCLRU scale is insensitive and useless when small extent must be evaluating. **Conclusion:** Good agreement was found between the CCLRU type and extent scale and the value calculated from this new quantitative method. The quantitative analysis was successful applied to study the behaviour of corneal staining.

LUBRICIN AS AN OCULAR SURFACE – CONTACT LENS BOUNDARY LUBRICANT: DOSE-DEPENDENT & SYNERGISTIC EFFECTS. S. Morrison[1], B. Snider[1], B.D. Sullivan[2], E. Truitt III[3], D.A. Sullivan[4], T. Schmidt[1] [1] University of Calgary, Calgary, Canada; [2] TearLab Corp., San Diego, CA; [3] Singularis, Inc., San Diego, CA; [4] Schepens Eye Research Institute and Harvard Medical School, Boston, MA.

Objective: Epithelial cells at the ocular surface are subject to significant shear forces generated during eyelid blinking and contact lens wear, especially in the presence of a compromised tear film. Given that ocular surface cells express Lubricin mRNA, a cartilage boundary lubricant, the objectives of this study were to 1) assess the boundary lubricating ability of Lubricin at a cornea-contact lens biointerface and 2) determine if Lubricin protein is present at the ocular surface. **Methods**: Human corneas and silicone hydrogel contact lens material were mounted on a biomechanical testing machine, forming a cornea-contact lens biointerface. Sample surfaces were articulated against each other at effective sliding velocities ranging from 0.3–30 mm/s under physiological loads of 15-20 kPa. Samples (total n=10) were tested serially in A) Lubricin @ 30, 100, 300 ug/ml; B) Aquify (CIBA Vision), Lubricin @ 300 ug/ml, then Aquify+Lubricin; or C) Systane (Alcon), Lubricin, then Aquify+Lubricin. SterilePlus Saline (Bausch & Lomb) was a negative control, and both static and kinetic friction coefficients were calculated. Extract from fresh porcine corneas was assessed for Lubricin by SDS-PAGE western blotting. **Results**: Lubricin functioned as an effective friction-lowering boundary lubricant at all sliding velocities. Kinetic friction values were greatest in Saline (0.34±0.02, mean±sem), decreased with increasing Lubricin concentrations, and were lowest in Aquify+Lubricin (0.15±0.01). Values in Lubricin @ 300 ug/ml (0.20±0.01) were lower than those in Aquify (0.35±0.05) and Systane (0.23±0.03). Western blotting revealed a high molecular weight Lubricin protein band in the porcine cornea extract. **Conclusion**: Lubricin protein exists at the ocular surface and functions as a dose-dependent boundary lubricant to reduce friction, alone and in combination with hyaluronan, better than currently available eye drops in this test model. Sullivan, Truitt, Sullivan & Schmidt: Singularis Co-Founders.

TEAR FLUID REGULATION OF GENE EXPRESSION IN CORNEAL EPITHELIAL CELLS. J. Mun[1], C. Tam1, D. Evans[1,2] and S. Fleiszig[1]. UC Berkeley, CA[1]. Touro University- CA[2].

Purpose: We previously showed that pre-exposing corneal epithelial cells to human tear fluid protects them against *P. aeruginosa* (PA) virulence strategies. Here, we explored how tear preexposure impacts cellular gene expression. **Methods**: RNA was extracted from human corneal epithelial cell cultures: 1) after incubation with human tears (or media) for 6 h, or 2) after incubation with tears (or media) for 16 h, then for 3 h with PA antigens. RNA was also extracted from mouse corneal epithelia *in vivo* (therefore tear pre-exposed) after the healthy surface was exposed to PA or media (6 h). First strand cDNA was synthesized. Generated fragmented cRNA was applied to Affymetrix GeneChip Human Genome U133 Plus 2.0 or Mouse Genome 430 2.0 Arrays or used for semi-quantitative RT-PCR. Genes up- or down-regulated by ~5- fold (human) or 3-fold (mouse) were grouped by function using Onto-Express. **Results**: Tears upregulated 209 genes and downregulated 40 genes in human corneal epithelial cells. Tear pre-exposure changed the cells' response to PA with 184 additional up- and 106 down-regulated genes. Upregulated genes encoded transcriptional regulators, tight-junction proteins, cytokines and receptors, proteases/inhibitors, microRNA and antimicrobials. With or without subsequent bacterial exposure tears increased expression of ST2 (an immunomodulator) 12- to 113-fold, and RNase7 (antimicrobial found in other tissues) 18- to 69-fold. RT-PCR confirmed ST-2 (2-fold) and RNase7 (4-fold) upregulation. Genes downregulated included Ran/Ras and hypoxia- and in bacterial exposed cells, cell cycle-related proteins. In mice *in vivo*, bacteria upregulated 31 and downregulated 14 genes, including transcriptional regulators, structural proteins, transporters and potential antimicrobials. **Conclusions**: Tear fluid modulates gene expression in corneal epithelial cells with or without subsequent bacterial challenge. Understanding how tears modulate cell responses to microbes *in vitro* and *in vivo* will help elucidate mechanisms involved in resistance to infection, and how they may be compromised to allow susceptibility. [Support: NIH EY11221, The Bill and Melinda Gates Foundation].

A GENETIC ASSOCIATION OF IL 6 AND IL 6R GENES IN KOREAN DRY EYE PATIENTS. Kyung-Sun Na,[1, 2, 3] Jee-Won Mok,[1,2] Choun-Ki Joo.[1, 2, 3]Laboratory of Ophthalmology and Visual Science, The Catholic University of Korea, [1]Korea Eye Tissue and Gene Bank, [2]Department of Ophthalmology and Visual Science, St. Mary's Hospital[3]Seoul, Korea

Purpose. To determinethe possibility of inflammation related genes, interleukin 6 (IL6) and interleukin 6 receptor (IL6) genes, as potential susceptibility candidate gene for Korean patients with dry eye, we investigated the association of the IL6 and IL6R polymorphisms in unrelated Korean patients with dry eye. **Methods**. Genomic DNA was extracted from blood samples of unrelated Dry eye patients with two symptoms of non-Sjogren's disease and Sjogren's disease, visited the Department of Ophthalmology at the Catholic University Medical Center. To screen genetic variations in rs1800795 of IL6 promoter region and Asp358Ala (rs8192284) of IL6R were performed using polymerase chain reaction, restriction fragment length polymorphism and direct sequencing. Control individuals were selected from the general population without dry eye. **Results**. In this study, we investigated rs1800795 of IL6 and rs8192284 of IL6R in Korean patients with dry eye.Genotypic and allelic distribution of the rs8192284 of IL6R was significantly

different between dry eye patients with non-Sjogren's symptom and controls; C alleleof dry eye patients was significantly difference compared with control subjects (p = 0.045, O.R. = 1.902). And also, Sjogren's disease patients had significantly higher C allele frequency, C allele had significantly higher than controls was significantly difference compared with control subjects (p<0.001, O.R.=2.959). But there were no statistically significant differences in the allele and genotype frequencies of rs1800795 of IL6 promoter region betweendry eye patients and controls. The genotype distributions of all polymorphisms of IL6 and IL6R among the control subjects and the affected individuals were in Hardy-Weinberg equilibrium. **Conclusions**. This is the first report of IL6 and IL6R gene variations screening in Korean dry eye patients. Significant differences in allelic frequencyin rs8192284 of the IL6R gene between dry eye, which is non-Sjogren's and Sjogren's diseases, and the control group suggest that IL6R polymorphisms may play a role in the susceptibility of unrelated Korean to develop dry eye, particularly, Sjogren's disease. [This research was supported by grants of Korea Health Industry Development Institute]

MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) PROMOTES *P. AERUGINOSA*-INDUCED OCULAR KERATITIS. Jill Nagashima[1], Tanweer Zaidi[1], Robert A. Mitchell[2], Gerald B. Pier[1], and Mihaela Gadjeva[1] Channing Laboratory[1], Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115; James Graham Brown Cancer Center, University of Louisville[2], Louisville, Kentucky 40202

Purpose*: Pseudomonas aeruginosa* causes severe sight-threatening corneal infections, with the inflammatory response to the pathogen being the major factor resulting in damage to the cornea that leads to loss of visual acuity. Here, we investigated the contribution of a host innate immunity factor – MIF – to regulate sensitivity to *P. aeruginosa* infection. **Methods**: Mice-sufficient for MIF and mice-deficient for MIF were compared for susceptibility to *P. aeruginosa*-induced ocular infection. **Results**: We found that mice-deficient for MIF had significantly reduced consequences from *P. aeruginosa*-induced acute keratitis. The improvement in the outcome was manifested as improved bacterial clearance, decreased neutrophil infiltration, and decreased inflammatory responses. MIF regulated the epithelial cell responses to infection by promoting bacterial invasion of corneal epithelial cells, a correlate of virulence in the keratitis model, and by enhancing synthesis of proinflammatory mediators in response to *P. aeruginosa* infection. MIF stimulated the formation of caveolin enriched lipid raft structures in the corneal epithelial cells that are known to facilitate bacterial invasion. **Conclusion**: Lack of MIF or inhibition of MIF reduced bacterial invasion, therefore restricting bacterial presence to the extracellular compartment. This made *P. aeruginosa* more amenable to clearance by PMN, particularly in the 24-48 hr post-infection period, hence PMN number and bacterial numbers in MIF KO mice were lower. Our results suggest that inhibition of MIF during infection may have a beneficial therapeutic effect. This work was supported by Grant-in-Aid (Fight for Sight) and R21 EY019944-01 to MG. Dr. R. A. Mitchell holds a patent on the use of 4-IPP and its derivatives.

COMPARISON OF DRY EYE PATIENT SIGNS IN ENVIRONMENTAL CONDITIONS TO STATIC AND MOBILE CONTROLLED ADVERSE ENVIRONMENT(CAE) MODELS. Joel Naor[1], Donna Welch[1], Gail Torkildsen[2], George W. Ousler III[1]1Ora, Inc, Andover, MA; [2]Andover Eye Associates, Andover, MA

Objective: The Controlled Adverse Environment (CAE) model was designed to exacerbate the signs and symptoms of dry eye in a controlled and reproducible manner. The specifications of the CAE were designed to emulate real-world environmental conditions with drying effects. The objective of this study was to compare the appearance of clinical signs of patients challenged in the CAE (both static and mobile units) to those experiencing everyday environmental exposure. **Method:** Thirty subjects diagnosed with dry eye were evaluated following exposure to the static CAE, mobile CAE, and natural environment. Subjects underwent clinical assessments using a photo slit-lamp, questionnaire completion, and several dry eye diagnostic tests. A panel of clinicians evaluated the photographs for distinguishable patterns of clinical signs (ocular surface staining, conjunctival redness, tear film break-up) between populations. **Result:** Subjects emerging from CAE exposure demonstrated a worsening of clinical signs. When compared across static CAE, mobile CAE, and environmental exposures, no significant between-group distinction was evident in clinician panel results. **Conclusion:** Exposure to the CAE demonstrated signs and symptoms consistent with those of patients with natural environmental exposures. These data provide critical validation of the CAE by demonstrating accurate duplication of real-world disease manifestation through clinical modeling.

LIPCOF IN THE DIAGNOSIS OF DRY EYE - MULTICENTER STUDY. Janos Nemeth, [1] Eszter Fodor, [1] Andras Berta, [2] Tímea Komar, [2] Igor Petricek, [3] Mohamed Higazy, [4] Pavel Nemec, [5] Marek Prost, [6] Galina Semak, [7] Hristina Grupcheva, [8] Ozlem Evren, [9] Petra Schollmayer, [10] Ameed Samaha, [11] Katarina Hlavackova. [12] Dept of Ophthalmology Semmelweis University Budapest, Hungary, [1] Debrecen, Hungary, [2] Croatia, [3] Egypt, [4] Czech Republic, [5] Poland, [6] Belarus, [7] Bulgaria, [8] Turkey, [9] Slovenia, [10] Lebanon, [11] Slovakia[12]

Purpose: The main aim of the study was to analyse the clinical application of lid parallel conjunctival folds (LIPCOF) as a diagnostic test for dry eye and compare the LIPCOF grade to other clinical test results and subjective symptoms. **Methods:** In 12 centers in 11 countries altogether 272 eyes of 272 dry eye patients (75 male and 197 female) were examined. Their age was on average 52.7 +/- 16.2 years (min: 17, max: 89 years). The LIPCOF was examined and graded according to the method of Höh. Tear film break up time (BUT), fluorescein staining and Schirmer test without anesthesia were measured. The subjective symptoms were detected using a written 16-item-questionnaire. **Results:** The LIPCOF grade showed significant positive correlations with the following parameters: age, severity, fluorescein staining (r>0.2; p<0.001), and inverse correlations with BUT, Schirmer test (r>0.2; p<0.000). The LIPCOF grade exhibited significant correlations with subjective symptoms (r=0.250, p<0.001). The sensitivity and specificity of LIPCOF were the best with the cut-off value of 1.5 between normal and dry eye. **Conclusions:** For the diagnosis of dry eye disease, the LIPCOF test showed a medium sensitivity and specificity with good positive and medium negative predictive value. These results support the use of LIPCOF as a screening test. It is a simple, quick and noninvasive method, which might be part of the routine ophthalmological examination protocol.

CONJUNCTIVAL FOLDS: SIGN OF AGE OR SIGNS OF LACRIMAL TEARS DYSFUNCTION. Johannes Nepp Ophthalmological Department, Medical University Vienna

Purpose: Folds of the conjunctiva (CF) are seen in patients with dry eyes. The unclear aetiology was discussed , if there exists a relation of these folds with dry eyes or if they are only signs of aging. Höh described a staging of folds in relation with severity of dry eyes. In a clinical study we wanted to evaluate correlations of these folds with subjective complaints, age and dryness. **Method**: In a retrospective

study dry eye patients were observed. 234 patients between 2003 and 2009 were included. Their mean age was 57 years. Observation included lid folds (4 stages), Schirmer's test, BUT, Lipid layer thickness (LIT) and lissamin-green staining. The epithel was observed in impression cytology, rated with 11 points maximum A scoring system (sicca-score; ssc) was used to compare all facts by Pearson correlation. **Results**: LF was 1,98 (1-4)average; the impression cytology 0,265 (Score 0-1), the sicca-score SSC 0,424 (average). There was no significant correlation of age and CF! (R23, p>0,05) The relationship of BUT and Lissamin green staining to LF was significant (R56/53) Destructions of intercellular connections were correlated to LF (R58) but not to the age (R: 19) **Conclusion**: Conjunctival folds are symptoms of epithelial intercellular destruction. They occur in some elder patients, but they are not an oblige function of age. The dysfunction of mucin with loss of the tear film stability may be causative.

TEAR FILM & OCULAR SURFACE SOCIETY: A REPORT FROM THE INTERNATIONAL WORKSHOP ON MEIBOMIAN GLAND DYSFUNCTION. Nichols KK, Nelson JD, Knop E, Green-Church K, Schaumberg DA, Tomlinson A, Geerling G and Asbell PA, on behalf of the International Workshop on Meibomian Gland Dysfunction members.

Purpose: Meibomian gland dysfunction (MGD) may well be the leading cause of dry eye disease throughout the world. However, although this condition influences the health and well being of millions of people, there is no global consensus on the definition, classification, diagnosis or therapy of MGD. To achieve such a consensus, the Tear Film & Ocular Surface Society (TFOS; http://www.TearFilm.org), a non-profit organization, launched the International Workshop on Meibomian Gland Dysfunction (www.tearfilm.org/mgdworkshop/index.html). The objectives of the MGD Workshop were to: (1) conduct an evidence-based evaluation of meibomian gland structure and function in health and disease; (2) develop a contemporary understanding of the definition and classification of MGD; (3) assess methods of diagnosis, evaluation and grading of severity of MGD; (4) develop appropriate norms of clinical trial design to evaluate pharmaceutical interventions for the treatment of MGD; (5) develop recommendations for the management and therapy of MGD; and (6) create an executive summary of recommendations for future research in MGD. **Methods:** The Workshop, which required more than 1.5 years to complete and finalized its report in mid-2010, involved the efforts of 50 leading clinical and basic research experts from around the world. These people were assigned to 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 MGD Workshop.

INTERNATIONAL DRY EYE WORKSHOP: UPDATE ON THE EPIDEMIOLOGY OF DRY EYE. Kelly K. Nichols, OD, MPH, PhD Ohio State University College of Optometry, Columbus, OH, USA

Objective: In 2007, the Report of the International Dry Eye Workshop was published. At the time, there had been significant advances in the understanding of the prevalence of dry eye, as well as the factors associated with the disease. Recent developments in the epidemiology of dry eye, including prevalence and associated factors are reviewed.**Results and Discussion**: Symptom-based definitions of dry eye have widely been used in population-based studies, including the Schaumberg criteria, the Schein criteria, and the short form CLDEQ. Over the past three years, there have been new population-based studies of dry eye disease across the world. The assessment of symptoms remains a challenge in clinical trials, and several survey instruments have been utilized in clinical trials and are reviewed including the OSDI, McMonnies Questionnaire, and the IDEEL survey, although achieving statistically significant changes in symptomatology over time or with treatment using existing techniques remains elusive. With the current interest in evaporative dry eye and meibomian gland dysfunction, further validation of symptom-based definitions against clinical diagnostic tests is warranted as blepharitis and dry eye are often co-morbid. **Conclusion**: Dry eye continues to be a very prevalent worldwide disease with a significant impact on quality of life.

COMPARISON OF MASS SPECTROMETRY LIPID PROFILES USING VISUAL AND COMPUTER-BASED TECHNIQUES. Kelly K. Nichols, OD, MPH, PhD;[1] Jianzhong Chen, PhD,[2] Kari B. Green-Church, PhD[2] College of Optometry;[1] Mass Spectrometry and Proteomics Facility;[2] The Ohio State University, Columbus, OH, USA

Objective: Unlike proteomic analysis where large protein data bases and sophisticated computer software programs are routinely used to compare human samples, identification and comparison of lipids remains laborious and is largely qualitative rather than quantitative. Existing bioinformatic software packages and techniques for comparison of lipids are explored. **Methods**: Electrospray time-of-flight mass spectrometry (ESI-MS) analysis (Waters, Q-TOF II) was performed on meibum samples collected from each of three groups: postmenopausal women with dry eye (n = 5), postmenopausal women without dry eye (n = 5), and patients with contact lens-related dry eye (n = 5). Visual comparison techniques, such as spectral "envelope" comparison and the presence/ absence of specific lipid peaks, are compared to existing software packages that perform principle component analysis, including Nonlinear Dynamics Progenesis MALDI software and Origin Lab. **Results**: Direct infusion ESI-MS provides hundreds of lipid peaks for comparison in both positive and negative ion mode, consistent with previous reports. Visual comparison of lipid profiles from normal, dry eye, and contact lens dry eye samples demonstrates similar spectral "envelopes" between the three groups. When the presence/ absence of specific lipid peaks are compared, minor differences within groups as well as between groups appear to be present. Initial analysis using the Nonlinear Dynamics Progenesis MALDI software responded poorly to the direct infusion ESI-MS data. One possible reason is that raw data conversion resulted in a reduction or "smoothing" of the rich spectral information in the original spectra and overall differences in the group spectra were not identified. **Conclusion**: At this time, existing software to compare lipid profiles is limited to the newest mass spectrometry instrumentation, and is not widely available. Visual comparison techniques, while valuable, are likely not sensitive enough to detect differences between spectra. Even with newer instrumentation, the software packages may not be optimized to compare the unique lipid profiles from meibum. New computer programming, perhaps specific to meibum, is needed. Disclosures: Grant funding NIH R01 EY015519, KN (C)

THE EFFECT OF RIGID GAS PERMEABLE AND SOFT CONTACT LENS WEAR ON OCULAR SURFACE TEMPERATURE. Sachiko Nishimura[1, 2], Paul J Murphy[1], Christine Purslow[1] [1]CardiffUniversity, School of Optometry and Vision Sciences, Cardiff, UK, [2]Menicon, Japan

Objective:Modern contact lenses exhibit excellent oxygen permeability but complications due to infection persist, especially in

soft contact lenses. Tear exchange beneath the lens varies between lens types and may be an important factor in infection. This study investigated the use of non-invasive ocular thermography to monitor temperature across the ocular surface when contact lenses are applied. **Methods**:10 healthy experienced contact lens wearers (3M, 7F; age 27.3±4.0 yrs) were randomly fitted with rigid gas permeable (RGP; Menicon Z-, Menicon) and soft hydrogel contact lenses (SCL; 1 day Acuvue, Johnson and Johnson, US) for one hour, at two sessions. Ocular surface temperature (OST) was recorded (FLIR A40, FLIR) at 2 and 60 minutes after lens application. The thermal information at each time point was analyzed specifically on the peripheral area of the contact lens (CLP) and the area beyond the lens edge (CLO). Average temperature differences between CLP and CLO for lens types were compared using paired t-tests. **Result**:With RGP contact lenses, the temperature of both the ocular surface and the contact lens significantly decreased over one hour of lens wear (-0.93±0.42°C and -1.04±0.54°C, respectively; p<0.001). However, with SCL wear mean temperature measured on the ocular and contact lens surfaces remained similar between lens application and one hour of lens wear (+0.06±0.68°C and +0.18±0.68°C respectively, 0.429<0.770). After one hour of contact lens wear, the disparity in lens and ocular surface temperatures was significantly greater with RGP lens wear compared to SCL wear (0.85±0.53°C versus 0.50±0.56°C; p=0.044). **Conclusion**:These results suggest that significantly more stabilization of temperature (via disruption and cooling of the tear film) occurs with RGP wear compared to soft lens wear. This is likely to result from the characteristic mobility and tear exchange associated with RGP lens fit. This may have important implications for ocular physiology and infection during contact lens wear. Commercial Relationship: none

ASSESSMENT OF *STREPTOCOCCUS PNEUMONIAE* CAPSULE IN CONJUNCTIVITIS AND KERATITIS *IN VIVO*: NEURAMINIDASE ACTIVITY INCREASES IN NONENCAPSULATED PNEUMOCOCCI FOLLOWING CONJUNCTIVAL INFECTION. Erin W. Norcross,[1] Nathan A. Tullos,[1] Sidney D. Taylor,[1] Melissa E. Sanders,[1] and Mary E. Marquart[1] Department of Microbiology[1], University of Mississippi Medical Center, Jackson, MS, USA

Purpose: This study aimed to determine the effect of capsule in pneumococcal keratitis and conjunctivitis in the rabbit. **Methods**: A capsule deficient isogenic mutant was created using homologous transformation. Parent and mutant strains were injected within the upper bulbar conjunctiva (conjunctivitis) or into the corneal stroma (keratitis) of New Zealand white rabbits. Clinical examinations were performed 24 and 48 hours postinfection at which time corneas or conjunctivae were removed to determine the recovered bacterial load. Whole eyes were examined by histology. The neuraminidase activity was determined following *in vitro* and *in vivo* growth. **Results**: There were no significant differences in clinical scores between the eyes infected with the parent or mutant for either infection, nor was there a difference in the amount of bacteria recovered from the cornea. The mutant strain, however, was cleared from the conjunctiva faster than the parent strain. Histological examination showed slightly more infiltrating PMNs and macrophages in the conjunctivae infected with the parent strain. The neuraminidase activity of both strains was not significantly different when the strains were grown *in vitro*. However, the neuraminidase activity of the parent was significantly less than that of the mutant at 3 and 12 hours post conjunctival infection. **Conclusions**: Although more outbreaks of pneumococcal conjunctivitis are tied to nonencapsulated *S. pneumoniae* strains, this study showed that an encapsulated strain was capable of establishing conjunctivitis in the rabbit and survive attack by the host immune system longer than its nonencapsulated isogenic mutant. Nonetheless, the nonencapsulated pneumococci had increased neuraminidase activity levels *in vivo* compared to the parent strain. Support: Public Health Services Grant R01EY016195, National Eye Institute, National Institutes of Health and the University of Mississippi Medical Center

CLINICAL AND HISTOLOGICAL CHANGES CAUSED BY SUGAR CANE BURNING EMISSIONS ON THE OCULAR SURFACE OF SUGAR CANE WORKERS. Priscila Novaes[1A], Monique Matsuda[1A], Maristela P. Rangel[1A], Ubiratan P. Santos[1B], Newton Kara-José[1A], Alejandro Berra[2], Paulo H. N. Saldiva[1CA]Ophthalmology, [B]Pneumology- INCOR, [C]Pathology, [1]University of São Paulo, São Paulo, Brazil; [2]Pathology, University of Buenos Aires, Buenos Aires, Argentina.

Objective: Assess the impact of sugar cane burning on the ocular surface of sugar cane workers. **Methods**: 101 sugar cane workers and 80 healthy controls were recruited from the rural city of Mendonça, São Paulo, Brazil. Clinical evaluation was performed during the burning and the non-burning periods. They underwent biomicroscopy; tear film break-up time (TBUT); corneal and conjunctival vital staining with fluorescein and Lissamine Green; and Schirmer's I test during both periods. Impression cytology samples were collected from inferior bulbar conjunctiva and stained with PAS and Alcian Blue (AB). **Results**: The sugar cane workers presented significantly lower TBUT values during the burning period (mean 8.5, sd±3.5), when compared to the non-burning period (mean 10.5, sd±6.1) (p=0.005), and a significant increase in hyperemia (p=0.009), meibomitis (p<0.001), papillae (p<0.001) and particulate material on eyelid margins (p<0.001) during the burning period. Schirmer I test values and vital staining patterns were within the normal range, in both periods for both groups. The number of PAS positive cells, AB cells and the total density of goblet cells in impression cytology samples of sugar cane workers was lower during the burning period, when compared to the non-burning period and to the control group. **Conclusions**: The presence of particulate matter on the eyelid margins of sugar cane workers indicates direct contact of the highly irritative air pollutants with the tear film and the ocular surface. The increase in the frequency of papillae in these individuals reinforces the presence of increased ocular irritation and inflammation. Our findings indicate that seasonal episodes of high levels of air pollution generated by sugar cane burning causes tear film instability, induce histological changes and may have toxic effects on the ocular surface.

BONE MARROW MESENCHYMAL STEM CELLS TRIGGER PATHOGENIC FIBROSIS IN CHRONIC GRAFT VERSUS HOST DISEASE. Yoko Ogawa[1,3], Shigeto Shimmura[1], Satoru Morikawa[2,4] Yo Mabuchi[2], Tomonori Yaguchi[3], Sadafumi Suzuki, Takaaki Inaba[1], Yutaka Kawakami[3], Hideyuki Okano[2], Yumi Matsuzaki[2] , Kazuo Tsubota[1] [1]Department of Ophthalmology, [2]Department of Physiology, [3]Institute for Advanced Medical Research, Division of Cellular Signaling, [4]Department of Dentistry and Oral Surgery, Keio University, School of Medicine

Purpose; Organ dysfunction due to excessive fibrosis is a prominent histologic feature of chronic graft-versus-host disease (cGVHD) following allogeneic hematopoietic stem cell transplantation. We have reported a subset of fibroblast originating from circulating donor-derived precursors may participate in the excessive fibrosis in patients with lacrimal gland cGVHD. To examine the cellular sources of chronic GVHD fibrosis and the pathogenic process of chronic GVHD, we evaluated how the bone marrow stem cells including mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs) contributed to the cGVHD fibrosis. **Methods**. Eight week-old male B10.D2 (H-2^d) and female BALB/c mice were used as donors and recipients, respectively. As a prospective study in vivo,

fresh MSCs were isloated by cell sorter (Morikawa S, JEM 2009) from male B10D2 donors, and SP cells (Matsuzaki Y 2004) from female Balb/c recipients and injected into female Balb/C mice. The occurrence and severity of cGVHD were compred with syngeneic control. **Results**. We found purified allogeneic MSCs contributed to chronic GVHD fibrosis in target organs including conjujnctiva and lacrimal gland. When we transplanted allogeneic MSC into Nude mice, the magnitude of fibrotic area was significantly decreased. In addition, the MSCs depletion transplantation also decreased the cGVHD fibrosis in various organs. **Conclusion**. These findings suggested that allogeneic MSCs may be a trigger of cGVHD fibrosis. In paticular, our findings may help elucidate the pathogenesis of chronic GVHD fibrosis and facilitate the development of novel anti-fibrotic therapies. The authors declare no commercial relationships. Grant supported by the Japanese Ministry of Education, Science, Sports, and Culture #20592058.

CLUSTERIN PROMOTE CORNEAL/LIMBAL EPITHELIAL GROWTH THROUGH EPITHELIAL-MESENCHYMAL INTERACTION. [1]Naoko Okada, [1,3]Tetsuya Kawakita,[2]Kenji Mishima,[2]Ichiro Saito,[1,3]Hideyuki Miyashita, [1,3]Shigeto Shimmura, [1,3]Kazuo Tsubota[1]Department of Ophthalmology, Keio University School of Medicine, Tokyo, JAPAN and [2]Department of Pathology, Tsurumi University, [3]Department of Ophthalmology, Tokyo Dental College, Chiba, JAPAN.

Objective: Cornea has high expression of clusterin (CLU), however the role of clusterin in cornea was poorly understood. This study was performed to investigate the role of clusterin in epithelial-mesenchymal interaction in cornea. **Methods:** CLU was overexpressed in 3T3 cells by transfection of the expression vector encoding full length CLU. Colony forming efficiency was compared in mouse corneal cell line (TKE2) and human primary corneal/limbal epithelial cells (HLECs) that were cultured using CLU-3T3 and mock-3T3 as feeder cells in direct contact or indirect contact through the culture medium (separate culture). To suppress the effect of secretory CLU in co-cultured TKE2 with CLU-3T3 cells, neutralizing antibody was used. The expression of cellular factors associated with limbal stem/progenitor cell maintenance and growth in CLU-3T3 were analysis by RT-PCR. **Results:**TKE2 cells co-cultured with mitomycin C treated CLU-3T3 and mock-3T3 cellsshowed a higher colony-forming ability and larger size colony population in comparisonwith those in mock-3T3 cells in direct contact. These increases in the CFE of TKE2 on CLU-3T3 cells were also found in separate culture with no need to contact directly each other, and which were significantly blocked by treatment with CLU neutralizing antibody. Furthermore, we found that HGF and N-cadherin was relatively higher expressed in CLU-3T3 cells than mock-3T3 cells. These results suggest that the promotion of colony-forming and cell proliferation by CLU-3T3 cells is partly mediated via induction of HGF and N-cadherin. **Conclusion:**Secretory clusterin promoted colony forming efficiency of corneal/limbal epithelial cells through epithelial-mesenchymal interaction.

BLOOD COAGULATION FACTOR XIII IN TEARS. Zsuzsanna Z. Orosz,[1] Éva Katona,[1] Andrea Facskó,[2] László Módis,[2] László Muszbek,[1,3]András Berta.[2] Clinical Research Center,[1] Department of Ophthalmology[2] and Thrombosis, Hemostasis and Vascular Biology Research Group of the Hungarian Academy of Sciences,[3] University of Debrecen, Medical and Health Science Center, Debrecen, Hungary

Objective. As blood coagulation factor XIII (FXIII) is of high importance in wound healing, we determined the concentrations of FXIII A and B subunits (FXIII-A and FXIII-B) and their complex ($FXIII\text{-}A_2B_2$) in normal tears with and without the stimulation of tear production and in tears from patients undergoing penetrating keratoplasty (PKP) or cataract surgery with phacoemulsification. **Methods**. Highly sensitive chemiluminescent ELISAs, most recently developed in our laboratory, were used to measure the concentrations of FXIII-A, FXIII-B and FXIII complex in tear samples from 60 healthy volunteers before and after stimulation of tear production with a spray of 80% ethanol. The above FXIII parameters were also determined in tears collected from 31 patients before and 1, 2, 4, 7 days after PKP. Tears from 100 patients before and 1 day after cataract surgery were also investigated. **Results**. In non-stimulated tears from healthy volunteers low, but consistent amounts of FXIII-A and FXIII-B (medians: 2.13 μg/L and 7.22 μg/L, respectively) were measured, mostly in non-complexed form. Following stimulation of tear secretion FXIII levels moderately decreased, but if normalized to protein concentration they did not change. One day after PKP FXIII levels became highly (15-25-fold) elevated, then gradually decreased, but even on day 7 significantly exceeded pre-surgery values. One day after cataract surgery FXIII levels became elevated, elevation correlated with the conjunctival hyperaemia. **Conclusions**. FXIII subunits are low concentration components of tear proteome and stimulation of tear production failed to increase their levels. The striking elevation of FXIII subunits and FXIII complex concentrations after PKP and phacoemulsification suggests the involvement of FXIII in corneal wound healing. Grant support: OTKA-NKTH CNK80776 from the Hungarian National Research Fund.

CELLULAR FACTOR XIII, A TRANSGLUTAMINASE, IS PRESENT IN THE CORNEAL STROMA. Zsuzsanna Z. Orosz,[1] Helga Bárdos,[2] Andrea Facskó,[3] András Berta,[3] Róza Ádány,[2] László Muszbek.[1,4] Clinical Research Center,[1] Department of Preventive Medicine,[2] Department of Ophthalmology[3] and Thrombosis, Hemostasis and Vascular Biology Research Group of the Hungarian Academy of Sciences,[4] University of Debrecen, Medical and Health Science Center, Debrecen, Hungary

Objective. Transglutaminases (TGs) are a family of enzymes that cross-link proteins by e(g-glutamyl)lysyl bonds and most of them have been implicated in the modulation of extracellular matrix. Here we investigated the presence of three TGs, keratinocyte TG (TG-1), tissue transglutaminase (TG-2) and the cellular form of blood coagulation factor XIII (cFXIII) in the corneal tissue. **Methods**. Frozen sections of normal human cornea obtained from enucleated bulbus were stained for cFXIII, TG-1 and TG-2 using poly-, or monoclonal antibodies. Detection of cFXIII was also combined with labeling for CD11b, CD34, CD45, CD68 and CD163 using double immunofluorescent staining. FITC-labeled or biotinylated secondary antibodies with Texas red-labeled streptavidin were used for the visualization of immunoreactions. **Results**. A significant part of keratocytes showed intensive staining for cFXIII, but not for TG-1 and TG-2. Neither epithelial nor endothelial cells were labeled by anti-cFXIII antibody. cFXIII positive keratocytes were unevenly distributed in the corneal stroma; they were abundant in the subepithelial tertile of stroma (120±10/visual field), while they were sparse (38±6/visual field) in the subendothelial tertile. cFXIII+ cells showed co-staining for CD34, however, a significant number of CD34+ cells were negative for cFXIII. CD34+ cells were evenly distributed throughout the stroma. Only a few cells were stained for CD11b and CD45, they were also labeled by anti-cFXIII antibody. No cell showed positivity for CD68 and CD163. **Conclusion**. This is the first report demonstrating the presence of cFXIII in the cornea. A significant part of CD34+ keratocytes contained cFXIII, their transglutaminase activity might play a role in the structural organization of normal corneal stroma. *Grant support: OTKA-NKTH CNK80776 from the Hungarian National Research Fund.*

THE ENHANCED CONTROLLED ADVERSE ENVIRONMENT (ECAE) SYSTEM INCREASES WITHIN-SUBJECT RELIABILITY. George W. Ousler III, Joel Naor, Donna Welch, Patrick Johnston, Keith J. Lane. Ora, Inc

Objective: The variability observed in the signs and symptoms of dry eye patients is a constant challenge when evaluating investigational therapies. The Controlled Adverse Environment (CAE) model was developed to better control this variability, and the model has been integral in the majority of dry eye clinical trials in the United States across thousands of patients. Research and technological advances emerging since the inception of the CAE facilitates continuous improvement to the model and study designs to emulate real-world dry eye challenges in a reproducible manner with consistent results. The purpose of this research was to evaluate the reliability of clinical findings in the most recent iteration, the Enhanced CAE (ECAE). **Method:** A retrospective analysis was performed on data from 608 dry eye subjects exposed to the ECAE for 90 minutes at two separate visits across 4 studies. Inter-class correlation (ICC) was calculated using a components of variance model to determine within-subject variability of results. Paired t-tests were used to compare post-ECAE means between visits. **Result:** Exposure to the ECAE demonstrated consistent exacerbation of signs (e.g. post-CAE ocular surface staining means = 2.14, 2.16 at visits 1, 2 [difference = 0.02 ± 0.02]). Symptom aggrevation was also observed (e.g. post-CAE ocular discomfort means = 3.13, 3.21 at visits 1, 2 [difference = 0.08 ± 0.04]). Importantly, exposure to the CAE increased within-subject reliability for both signs (e.g. staining, ICC pre- and post-CAE = 0.55 and 0.58) and symptoms (e.g. discomfort, ICC pre- and post-CAE = 0.32, 0.43). **Conclusion:** These findings demonstrate the overall reduction in the inherent variability within dry eye patients provided by the ECAE. Furthermore, these results validate the evolution to the ECAE, confirming the benefit of applying technological and design advances in a continuous endeavor to efficiently demonstrate accurate, consistent, and meaningful clinical research results.

MODELLING MEIBOMIAN LIPID FILM STRUCTURE USING X-RAY REFLECTIVITY. Chendur K. Palaniappan[1], Shiwani R. Raju[1], Michael James[2] and Thomas J. Millar[1] School of Natural Sciences, University of Western Sydney[1], Bragg Institute, Australian Nuclear Science and Technology Organisation, Sydney[2]

Purpose: Our understanding of the lipid layer structure of the tear film is limited to interference patterns and interference microscopy. At the molecular level, structural models based on lipid composition have been made, but never tested. Here, X-ray reflectivity has been used to investigate the structural organisation of spread meibomian lipid films at the molecular level. X-ray reflectivity was also used to measure films of wax esters, cholesterol esters or their mixtures that could emulate the structure of meibomian lipid films. **Methods**: Human meibomian lipids were spread on a buffered aqueous subphase in a Langmuir trough. X-ray reflectivity spectra were collected from these films at various surface pressures. Reflectivity spectra were also collected from: dipalmatoyl phosphatidyl choline (DPPC), used to emulate surfactant molecules of meibum; cholesterol ester films; wax ester films; and mixtures of these to model the non-polar components of meibum. **Results**: X-ray reflectivity profiles from meibomian lipid films were best fitted by a structural model having three phases (multilayered). This was the case at all pressures, except for an increase in total thickness with high surface pressure and water being squeezed out of the layer adjacent to the subphase. By contrast, DPPC films could be modeled as a single lipid layer with two phasesrepresenting the polar headgroup and hydrocarbon tails. Compression of pure non-polar films led to multilayer structures being formed, with Bragg peaks being present in the reflectivity data. A film of a 1:1 mixture of the above esters showed little structure, suggesting micelle formation. **Conclusions**: Meibomian lipid films have unique characteristics and show a 3-phase structure. Emulating a meibomian lipid film as a mosaic of its major chemical constituents was unsuccessful. A more complex system that includes a mixture of esters of varying chain lengths, or the inclusion of minor, non-polar components may be necessary to better model the structure and behaviour of a meibomian lipid film. [This research was supported by UWS graduate fellowships and by an AINSE grant]

CHARACTERIZATION OF THE PATHOGENIC MECHANISM OF ACANTHAMOEBA KERATITIS - THE PROTECTIVE ROLE OF TEAR FLUID. Noorjahan Panjwani, Departments of Ophthalmology and Biochemistry, and The New England Eye Center, Tufts University School of Medicine, Boston, Massachusetts.

Purpose: *Acanthamoeba* keratitis (AK) is a serious infection of the cornea. At present, diagnosis of the disease is not straightforward and treatment is very demanding. The first critical step in the pathogenesis of infection is the adhesion of the microbe to the surface of the host tissues. The goal of the current study was to characterize the molecular mechanism by which of *Acanthamoeba* adhere to the surface of the cornea and produce cytopathic effect. **Methods and Results:** Cell adhesion assays revealed that *Acanthamoebae* express a major virulence protein, the mannose-binding protein (MBP), which mediates the adhesion of amoebae to the surface of the cornea. The MBP is a transmembrane protein with characteristics of a typical cell surface receptor. Subsequent to the MBP-mediated adhesion to host cells, the amoebae produce a contact-dependent metalloproteinase and several contact-independent serine proteinases. These proteinases work in concert to produce a potent cytopathic effect (CPE). In the hamster animal model, oral immunization with rMBP protects against AK, and this protection is associated with an increased level of anti-MBP IgA in tears of protected animals. Normal human tear fluid contains IgA antibodies against *Acanthamoeba* MBP that is likely to provide protection by inhibiting the adhesion of parasites to host cells. Indeed, in in vitro CPE assays, even a low concentration of tears (10µL of undiluted tears per milliliter of media) almost completely inhibits *Acanthamoeba*-induced CPE. In addition to adherence-inhibiting, IgA-mediated protection, human tears also contain IgA-independent factors that provide protection against *Acanthamoeba*-induced CPE by inhibiting the activity of cytotoxic proteinases. **Conclusions:** Characterization of the CPE-inhibitory factors of human tears should lead to a better understanding of the mechanism by which the tissues of the host resist the infection and also help decode circumstances that predispose to *Acanthamoeba* infections. Grant Support: NIH EY09349; RPB Challenge grant. Commercial relationship: None

LONGTERM CHANGES OF BUT AND CORNEAL SENSITIVITY FOLLOWING LASIK AT MIDDLE AGE. Woo Chan Park, Jae Kwan Park, Ki Sung Park, Byung Moo Min. Dept. of Ophthalmology, Dong-A University, Busan, Korea

Purpose: To evaluate the long-term changes of BUT and corneal sensitivity following LASIK surgery at aged over 45. **Methods:** The study consisted of 106 eyes of 56 patients aged over 45 who received presbyopia LASIK surgery. Mean age was 52.4 years. Hansatom microkeratom (140um, 8.5mm flap) and Kera Excimer laser were used. BUT and corneal sensitivity using Cochet-Bonnet aesthesiometer were measured at before and 1, 3, 6, 12 and 18 months after LASIK. **Results**: Preop 1 Mo 3 Mo 6 Mo 12 Mo 18

Mo BUT 5.5±2.0 4.7±1.7 4.9±1.8 4.8±1.9 4.8±1.3 4.7±1.3 sec. Sensitivity 5.6±0.6 4.1±1.4 4.7±1.1 4.7±0.8 4.7±0.8 4.9±0.7cm. The BUT and corneal sense were decreased during the first 1 months and then both of them returned to its 85% of preoperative value until postop 18 months. **Conclusions**: The BUT and corneal sense of middle aged LASIK patient show similar pattern with young patient. But they are not complete returned.

THE INTERACTION BETWEEN EYE MAKE-UP REMOVERS AND THE TEAR FILM. Edward Ian Pearce, Madeline Harvey-Brown & Claire Higginson Glasgow Caledonian University, Glasgow, Scotland UK

Objective :To investigate the effect of eye make-up removers on the human tear film **Method**: Twenty young healthy subjects (10M, 10F, age 20-24 years) were recruited. Three eye make-up removers were chosen due to their disparate composition and mode of action: SimpleTM (Accantia, UK) eye make-up remover, an oil and alcohol free product; Johnson's 3in1 (J&J Germany), an oil based microemulsion; Blephaclean (Théa Pharma, France) a hyaluronic acid containing micelle solution designed primarily for lid hygiene. Tear film parameters assessed before and after treatment were: evaporation rate, lipid layer pattern, noninvasive tear stability (NITBUT), tear production (phenol red thread) and symptoms. The order of product testing was randomized using a Latin square. **Results**: All three products significantly increased evaporation rate ($p<0.05$), but the increase was greatest for Johnson's (33.8g/m2/h-1) compared with Simple (10.3g/m2/h-1) and Blephaclean (11.0g/m2/h-1). Simple and Blephaclean did not change lipid layer pattern whilst Johnson's did disrupt the lipid layer ($p=0.001$). Tear stability was unchanged with use of Simple but reduced significantly following use of Blephaclean (1.4sec) and Johnson's (2.0sec) ($p<0.05$). Tear production was significantly reduced only following the use of Simple ($p=0.031$). Most symptoms (dryness, grittiness, pain and visual acuity) were unchanged following treatment. Symptoms of stinging were found to increase following the use of Johnson's ($p=0.001$). Itchiness was found to be increased by Blephaclean use ($p=0.019$). **Conclusion**: Eye make-up removers are widely used products and this study shows that they can adversely interact with the tear film. All the products tested increased evaporation rate but it was apparent that oil containing products have the most detrimental effects on a range of tear parameters. As these effects are seen in healthy individuals, it is likely that the impact on a deficient tear film will be greater and that oil containing products in particular should be avoided by this group.

EFFECT OF MELATONIN AND ANALOGUES ON CORNEAL WOUND HEALING: INVOLVEMENT OF MT_2 MELATONIN RECEPTOR. Assumpta Peral, Ana Guzmán-Aranguez, Almudena Crooke and Jesús Pintor University Complutense of Madrid, School of Optics

Objective: Study the implication of the melatoninin the corneal re-epithelialisation of New Zealand white rabbits and in a rabbit corneal epithelial cell-line. **Method:**New Zealand white rabbits were used. Animals were kept under controlled light cycles (12h/12h). Experiments were carried out in accordance with the statement of ARVO on the Use of Animals in Ophthalmic and Vision Research. Cell line (SIRC, Statens Seruminstitut rabbit cornea from ATCC); reagents and antibodies were used. Compounds used were melatonin, luzindole, DH97 and prazosin. Corneal wounds were made in both eyes after anesthetising the animals with propofol and with topical ocular anestesia. Wounds were made by applying a 3mm disc of Whatman nº1 paper soaked in n-heptanol for 30 seconds. After that, eyes were washed with saline solution. A dose of 10 nmol of melatonin was applied every 6 hours, eight hours after the wound, until midnight. The wounds were staining and pictures were taken by a slit-lamp from 8 am to midnight. *In vitro* assays were performed as described by Mediero 2006 (IOVS 2006(10):4500-4506) by the application of melatonin with a dose of 100µM. Studies with antagonists were also performed. Paired Student's t-test was used for the *in vivo* experiments and ANOVA test for *in vitro*. **Results:** *In vivo*, Estimated Migration Rate(EMR) and Estimated Healing Time(EHT) for controls were 75±5µm/hour and 29.8±1.9hours, for melatonin were 110±7µm/hour and 20.4±1.5hours. The *in vitro* assays confirmed the results obtained on living animals. **Conclusions:**Melatonin seems to be responsible of the control of epithelial cell migration. Grant Support: SAF2007-60835 SANTANDER-COMPLUTENSE PR1/07-14890

EPITHELIAL IRREGULARITY FACTOR (EIF): A NEW DIAGNOSTIC CRITERION FOR THE DIAGNOSIS OF DRY EYE SYNDROME. Victor L. Perez, Mohamed Abou Shousha, William Feuer, Anat Galor and Jianhua Wang. Bascom Palmer Eye Institute, University of Miami Miller School of Medicine

Purpose: To evaluate the use Epithelial Irregularity Factor (EIF) obtained using ultra high resolution optical coherence tomography (UHR-OCT) as a qualitative and quantitative criterion for the diagnosis of dry eye syndrome. **Methods:** UHR-OCT images as well as dry-eye symptom questionnaire scores, corneal and conjunctival fluorescein staining scores, tear break-up time (TBUT) and Schirmer test were obtained in 21 dry eye patients. EIF was calculated using custom made software as the standard deviations of the corneal epithelial thicknesses measured along the central 3 mm zone of the UHR-OCT images. Averaging of measurements was done for patients who contributed both eyes to the study. Pearson correlations were used to assess the correlations between obtained scores and EIF. **Results:** Pearson correlations demonstrated that dry-eye symptom questionnaire scores correlated (all $p\leq0.005$) with cornea fluorescein staining ($r=0.594$) and most highly with EIF ($r=0.877$) When all variables were allowed stepwise inclusion in a multiple regression model, significant ones were EIF ($p<0.001$) and TBUT ($p=0.046$). TBUT by itself had no significant correlation. Fluorescein stainingdid not enter the model ($p=0.527$). **Conclusions:** Epithelial Irregularity Factor (EIF) is a novel quantitative and qualitative criterion for the diagnosis of dry eye syndrome that correlates accurately with patients' subjective symptoms and could be used to study patients with pain and no clinical signs. (This work was partially sponsored by a research grant from Alcon Ltd)

THE EFFECT OF OCULAR SURFACE LUBRICANT EYEDROPS ON LID PARALLEL CONJUNCTIVAL FOLDS (LIPCOF) AND OTHER SIGNS AND SYMPTOMS OF TEAR FILM DYSFUNCTION. Igor Petričeк[1], Snježana Lovrinčević[2], Sanja Njirić[3], Goranka Petričeк[4], Petar Rašregorac[5], Iris Urlić[6], Martina Tomić[7] Zagreb University Hospital Eye Department, Zagreb, Croatia[1] Croatia insurance, Zagreb, Croatia[2] Ophthalmology Polyclinic "dr Luciana Pavićević", Rijeka, Croatia[3] Zagreb University Medical School Family Medicine Department, "Andrija Štampar" School of Public Health, Zagreb, Croatia[4] Private Ophthalmology Practice, Samobor, Croatia[5] Ghetaldus Ophthalmology Polyclinic, Zagreb, Croatia[6] Clinical Hospital for Diabetes „Vuk Vrhovac", Zagreb, Croatia[7]

Purpose: The aim of this study was to investigate the effect of topical therapy with lubricating eyedrops on the signs and symptoms of dry eye, with the special emphasis on conjunctival folds (LIPCOF). **Methods:** During summer and fall of 2009, 229 patients were enrolled in study by 16 ophthalmologists from various parts of Croatia. Enrollment criteria were symptoms of dry eye as ranked by the

standardized questionnaire. Upon enrollment, TBUT, fluoresceine staining, assessment of conjunctival hyperemia using CCLRU Grading Scale and LIPCOF were assessed . After the examination, every enrolled patient was given one bottle of rewetting and lubricating eyedrops containing hydroxypropyl-guar, and was instructed to instil them in each eye twice a day during 14 days. **Results:** After 14 days of therapy with lubricating eyedrops (hydroxypropil-guar), statistically significant reduction of LIPCOF score was observed, as well as the reduction of symptoms and other signs of tear film dysfunction (TBUT, Schirmer test, conjunctival hyperemia). **Conclusions:** The results of this study stress the influence of the increased ocular surface friction during blinking on symptoms and signs of dry eye, its possible influence on appearance of conjunctival folds (LIPCOF), as well as their reversibility after lubricating therapy. Also, they stress that, along with rehydration, lubrication plays a significant role in treating tear film dysfunction, and the importance of choosing therapy that has proven lubricating effect.

EXTERNAL EYE DISEASES GROUP. Petricek Igor[1], Andras Berta[2], Janos Nemeth[3], Mohamed T Higazy[4], Marek Prost[5], Pavel Nemec[6]Head of Electrophysiology and Ultrasound Laboratory, Department of Ophthalmology, Zagreb University Hospital, Zagreb, Croatia[1] Professor, Chairman, Department of Ophthalmology, University of Debrecen, Debrecen, Hungary[2] Professor, Chairman, Department of Ophthalmology, Semmelweis University, Budapest, Hungary[3] Professor of Ophthalmology, Benha University, Heliopolius, Cairo, Egypt[4] Professor, Chairman, Department of Ophthalmology, Military Institute of Aviation Medicine, Warsaw, Poland[5] Department of Ophthalmology, Faculty of Medicine, Charles University, Prague, Czech Republic[6]

Together with refractive errors, red eye (non-traumatic inflammation of the external eye) is the most frequent ophthalmic reason for visiting family medicine doctors, pediatricians or ophthalmologists. Comparatively few groups of physicians in the world deal specifically with this issue. Furthermore, many current advances in ophthalmology are not of much use in parts of the world where conditions in eye care are different than in those where those advances are made. In order to address this problem, The External Eye Disease Group, or EED Group, has been formed in 2004. Currently, it includes ophthalmologists from the Southeastern Europe and the Middle east: Belarus, Bosnia, Bulgaria, Croatia, Czech Republic, Egypt, Hungary, Israel, Lebanon, Poland, Romania, Russian Federation, Slovenia, Slovakia, Turkey and Ukraine. Main interests of the EED Group are non-traumatic inflammation of the external eye, mainly caused by bacterial and viral infection, dry eye or allergy. The group is presided by the Key Faculty, which comprises of Prof. Andras Berta (Hungary), Prof. Janos Nemeth (Hungary), Prof. Marek Prost (Poland), Prof. Mohamed Higazy (Egypt), Dr. Igor Petriček (Croatia) and Dr. Pavel Nemec (Czech Republic). Main goal of the group is education of ophthalmologists and other physicians in the region of country members involved in treating external eye diseases by disseminating most current knowledge in the field, adapted to local situation. By doing this, they form a vital and unique link between current science and local needs.

EVALUATION OF TEAR FILM QUALITY WITH A DOUBLE-PASS SCATTERING INDEX. Pisella Pj, Habay T, Nochez Y. CHU Bretonneau, Tours, France Faculté de Médecine François Rabelais, Tours, France.

Purpose: To compare clinical evaluation, break-up time analysis (BUT), biological evaluation (tear osmolarity), and aberrometric evaluation (Ocular Scattering Index) of the tear film. **Methods:** This prospective study included 20 eyes: 10 eyes with normal BUT and 10 eyes with dry-eye syndrome. An Objective Scatter Index (OSI),estimated from the double pass images, quantifies scattering. We compared OSI as a function of time, between each blinking, during 10 seconds. **Results:** Patients with dry-eye syndrome have higher amounts of OSI compared to normal eyes (preliminary results : respectively OSI=5,6 +/- 4 versus 1,2 +/- 2,3 with p<0,05). We analyse the slope of the line describing OSI evolution during 20 seconds. This slope coefficient is greater in patients with dry-eye syndrome than in normal eyes (1,8 +/- 1,2 versus 0,4 +/- 0,5 with p<0,05). This new objective method may quantify the blurry vision associated with dry-eye syndrome. Moreover, this dynamic analysis of the tear film could evaluate the effect of eye drops on tear-film quality and stability. **Conclusion:** We can understand the optical and visual impact of tear-film breakup in normal and dry eyes. Correlation between tear film dynamics and tear film osmolarity will be considered. No Financial Disclosure.

TRANSGLUTAMINASE-2 DEPENDENCE IN HYPEROSMOLARITY-INDUCED MITOCHONDRIAL DYSFUNCTION. Evelyn Png,[1] Shyam S. Chaurasia,[1] Louis Tong.[1,2,3] Singapore Eye Research Institute,[1] Singapore National Eye Center,[2] Duke-NUS Graduate Medical School, Singapore[3]

Purpose: Hyperosmolar tear is a feature of dry eyes. It is a final common pathway for epithelial damage resulting in apoptosis, so understanding of mediators in this pathway is clinically useful. Transglutaminase (TGM)-2 is a multifunctional protein expressed in corneal epithelial cells and regulates stress response. Although hyperosmolarity is known to activate the mitochondrial apoptotic pathway, it is not known if TGM-2 is required for this process. We aimed to determine if TGM-2 is upstream of mitochondrial damage in hyperosmolar stimulated cells. **Methods**: HCE-T expressing either shRNA targeting TGM-2 or scrambled shRNA were constructed by stable transfection. Over-expression of TGM-2 was performed by electroporation and transfection with pSG5 Tgase. Sodium chloride was introduced to achieve hyperosmolarity (560-570 mOsm), and was confirmed by VAPOR Pressure Osmometry. Mitochondrial membrane potential determination was performed using the JC-1 assay and percentage of cells with depolarization quantified by a mini-flowcytometry method. Cell proliferation was determined by a real time impedance method. A calorimetric method was used to measure transamidase activity.**Results**: Hyper-osmolar conditions induced a significant decrease in mitochondrial potential in HCE-T, and caused a reduced cell proliferation. Over-expression of TGM-2 increased cellular transamidase activity, and also induced a reduction in cell proliferation. Importantly, compared to pSG5 (vector alone) transfection, TGM-2 over-expression also increased the proportion of cells with mitochondrial depolarization. Hyperosmolar conditions induced mitochondrial depolarization in cells transfected with shRNA control and shRNA targeting TGM-2, but to a significantly greater extent in the former. **Conclusions**: TGM-2 mediates the hyperosmolar induced mitochondrial cell death. Strategies targeting TGM-2 may be useful to protect corneal epithelial cells in dry eye disease. This research was supported by grants from Singapore National Medical Research Council NMRC/NIG/0002/2007, NMRC/1206/2009 and NMRC/CSA/013/2009

RANDOMIZED STUDY OF THE EFFICACY OF 0.05% CYCLOSPORINE OPHTHALMIC EMULSION IN THE TREATMENT OF MEIBOMIAN GLAND DYSFUNCTION. Pinnita Prabhasawat, Nattaporn Tesavibul, Wannaree Mahawong, Department of Ophthalmology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand

Purpose: To compare the efficacy of topical cyclosporine A 0.05%

(CsA) and non-preservative artificial tear in the treatment of meibomian gland dysfunction. **Methods**: Prospective, randomized, double-masked, parallel-group, 3-month controlled trial study. Seventy patients with symptomatic meibomian gland dysfunction and unstable tear film (TBUT< 8 sec) were randomized to either topical CsA 0.05% (group A) or placebo (group B, 0.5% carboxymethylcellulose), twice daily for 3 months. They were evaluated at baseline and 1, 2, and 3 months. Main outcome measures: Efficacy: ocular surface index (OSDI), lid margin inflammation, meibomian gland expression, conjunctival injection, corneal and interpalpebral dye staining, non-invasive and invasive tear break-up time, and Schirmer tear test (without anesthesia). Safety: occurrence of adverse events. **Results** : Sixty-four patients completed the study, 31 in group A and 33 in group B. All baseline parameters were not statistically different between both groups. At 3 month follow up, mean OSDI, noninvasive tear break up time (tear scope®) and fluorescein tear break up time (NTBUT and FTBUT), lid margin inflammation, meibomian gland expressibility and tarsal injection significantly improved in group A ($p < 0.01$, $p<0.01$, $p<0.001$, $p<0.05$, $p<0.001$ respectively). In group B, only OSDI improved significantly at 3 month ($p = 0.003$). Tear breakup time (NTBUT, FTBUT) was statistically longer in group A as compare with group B at any time point of visit and the mean change of tear breakup time from baseline was also significantly greater in group A as compare with group B at the last visit ($p<0.001$). **Conclusions** : Topical CsA 0.05% twice daily may be helpful in the treatment of meibomian gland dysfunction superior effect over a non-preservative artificial tear by decreased inflammation of lid margin and meibomian gland and improved tear film stability.

BLEPHASTEAM®: A NOVEL EQUIPMENT TO TREAT MEIBOMIAN GLAND DYSFUNCTION (MGD). A CLINICAL AND LABORATORY STUDY. V Profazio, P. Versura, MG Tedeschi, C. Coslovi, M. Cellini, E C Campos Ophthalmology Unit, Alma Mater Studiorum University of Bologna

Purpose: Application of heat and humidity on the eyelids has a beneficial effect upon various MGD conditions. We report here a pilot study on a device which delivers latent heat and controlled humidity to the surface at open eyes. **Methods:** Five low delivery MGD patients were enrolled. Blephasteam® was applied twice/day/10'each session for twenty days. A symptom questionnaire provided by the manufacturer was filled by the patient at day 1, 2, 4, 6, 12, and endopint. Schirmer test I, Break Up Time (BUT), corneal and conjunctival fluo staining (Oxford score), tear osmolarity (Tearlab, Ocusense), Direct Meibometry, (DM, Meibometer MB550-Courage-Khazaka) conjunctival scraping and imprint cytology were performed. at baseline (day 0) and endpoint.(day 21). Statistical analysis was carried out by using SPSS 14.0 and MedCalc 5.0 software. **Results:** A significant reduction was shown at the endpoint-vs-baseline in symptom questionnaire (score 6,4±3,2 vs 1,4±1,5, p=0,003), Oxford staining score (0,9±0,8 vs 0, p=0,01), scraping cytology score (3,4±0,5 vs 4,6±1,2, p=0,005), imprint cytology score (1,15±0,2 vs 1,35±0,2, p=0,03), tear osmolarity and Schirmer I did not appear to change. A significant improvement was shown endpoint-vs-baseline in BUT (9,4±0,9 vs 7,1±1,9, p=0,001) and in casual lipid level/lower lid margin (DM-Arbitrary Unit 350,2±139,2 vs 250,5±89, p=0,01). All patients reported an high satisfaction degree upon Blephasteam use from day 12. **Conclusions:** Blephasteam® was reported as a comfortable device to relief discomfort symptoms in MGD disease by all subjects. Its efficacy was also found to improve ocular surface features related to amelioration of meibum outflow after treatment.

A NEW MODIFIED FLUORESCEIN STRIP: IT'S REPEATABILITY AND USEFULNESS IN TEAR FILM BREAK-UP TIME ANALYSIS. Heiko Pult[1,2], Britta Riede-Pult[1] [1]Optometry and Vision Research, Weinheim, Germany [2]Contact Lens & Anterior Eye Research Unit (CLAER), School of Optometry and Vision Sciences, Cardiff University

Purpose: To (i) analyse the repeatability of fluorescein instillation from a modified fluorescein strip (MFS) compared to a standard fluorescein strip (FS), and to (ii) observe its usefulness in the measurement of the fluorescein break-up time (FBUT) in comparison to the Tearscope. **Methods:** *In-vitro*: Intra- and inter-observer repeatability in fluorescein instillation from the MFS and FS was evaluated by fluorescence analysis (n=10, each). *In-Vivo*: BUT of the right eye of 20 randomly selected subjects (mean age 43.3±11.5, range= 21-60 years, 8 males, 12 females) was measured by use of the Tearscope and MFS. Subjects were grouped into OSDI+/- (n=8/12) by the Ocular Surface Disease Index (OSDI). Repeatability was assessed by Bland-Altman plots. Correlations between tests were evaluated by Pearson`s, predictive ability, and cut-off values for dry eye by receiver operative characteristic (ROC) curve and area under the ROC (AUC). **Results:** *In-vitro:* Inter-observer 95% limit of agreement (LoA) of the MFS was similar to the FS LoA in observer 1 (O1), but better than the FS LoA in observer (O2) (MFS: O1: LoA=1.98(mW)/p=0.179; O2: 2.71/0.442; FS: O1: 1.71/0.246; O2: 4.11/0.512). Intra-observer LoA in fluorescence was better in MFS (1.42/0.111) than in FS (3.71/0.003). *In-vivo*: MFS FBUT was significantly shorter than the non-invasive BUT (NIBUT) (p=0.002), but significantly correlated (r=0.864, p<0.001). NIBUT and FBUT were significant discriminators (p<0.001) of OSDI+/- (0.948/8 (sec) and 0.938/5 [AUC/cut-off value]; NIBUT and FBUT, respectively). The difference between NIBUT and FBUT can be described as: 'NIBUT=3.665 + 0.817 x FBUT' (log. regression analysis). **Conclusions:** FBUT is significantly correlated to NIBUT, but significantly shorter. Both methods are good discriminators of dry eye symptoms, but differ in their cut-off values. To improve repeatability, controlled instillation of fluorescein by use of the new MFS should be preferred over the use of a standard FS.

REPEATABILITY OF GRADING OF REAL EYES VERSUS GRADING OF PHOTOGRAPHS. Heiko Pult[1,2], Christine Purslow[2], Paul J Murphy[2], Russels L Woods[3] [1]Optometry and Vision Research, Weinheim, Germany, [2]Contact Lens & Anterior Eye Research Unit (CLAER), School of Optometry and Vision Sciences, Cardiff University, UK, [3]Schepens Eye Research Institute, Harvard Medical School, Boston, USA.

Purpose: To investigate inter- and intra-observer agreement of bulbar hyperaemia grading between real eyes and photographs. **Method:** The bulbar hyperaemia was graded twice for (1) the right eye of 20 subjects (median 54y; 8 female) from the patient pool of the Horst Riede GmbH, Weinheim, Germany, and (2) an unrelated 50 anterior segment photographs. The two trained optometrists (O1 and O2) used the CCLRU grading system, interpolated in 0.1 unit increments. Limits of agreement were calculated from the distributions of differences between observer, observations and sources (real eye vs. photographs). Limits of agreement were compared using O`Briens Test of homogeneity of variances. **Results:** No significant differences were found between observers or observations ($t20<1.48$; $p>0.16$). For real eyes, the 95% inter-observer limits of agreement (O1=0.87 units, O2=0.74) was slightly, but not significantly ($F3,76=1.00$; $p=0.42$), worse than intra-observer-agreement (O1=0.56, O2=0.56). For photographs, inter-observer limits of agreement (O1=0.78, O2=0.61) was slightly, but not significantly ($F3,216=1.25$; $p=0.28$), worse than intra-observer-agreement (O1=0.66, O2=0.60). No significant differences were found between the limits of agreement of real-eyes and photographs ($F3,146=1.20$; $p=0.31$, $F149=0.19$; $p=0.90$, inter-

observer agreement and intra-observer agreement, respectively). **Conclusion:** Intra-observer agreement was marginally better than inter-observer agreement in real eyes and photographs, albeit these differences were not significant. We conclude that grading of photographs is a repeatable measurement and comparable to the grading of real eyes. These limits of agreement indicate that a change in bulbar hyperemia grade ≥0.9 units should to be considered as abnormal.

THE LONGITUDINAL IMPACT OF SOFT CONTACT LENS WEAR ON LID WIPER EPITHELIOPATHY AND LIDPARALLEL CONJUNCTIVAL FOLDS. Heiko Pult[1,2], Paul J Murphy[2], Christine Purslow[2] [1]Optometry and Vision Research, Weinheim, Germany, [2]School of Optometry and Vision Sciences, Contact Lens and Anterior Eye Research (CLAER) Unit, Cardiff University, Wales, UK.

Purpose: To investigate differences in the ocular signs, hyperaemia, staining, lid-parallel conjunctival folds (LIPCOF) and lid-wiper epitheliopathy (LWE), between three subject groups: non-contact lens wearers (Non-CL), new CL (New-CL) and experienced CL (Exp-CL). **Method:** Retrospective audit of patient records from Horst Riede GmbH, Weinheim, Germany for three groups was performed to facilitate cross-comparison of LIPCOF, LWE, bulbar and limbal hyperaemia and corneal staining, analyzed using Kruskal-Wallis and Mann Whitney U-Test. Groups: Non-CL (mean age=31.3; n=39), New-CL (mean age=31.0; n=65; SiHy/Hyd 32/33), Exp-CL (>1y. experience; mean age=32.1; n=61; SiH/Hyd=15/46). **Results:** Nasal LIPCOF was similar in all groups (p=0.811). Temporal LIPCOF scores were significantly higher in Exp-CL (p<0.001), but not in New-CL (p=0.104). LWE grade was significantly higher amongst lens wearers (p=0.001), and higher still in Exp-CL (p<0.01). Levels of bulbar and limbal hyperaemia were similar between Non-CL and New-CL (p=0.903 and p=0.379 respectively), but Exp-CL showed higher levels of bulbar hyperaemia compared to Non-CL and New-CL (p<0.05), and higher levels of limbal hyperaemia compared to Non-CL (p<0.05). Differences in limbal hyperaemia amongst experienced SiHy lens wearers were similar to Non-CL (p=0.590). Levels of corneal staining were significantly higher amongst lens wearers (p<0.01), but similar between New-CL and Exp-CL (p=0.15). The power calculation of the completed study resulted in a power of >0.89.
Conclusion: While LWE and corneal staining ncrease in initial CL, nasal LIPCOF appears unaltered and temporal LIPCOF only increases with longer experience of lens wear. Longer experiences in CL are also related to a significant increase in ocular hyperaemia; however, in silicone hydrogels limbal hyperaemia is not significantly different to normal individuals. LWE and staining might be the first indicators of changes to the ocular surface due to contact lenses.

NORMAL VALUES FOR LISSAMINE GREEN STAINING OF THE OCULAR SURFACE.
Christine Purslow & Rachel Tinsley School of Optometry & Vision Sciences, Cardiff University, Cardiff, United Kingdom

Objective: Lissamine Green (LG) has received renewed interest as an ocular surface staining agent in dry eye but the prevalence of staining in healthy eyes is unclear. This study investigated the prevalence and the grade of LG staining of the cornea and conjunctiva in a large group of normal subjects. **Method:**LG staining of the cornea and conjunctiva (nasal, temporal) was graded on the right eyes of 94 healthy subjects (48M, 46F; median age 20yrs, range 18-35) using an interpolated Oxford Grading Scale. LG was applied using a moistened impregnated strip (1.5mg; Lissamine Green Ophthalmic Strips, Contacare) according to a standardised protocol. Staining was observed using a slit lamp biomicroscope (white light, 16x magnification) combined with a red filter (Wratten n25 equivalent; Ocular Solutions, UK). Non-parametric statistics were used to examine differences across the ocular surface (Friedman), and between genders (Mann Whitney U tests). **Result:**The prevalence of LG staining of the cornea was 13.5% and of the conjunctiva was 84.3% in this cohort. Median values for LG staining scores were significantly different (p<0.001) across the ocular surface: nasal 0.75 (range 0-3.50); cornea 0.00 (range 0-1.75) and temporal 0.00 (range 0-2.00). No significant differences were observed between males and females in any area (0.188 <0.418).**Conclusion:**In normal subjects LG staining of the cornea is relatively uncommon but conjunctival staining is observed in over 80% of young subjects. There is significantly more staining seen nasally in normal eyes than elsewhere on the ocular surface. Therefore, it is recommended that nasal and bulbar conjunctiva should be graded separately.No commercial relationships

POTENTIAL LOCALIZATION OF PUTATIVE STEM/PROGENITOR CELLS IN HUMAN BULBAR CONJUNCTIVAL EPITHELIUM Hong Qi[1,2], Xiaofen Zheng[1], Xiaoyong Yuan[1], Stephen C. Pflugfelder[1],De-Quan Li[1*] [1]Ocular Surface Center, Cullen Eye Institute, Department of Ophthalmology, Baylor College of Medicine, Houston, Texas [2]Peking University Third Hospital, Department Ophthalmology,Beijing, China

Although the conjunctival fornix appears to contain the greatest proportion of stem cells, it is likely that pockets of conjunctival epithelial stem cells may also exist throughout the conjunctival epithelium. This study was to investigate the potential localization of putative stem/progenitor cells in the human bulbar conjunctival epithelium by evaluating 6 keratins and 13 molecules that have been previously proposed stem cell associated or differentiation markers. We found that cornea specific cytokeratin (CK) 3 was not expressed by the bulbar conjunctival epithelialcells. In contrast, CK4 and CK7 were expressed by the superficial cells of bulbar conjunctival epithelium. CK14 and CK15 were confined to the basal cell layer. CK19 was stronglyexpressed by all layers of the bulbar conjunctival epithelium. The expression patterns of molecular markers in the basal cells of human bulbar conjunctival epithelium were found to be similar to the corneal epithelium. Basal conjunctival epithelial cells strongly expressed stem cell associated markers, including ABCG2, p63, nerve growth factor (NGF) with its receptors tyrosine kinasereceptor A (TrkA) and neurotrophin low-affinity receptor p75NTR, glial cell-derived neurotrophic factor (GDNF) with its receptor GDNF family receptor alpha 1 (GFR-1), integrin 1, -enolase andepidermal growth factor receptor (EGFR). The differentiation associated markers nestin, E-cadherin and involucrin were not expressed by these cells. These findings indicate that the basal cells of bulbar conjunctival epithelium shares a similar expression pattern of stem cell associated markers to the corneal epithelium, but has a unique pattern of differentiation associated cytokeratin expression.

THE ENVIRONMENTALLY INDUCED DRY EYE – EXISTING FINDINGS AND CURRENT ASPECTS. Dieter F. Rabensteiner, Jutta Horwath-Winter, Otto Schmut. Department of Ophthalmology, Medical University of Graz, Austria.

Purpose. A worldwide increase in the sicca-syndrome can be observed. Multiple different in- and extrinsic factors are known to induce or at least promote dry eye. One reason why it is affecting so many people seems to be the ongoing problematic change of our environment. Hence the tear film and the ocular surface are increasingly exposed to a variety of external influences and noxa,

leading to the development of an environmentally induced dry eye. We want to summarise our existing findings and present current aspects. **Methods.** In former studies we investigated the influence of ultraviolet light, ozone, exhaust emissions and cigarette smoke onto the human tear fluid and the ocular surface. Lately we analysed the influence of pollen and fine dust on the anterior part of the eye. Human tear fluid was incubated with pollen extracts or fine dust suspensions, respectively. By polyacrylamidegel-electrophoresis the effects on tear fluid proteins were studied. In addition cultivated conjunctival cells were incubated with pollen extracts or fine dust suspensions, respectively. Cytomorphological changes were analysed using the CASY1 Cell Counter. Via MTS-assay the cell viability was quantified and compared to the viability of control cells. **Results.** Some time ago we proofed, that ultraviolet light, ozone, exhaust emissions and cigarette smoke are able to destroy human tear fluid proteins and to promote cell damage to the ocular surface. Our current experiments showed significant changes of the tear fluid protein pattern and the induction of cell damage in cultures of conjunctival cells, following the incubation with pollen extracts and fine dust suspensions. These effects increased especially when tear fluid was incubated with fine dust suspensions and exposed to UVA light simultaneously. **Conclusions.** The tear film, the ocular surface, the main lacrimal gland and the interconnecting neural reflex loops comprise a functional unit. Disruption of this functional unit due to environmental influences may be an additional cause in the pathogenesis of the sicca-syndrome. The term environmentally induced dry eye seems to be suitable.

VISCOELASTICITY OF HUMAN MEIBOMIAN LIPID FILMS AT THE AIR-LIQUID INTERFACE. Shiwani R. Raju, Chendur K. Palaniappan and Thomas J. Millar. Schoolof Natural Sciences, University of Western Sydney, Australia

Purpose:The meibomian lipid layer withstands the enormous stresses that occur during a blink. Its resistance to this stress is because of both its viscous (fluidity) and elastic (rigidity) properties. While elasticity provides the lipid film with strength, viscosity allows flexibility. Therefore, the viscoelasticity of meibomian lipid films was determined and this was compared with films that were seeded with particular lipids associated in disease states. Since proteins from the aqueous layer are known to interact with the lipid layer *in vivo*, it is likely that major tear proteins contribute to the viscoelasticity of meibomian lipids and hence, this was also tested.
Methods:Meibomian lipid films alone or seeded with 1% cholesterol or 1% -carotene were spread on an aqueous pendant drop and viscoelasticity was measured as a function of oscillation frequencies. Alternatively, protein solutions (lysozyme, lactoferrin, mucin, albumin) were injected into the drop and adsorbed to the lipid film. Temperatures used were 20°C and 37°C. **Results**:Pure meibomian lipid films showed greater elasticity (E') than viscosity (E") indicating that the film is more solid-like. Seeding with cholesterol or b-carotene had no effect on these properties. Adsorption of proteins increased both E' and E" giving the film more gel-like characteristics. These trends were seen at both 20°C and 37°C. **Conclusion**:The viscoelasticity of human meibomian lipid films is resistant to changes in lipid composition, but strongly enhanced by proteins. Therefore, a role for proteins in the lipid layer of the tear film could be to maintain its structure during a blink cycle. [This research was supported by UWS graduate fellowships]

REGENERATIVE MEDICINE OF THE OCULAR SURFACE. Paolo Rama, M.D., Stanislav Matuska, M.D., Giorgio Paganoni, M.D., Alessandra Spinelli, M.D., Michele De Luca, M.D., and Graziella Pellegrini, Ph.D. San Raffaele Scientific Institute, Ophthalmology Unit, Milan (P.R., S.M., G.P., A.S.); and the Center for Regenerative Medicine Stefano Ferrari, University of Modena and Reggio Emilia, Modena, Italy (M.D.L., G.P.).

Background: Corneal renewal and repair are mediated by stem cells of the limbus, the narrow zone between the cornea and the bulbar conjunctiva. Ocular burns may destroy the limbus, causing limbal stem-cell deficiency. We investigated the long-term clinical results of cell therapy in patients with burn-related corneal destruction associated with limbal stem-cell deficiency, a highly disabling ocular disease. **Methods:** We used autologous limbal stem cells cultivated on fibrin to treat 112 patients with corneal damage, most of whom had burn-dependent limbal stem-cell deficiency. Clinical results were assessed by means of Kaplan–Meier, Kruskal–Wallis, and univariate and multivariate logistic-regression analyses. We also assessed the clinical outcome according to the percentage of holoclone-forming stem cells, detected as cells that stain intensely (p63-bright cells) in the cultures. **Results:** Permanent restoration of a transparent, renewing corneal epithelium was attained in 76.6% of eyes. The failures occurred within the first year. Restored eyes remained stable over time, with up to 10 years of follow-up (mean, 2.91±1.99; median, 1.93). In post hoc analyses, success — that is, the generation of normal epithelium on donor stroma — was associated with the percentage of p63-bright holoclone-forming stem cells in culture. Cultures in which p63-bright cells constituted more than 3% of the total number of clonogenic cells were associated with successful transplantation in 78% of patients. In contrast, cultures in which such cells made up 3% or less of the total number of cells were associated with successful transplantation in only 11% of patients. Graft failure was also associated with the type of initial ocular damage and postoperative complications. **Conclusions:** Cultures of limbal stem cells represent a source of cells for transplantation in the treatment of destruction of the human cornea due to burns.

CONCENTRATION-BASED FLUORESCENT OBSERVATIONS OF TEAR FILM BREAKUP P. Ramamoorthy, P.E. King-Smith, J.J. Nichols The Ohio State University College of Optometry

Objective: To assess tear break up time (TBUT) characteristics using low and high fluorescein (Fl) concentrations. For high Fl concentrations, TBUT should be reduced due to the effects of evaporation on fluorescence from "quenching." For low Fl concentrations, quenching should be small, so breakup should be caused mainly by tangential flow; because evaporation should have little effect, breakup time was expected to be increased. **Methods:**1 µl of 0.1% Fl solution was instilled in one eye of subjects. Each was asked to blink a few times, followed by holding the eyes open for up to one minute. Video recordings were made while illuminating the whole cornea. Ten minutes later, 1 µl of 5% Fl was instilled and recordings were made as above. Videos were examined by a masked examiner who determined TBUT values (the time to the observation of the first black spot following complete eyelid opening), which were then compared using the Wilcoxon signed ranks test. **Results:** Twenty subjects (35% female, 34.8 ± 14.0 years) completed the study. Average TBUT values with the 0.1% and 5% fluorescein were 11.5 ± 19.0 sec (n = 18, 2 videos too dim to evaluate) and 3.4 ± 3.2 (n = 20) sec (p = 0.01). **Conclusion:** TBUT values were significantly reduced for the high Fl concentration as expected from the greater contribution of quenching due to evaporation in this condition.

DETECTION OF TEAR GLYCOPROTEINS AND GLYCOSYLATION MOITIES P.Ramamoorthy, J.J.Nichols College of Optometry, The Ohio State University

Purpose: To detect and analyze glycoproteins and glycosylation

moieties in tear samples, using Periodic acid Schiff (PAS) staining, that has been used previously on tissue sections for histochemical staining of glycoproteins and mucus derivatives from systemic mucosal epithelia. **Methods:** Tear samples were obtained from 5 normal subjects by microcapillary tube extraction from the inferior tear meniscus. 10 µl of pooled tear samples, horse radish peroxidase (HRP) as positive control and a molecular weight marker were subjected to electrophoresis in a 3-8% tris acetate gradient gel at 150 V for ~ one hour. Samples were run in duplicate and one half of the gel was stained by a PAS based staining protocol adapted from Thornton and colleagues (1994) and the other half by a Coomassie based staining protocol for comparison of staining profiles. Relevant Coomassie stained bands were cut out for trypsin digestion and liquid chromatography mass spectrometry (LC-MS) for proteomic analysis. **Results:** PAS stained tear and HRP bands but not lysozyme and albumin (tested in a separate gel). Tear bands were detected near 500, 97, 66 and 55 kDa. Coomassie stained similar bands, although with small differences in intensity and band migration. LC-MS analysis revealed several proteins including immunoglobulins and glycoproteins such as zinc-alpha2-glycoprotein, lacritin precursor, glycoprotein 340 and heparin sulfate proteoglycan perlecan. **Conclusions:** PAS-based staining of tris acetate gels is a novel method for detection of glyco-moieties in tears. Analysis of differences in glycosylation properties may offer insights into mechanisms underlying ocular surface disorders such as dry eye.

DRY EYE MODULATES THE EXPRESSION OF ANTIMICROBIAL PEPTIDES ON THE OCULAR SURFACE. R. L. Redfern[1], W. Farley[2], C. S. De Paiva[2], S. C. Pflugfelder[2] and A. M. McDermott.[1] College of Optometry, University of Houston, Houston, Texas,[1] Baylor College of Medicine, Ocular Surface Center, Cullen Eye Institute, Houston, Texas[2]

Purpose: Severe dry eye increases the risk for corneal infection and vision loss. Here we examined the expression of antimicrobial peptides (AMPs) such as defensins and LL-37 in human and mouse dry eye samples. **Methods:** EDE was induced in 6-8 week old C57Bl/6 mice by subcutaneous scopolamineinjection (2.5 mg/ml)four times a day, exposure to low humidity and an air draftfor 5 days (n=3). Eyes were removed and immunostained for AMPs (n=2). RNA was extracted from the corneal epithelium, conjunctiva and lacrimal gland from untreated (UT) and EDE mice and from conjunctival impression cytology (CIC) samples from dry eye (n=3) and age-matched normal subjects. Real-time PCR compared the expression between normal/UT and dry eye samplesfor mBD-3, mBD-4 and cathelin-related antimicrobial peptide (CRAMP) in the mouse; human (hBD)-2 and LL-37 in the human. **Results:** In the corneal epithelium, CRAMP (mouse homologue to LL-37) mRNA was significantly decreased by 1.83±0.29 fold, mBD-3 was upregulated by 1.4±0.28 fold and there was no significant change in mBD-4. Immunostaining revealed a decrease in CRAMP but no change in mBD-3 or -4 protein. In the conjunctiva, there was a significant decrease in only mBD-4 mRNA by 1.59±0.09 and none of the mouse AMPs were modulated in the lacrimal gland. CIC samples revealed no significant change in LL-37 but hBD-2 was upregulated by 7.3fold in the two moderate dry eye subjects and 33fold in the severe dry eye patient compared to the age-matched normal subject. **Conclusion:** A balance of expression between AMPs in both the mouse and humans may exist to provide constant protection against microbial infections during dry eye. Support:NIH grants EY13175 (AMM), EY18113 and NIH Loan Repayment(RLR), EY07551 (UHCO CORE grant). Commercial Relationships:R.L. Redfern, None; W. Farley, None; C.S. De Paiva, None; A.M. McDermott, None;S.C. Pflugfelder, None.

TRANSPLANTATION OF CONJUNCTIVAL EPITHELIAL CELLS CULTIVATED EX VIVO INPATIENTS WITH TOTAL LIMBAL STEM CELL DEFICIENCY. Jose RS Ricardo[1,2,] Jose AP Gomes[1,2,] Ocular Surface Advanced Center (CASO),[1] Cornea and External Disease Service, Department of Ophthalmology, Federal University of São Paulo, São Paulo, Brazil2

Objective: To report the clinical and anatomopathologic results of transplantation of conjunctival epithelial cells cultivated ex vivo in patients with total limbal stem cell deficiency (TLSCD). **Methods**: Twelve eyes of 10 patients with TLSCD was submitted to autologous conjunctival epithelial cells transplantation cultured ex vivo in amniotic membrane. The cultivated tissue was transplanted to the recipient eye after superficial keratectomy. Impression cytology, immunocytochemistry and confocal microscopy were performed in the preoperatively and 6 months postoperatively. Complete success was defined as improvement in clinical parameters (corneal opacity, epithelial integrity and surperficial neovascularization) and cytological findings. Main Outcome Measures: clinical parameters of TLSCD (cornea opacity, superficial corneal neovascularization, epithelial integrity), visual acuity, impression cytology and cytokeratin profiles, and in vivo corneal confocal microscopy. Three patients were submitted to penetrating keratoplasty and histopathologic features of the recipient corneal buttons were studied with special attention to epithelial status. **Results**: The overall success rate for this treatment in our cohort was 10/12 (83.3%), where complete success was achieved in 8 patients (66.7%) and partial success in 2 patients (16.7%) in a mean follow-up time of 12.1 months (range, 6-20 months). Visual acuity improved in 7 of 12 eyes (58.3 %) to the range of hand movements to 0.5. Clinical outcomes (corneal opacity, epithelial integrity and surperficial neovascularization) improved respectively from 3.67 ± 0.49 to 2.42 ± 0.79 ($p<0.01$), 3.67± 0.49 to 1.67 ± 0.98 ($p<0.01$) and 3.67 ± 0.49 to 1.83 ± 0.57 ($p<0.01$). In postoperative evaluation, 3/8 eyes (37. 5%) showed the corneal phenotype and 5/8 (62. 5%) displayed a mixture of both conjunctival and corneal phenotypes. CK3 expression was positive in 38.27% preoperatively and 50.97% postoperatively, and CK19 expression in 46.58% preoperatively and in 41.61% postoperatively. In vivo confocal analysis and anatomopathologic features confirmed the clinical and cytological findings. **Conclusions**: We demonstrated the effectiveness of transplantation of conjunctival epithelial cells cultivated ex vivo for corneal surface reconstruction in cases with TLSCD. Future studies are needed to further assess the long-term efficacy of this procedure. Financial Disclosures(s): The authors have no proprietary or commercial interest in any materials discussed in this article.

CONJUNCTIVAL INFLAMMATION IN PATIENTS UNDER TOPICAL GLAUCOMA TREATMENT. Maria L. Veronese Rodrigues, Joao Marcello F.Furtado, Jayter S.Paula, Régia P. Lira, Edson G. Soares, Eduardo A. Donadi, Eduardo M. Rocha Medical School of Ribeirão Preto, University of São Paulo, Brazil.

Objective: To compare the frequency of conjunctival inflammation in eyes treated with topical prostaglandin analogues, versus eyes treated with other antiglaucomatous drugs. **Methods**: The study searched the inflammatory marker HLA-DR, in biopsies of the bulbar conjunctiva from 32 eyes (32 patients), 24 treated with prostaglandin analogues (Group 1) and 8 receiving other pharmacological agents (Group 2). To detect the amount of cell staining in areas of conjunctiva it was used the Image J software (version 1.41, National Institutes of Health, USA). The data were expressed as percentage of positive cell staining. **Results**: Of the 32 eyes, 13 from de Group 1 (54.1%) and 2 from de Group 2 (25%) were positive for the inflammatory marker HLA-DR. The percentage of stained cells ranged from 15.48 to 48.09% (median: 33.43) in

Group 1. In Group 2 the percentages were 18.35 and 28%. The differences were not statistically significant (p=0.23). **Conclusions**: This study suggests that conjunctival inflammation may be attributed to both, prostaglandin analogues and vehicle of drugs used in glaucoma treatment. Large samples studies are necessary to confirm the trend of higher frequency of inflammation in eyes of prostaglandin analogues users.

LACRIMA*: THE ITALIAN REGISTER OF PATIENTS WITH TEAR DYSFUNCTION

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Objective. Tear Dysfunction (TD) is one of the most frequent pathological conditions in Ophthalmology. The aim of the Register is that of taking a census of patients suffering from TD, in an on-line case history. The foundation of a Register, acquires then a social relevance, beyond the clinical one: for the first time in Europe and in the World, an institution wants to photograph, monitor and manage this phenomenon over time. **Method.** The web-based Case History Chart is aimed to manage the online patients case-history, enabling the Ophthalmologists to manage their patients database using any personal computer and an internet connection everywhere and every time they need. **Result.** The structured Case History Chart provides a guide to step by step input of clinical as well as anamnestic information about life style environment or medical conditions according to the latest indications of scientific knowledge. This leads to shareable homogeneous data available for consultation and statistical evaluations. With patient permission, the data will be easily available to other ophthalmologists to obtain a second opinion from a colleague afferent to Lacrima* network. **Conclusion.** The purpose is to create a network of centers across Italy and possibly world-wide that will operate accordingly the most up-to-date guidelines on the approach to the patient affected by TD [The authors thank Hippocrates® Research S.r.l. and Nidek Technologies for the project management. This project was supported by grants from Farmigea S.p.A.]

MICRO-ENGINEERED SILK BIOMATERIALS FOR OCULAR SURFACE RECONSTRUCTION.

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Objectives: Silk biomaterials are amenable for use in tissue engineering due to highly controllable material properties and lack of antigenicity. We investigated the ability for micro-engineered silk films to serve as substrates for the growth of immortalized and primary corneal epithelial cells, as well as the ability of surface modifications to direct epithelial cell movement. **Methods:** Micro-sized gratings (2 um pitch, 2 um width, 1.5 um depth) were fabricated on the silk film surfaces by using PDMS molds with different patterns (lines, rings and spiral). An immortalized cell line (HCLE) and primary human and rabbit corneal epithelial cells were plated on silk films and the attachment and proliferation of cells monitored by microscopy (light and electron) and cell proliferation assays. Time-lapse imaging was performed on cells plated at varying densities and the cell polarity and migration vectors measured. Cells were analyzed by immunohistochemistry to localize effect of substrate surface topography on the orientation of actin and the distribution of focal adhesions. To correlate the dynamic interactions between cell movement and the reorganization of cell adhesions and the cytoskeleton, we transfected cells with expression plasmids encoding actin and vinculin fused to green fluorescent protein spectral variants. These fusion protein expressing epithelial cells were analyzed on silk film substrates via live cell imaging. Unmodified silk films were applied to anesthetized rabbits as corneal onlays and the tissue response to these films monitored. **Results:** Silk film substrates with all topographies supported the adhesion and proliferation of immortalized and primary corneal epithelial cells, albeit at reduced levels compared to tissue culture plastic. Epithelial cells grown on silk films retained their epithelial morphology. Single cells were found to orient themselves along the axis of grooves. Cells grown in sheets not only demonstrated the orientation of cell polarity, but also of cell migration. Notably, each geometric pattern imbued distinctive effects on collective cell migration. Immonohistochemical and live cell time-lapse imaging revealed and increase of intracellular actin and focal adhesions along the patterned grooves in primary cell cultures. In vivo application of silk films was well tolerated with minimal tissue reaction to silk applied on the ocular surface or within the stroma. **Conclusions:** Micro-engineered silk films are well tolerated in vivo and can alter cell migration in vitro. Further evaluation of these films may produce improved materials for ocular surface reconstruction. Commercial relationships: M. Rosenblatt, Bombyx Technologies, Inc.; B.D. Lawrence, Bombyx Technologies, Inc.; Z. Pan, None. Support: NIH K08EY015829, R21EY019561, T32EY007138 and R24EY015656. Research to Prevent Blindness Career Development Award. Tri-Institutional Stem Cell Initiative.

MULTICENTER, RANDOMIZED, CONTROLLED, DOUBLE-MASKED, CROSSOVER STUDY ON EFFICACY AND SAFETY OF CYCLOSPORINE A EYE-DROP TREATMENT IN VERNAL KERATOCONJUNCTIVITIS (VKC).

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Purpose: to evaluate the efficacy of Cyclosporine A (CsA) 0.05% vs ketotifen fumarate (KF) 0.025% eye drops in preventing VKC relapses and to evaluate the efficacy of CsA 0.1% vs Dexametasone (D) 0.15% eye drops in the active VKC. **Methods:** a multicenter, randomized, double-masked, controlled study was performed on 34 patients (28M, 6F, 6-33 years old) with VKC in non-active phase. Patients were randomized to receive either CsA 0.05% or KF bid for 6 months during the spring season followed by a cross-over study in the second year. Patients were evaluated at baseline and after 1, 3, 6 months. Primary outcome was the number of VKC relapses. During the relapses of disease, VKC patients were randomized to receive either CsA 0.1% or D qid for one week. Signs (hyperemia, secretion, tarsal and limbal papillae, Trantas dots, superficial punctuate keratitis and ulcer) and symptoms (itching, photophobia, redness, tearing, secretion and decreased vision) were evaluated. Safety was assessed by the number of adverse events and drop-out. **Results:** Twenty-five patients completed the study, with 9 drop-outs (n=3 CsA, n=6 KF group). A significant reduction in the number of VKC relapses was observed in CsA vs KF treatment (15 vs 32 relapses; p =0.03). Itching, photophobia and conjunctival hyperemia scores were significantly lower during CsA vs KF treatment ($p<0.05$). Treatment with D showed significant amelioration of conjunctival hyperemia ($p<0.001$), secretion ($p=0.01$), Oxford scores ($p<0.01$) and all symptoms evaluated ($p<0.05$) when compared to CsA 0.1% eye drops. **Conclusion:** Compared to KF, CsA treatment bid provided significant decrease of seasonal relapses in patients with VKC. Both treatments were well tolerated. CsA eye drops may be used as a steroid-sparing agent in patients with VKC. Topical steroid treatment resulted in a better control of VKC active-phases than CsA. Financial disclosure: none

DIURNAL, DIFFERENTIAL CONTROL OF BIOACTIVITY OF PR0-INFLAMMATORY CYTOKINES AND CHEMOKINES. R Sack, B Cooper, S Sathe, A Beaton, P Iserovich. SUNY

TEXT:Objective: Overnight eye closure induces inflammation and the accumulation of pro inflammatory and angiogenic chemokines and cytokines in tears at levels that can exceed those in open eye tears (O) in severe allergic and autoimmune diseases (Sack et al. Prog.Ret Eye Res 2000, ARVO 2010). This study was designed to identify what factors down regulate cytokine induced inflammatory damage during overnight eye closure. **Method:** Matched O and C were assayed using ultra sensitive micro well arrays for >80 proteins. Samples were separated by size exclusion HPLC and then analyzed. Separated complexes were characterized using a modified immuno pull-down technique. **Result:** O fluid contains trace levels of pro-inflammatory cytokines including TNF, INF, IL1 and much higher levels of a wide range of decoy receptors and receptor antagonists. Most of these factors increase in concentration >10x in C fluid. Several chemokines, soluble integrins and selectins also increase in C in a selective manner consistent with a functional role in PMN recruitment and activation. HPLC reveals that virtually all cytokines in C are present in the form of inactive macromolecular complexes the composition have been partially characterized and are thus tagged for degradative processing while nearly all chemokines are present as bioactive monomers. **Conclusion**; Pro-inflammatory cytokine activity in tears is tightly controlled by formation of bio-inactive complexes. These complexes could mask epitopes that are employed for some immunological assays. C exhibits excess buffering capacity preventing inappropriate targeting of the ocular epithelium

TREATMENT OF PERSISTENT CORNEAL EPITHELIAL LESIONS AFTER VITREOUS SURGERY BY PUNCTAL PLUG OCCLUSION. Miki Sakata, [1,2] Hirotsugu Ogura[2], Wakita Eye Clinic[1] ,Tokyo, Kozawa Eye Hospital and Diabetes Center [2], Mito, JAPAN

Purpose . Persistent epithelial corneal lesions after vitreous surgery are caused by multiple factors including decreases in corneal sensitivity, dry eye and eye drop toxicity, Treatment by punctal plugs are thought to promote epithelial healing by improving the dry eye state through cytokines such as EGF, TGF-, which are reported to be present in the tear fluid. **Methods** . Ten eyes with persistent corneal epithelial lesions after vitreous surgery were treated by punctal plug occlusion(7 eyes with diabetic keratoepitheliopathy, 1 eye with dry eye complicated by rheumatoid arthritis and 2 eyes with other pathologies). The corneal epithelial lesions were evaluated by area and density and Schirmer tests and corneal sensitivity was examined before treatment. **Results**. Epithelial lesions improved in all eyes. Four eyes with complete remission and 6 eyes with milder staining compared to prior treatment. **Conclusions**. Treatment of persistent corneal epithelial lesions after vitreous surgery by punctal plug occlusion was found to be effective even in cases with only mild dry eye. This improvement is probably due to increased availability of growth factors in the tear film to the ocular surface. Punctal occlusion is an easy and effective treatment in such cases and therefore should be considered one of the primary options in their treatment.

MOXIFLOXACIN AND CHOLESTEROL COMBINED TREATMENT OF PNEUMOCOCCAL KERATITIS. Melissa E. Sanders[1], Nathan A. Tullos[1], Sidney D. Taylor[1], Erin W. Norcross[1], Lauren B. King[1], Isaiah Tolo[1], and Mary E. Marquart[1]. [1]Department of Microbiology, University of Mississippi Medical Center, Jackson, MS 39216.

Purpose: To compare the efficacy of treatment of pneumococcal keratitis with cholesterol alone, moxifloxacin alone, or a mixture of moxifloxacin and cholesterol (moxifloxacin/cholesterol). **Methods:** New Zealand white rabbits were injected intrastromally with 106 colony-forming units (CFU) of a clinical keratitis strain. The eyes were scored to determine severity of infection at 24 hours post-infection (PI), treated once every two hours from 25 to 47 hours PI, then scored again at 48 hours PI. Corneas were harvested at 24 hours PI for baseline log10 CFU and 48 hours PI for treatment groups to quantitate bacterial CFU. Myeloperoxidase (MPO) activity was measured at 48 hours PI. Eyes were extracted for histology. **Results:** Eyes treated with moxifloxacin/cholesterol had a significantly lower mean SLE score than eyes treated with PBS, moxifloxacin alone, or cholesterol alone ($P \leq 0.02$). A significantly lower log10 CFU was recovered from corneas of eyes treated with moxifloxacin/cholesterol and eyes treated with moxifloxacin alone as compared to corneas of eyes treated with PBS or cholesterol alone ($P < 0.01$). At 48 hours PI, significantly lower MPO activity was observed in eyes treated with moxifloxacin/cholesterol as compared to eyes treated with cholesterol or moxifloxacin alone ($P \leq 0.046$). Eyes treated with moxifloxacin/cholesterol had fewer PMNs and less corneal destruction than eyes from all other treatment groups. **Conclusions:** Treatment with a mixture of moxifloxacin and cholesterol significantly lowers the severity of infection caused by pneumococcal keratitis as compared to treatment with moxifloxacin alone, cholesterol alone, or PBS. This treatment mixture eradicates the bacteria in the cornea, unlike treatment with PBS or cholesterol alone. Using cholesterol with moxifloxacin as a treatment for bacterial keratitis could help lower the clinical severity of the infection. This research was supported by Public Health Services Grant R01EY016195, National Eye Institute, National Institutes of Health.

PHOSPHOLIPIDS IN TEARS, CONTACT LENSES AND MEIBUM? Jennifer T. Saville[1], Zhenjun Zhao[2], Mark D.P. Willcox[2,3], Todd W. Mitchell[4] and Stephen J. Blanksby[1]. [1]School of Chemistry and [4]School of Health Sciences, University of Wollongong, NSW 2052, [2]Brien Holden Vision Institute and [3]School of Optometry and Vision Science, University of New South Wales, NSW 2052, Australia.

Purpose: Phospholipids are proposed to play a critical role in the stability of the tear film. We have recently characterised 23 phospholipids in tears, many of which are also present in contact lens deposits. Meibum has been suggested as a potential source of lipids in tears, however the presence of phospholipids in this secretion has recently been questioned. Herein, we undertake an expanded examination of the tear phospholipidome and also examine meibum using contemporary lipidomic methods. **Methods:** Lipids were extracted from basal tears, worn contact lenses and meibum by standard bi-phasic extraction. Extracts were analysed by mass spectrometry-based lipidomic techniques and quantified using internal standards. **Results:** Fifty-one individual phospholipid molecules were detected in tears, including 21 distinct phosphatidylethanolamine (PE) and phosphatidylserine (PS) molecules. Preliminary results from meibum extracts suggest the presence of a number of phosphatidylcholine and sphingomyelin molecules. **Conclusions:** We were able to detect a number of new phospholipids in tears, including those from previously undetected PE and PS classes. Preliminary results also suggest the presence of phospholipids in meibum, with their profile showing strong homology to that in tears. [This research is supported by the Australian research Council (Linkage Project Grant LP0989883) and the Brien Holden Vision Institute.]

GENDER DIFFERENCES IN DRY EYE DISEASE IMPACT, MANAGEMENT, PATIENT SATISFACTION, AND COMORBID CONDITIONS Debra A. Schaumberg,[1] Jim Li[2] [1]Div of Preventive Med, Brigham & Women's Hospital, Harvard Medical School, Boston MA; [2] Outcomes Research, Pfizer, Inc., San Diego CA

Purpose: Dry eye disease (DED) affects women about twice as often as men, but there is little information on the impact of DED on patients' lives and vision, how they are treated, what impact treatments may have, and whether such factors differ by gender. **Methods**: We surveyed 4000 participants from the Women's Health Study of 39,876 female health professionals and the Physicians' Health Studies I and II of ~26,000 male physicians. Participants were selected based on a previous report of a diagnosis of DED or severe symptoms. The questionnaire focused on symptoms, treatments, comorbid conditions, certain medications, patient satisfaction, and impact of the disease. This report is based on completed questionnaires from the first 1925 participants who reported a DED diagnosis. **Results**: The study population consisted of 1,390 women (mean age 70.7 y) and 535 men (mean age 76.7 yr), with a mean duration of DED of 10.5 yr and 10.1 yr, respectively. The frequency and severity of DED symptoms were higher among women (each P<0.0001), and had a greater impact on everyday activities (P<0.0001). Women were more likely to use artificial tears (P<0.0001) use them more often (P=0.0003), and use Restasis® (P<0.0001), omega-3 fatty acids (P<0.0001), and have punctal plugs (P=0.0005). On average, women spent more money per month on dry eye treatments (P<0.0001), but reported less satisfaction with their treatments (P=0.006). Women were more likely to have lupus (P<0.02), Sjogren's syndrome (P=0.0004), rosacea (P=0.01), depression (P<0.0001), anxiety (P<0.0001), hayfever (P<0.0001), and dry mouth symptoms (P<0.0001), but less likely to have blepharitis (P=0.0004), and meibomian gland disease (P<0.0001). Women were also more likely to use antihistamines (P=0.01), and antidepressants (P<0.0001), but less likely to use glaucoma medications (P=0.0003), all previously shown to increase risk of DED. **Conclusions**: These data are the first to show that DED is generally more severe among women, having a greater impact on well-being. This work was sponsored by Pfizer, Inc.

OCULAR SURFACTANT PROTEINS AND THEIR REGULATION IN DRY EYE DISEASE.
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Purpose. Surfactant proteins (SPs) are originally known from lung tissue and have been detected in the meantime in a bulk of different extrapulmonary human tissues. SPs are surface-active agents and important players of the innate immune system. SP-A and SP-D have been shown to immobilize microorganisms by binding to bacterial surfaces. This in turn triggers the opsonization processes. In contrast, SP-B and SP-C are able to lower surface tension of interphases and thus support the rheology and disassembly of mucous fluids. Aim of the present study was to investigate the expression pattern of SPs within tear fluid of patients suffering from dry eye disease in comparison to healthy volunteers. **Methods**. Tears were collected from altogether 447 persons by Schirmer's strip method. Of these 307 persons suffered from different forms of dry eye disease, whereas 140 volunteers were free of dry eye related symptoms as well as other ophthalmological diseases or traumata. The obtained tear fluid was extracted and quantitatively analyzed for SP-A, -B, -C and -D by means of ELISA. **Results**. In cases of dry eye disease the protein concentration of all four surfactant proteins was significantly increased (p>0,05) compared to samples from healthy volunteers. **Conclusion**. The results suggest possible immunmodulatory effects of SP-A and SP-D at the ocular surface. Furthermore, SP-B and SP-C seem to reduce the surface tension of the tear film. In this context we suppose that surfactant proteins might be considered as players of the innate immune system at the ocular surface and thus could be of special interest for therapeutical approaches for the treatment of dry eye.

EFFICACY OF NOVEL THIOLATED BIOPOLYMER IN THE TREATMENT OF DRY EYE SYNDROME. Leopold Schmetterer, Sonja Hoeller, Margit Hornof Medical University of Vienna, Croma Pharma

Objective: To demonstrate the safety and efficacy of a new eye drop formulation containing a novel thiolated biopolymer, namely chitosan-N-acetylcysteine, in a number of pre-clinical tests. It is postulated that interaction between thiol groups of the topically applied chitosan-N-acetylcysteine and cysteine-rich mucin (MUC5AC) increases polymer residence time on the ocular surface and tear film stability. **Methods**: The efficacy of isotonic and buffered chitosan-N-acetylcysteine eye drops was evaluated in two different dry eye mouse models. The dosage dependent ocular residence time and biodistribution were investigated in a rabbit model using microPET technology. Long-term irritation and delayed-type hypersensitivity tests with chitosan-N-acteylcysteine eye drop formulation were conducted in rabbits. **Results**: In both dry eye studies mice treated with chitosan-N-acetylcysteine showed decreased expression of ocular surface mRNA of IL-, IL-10, IL-12, and TNF, indicating that the formulation may have protective ocular surface properties. The residence time of chitosan-N-acetylcysteine eye drops on the ocular surface of rabbits was increased (detection up to 22h). Results of a long-term ocular irritation study in rabbits demonstrate that the novel formulation is well tolerated and non-irritant to the eye. **Conclusion**: In animal models buffered chitosan-N-acetylcysteine eye drops showed good efficacy and no adverse reactions. Based on these promising pre-clinical study results a phase I clinical trial is scheduled in the near future. Commercial Relationships: The studies have been sponsored by Croma pharma

DEVELOPMENT OF A SERUM FREE AND XENOBIOTIC FREE SURROGATE CULTURE SYSTEM FOR HUMAN LIMBAL EPITHELIAL STEM CELL THERAPY. Schrader S[1,2,3], Tuft SJ[2], Beaconsfield M[2], Geerling G[4], Daniels JT[1,2], Notara M[1]. [1]UCL Institute of Ophthalmology, London, UK, [2]Moorfields Eye Hospital NHS Foundation Trust, London, UK, [3]Department of Ophthalmology, University of Luebeck, Germany, [4]Department of Ophthalmology, Julius-Maximilian-University Wuerzburg, Germany

Purpose: To establish and evaluate a serum-free and xenobiotic-free co-culture system that acts like a surrogate in-vitro niche environment for human limbal epithelial cells (HLEC) and can sustain their progenitor cell characteristics. This is achieved by co-culturing HLEC with mitotically active human limbal fibroblasts (3.1SF system). This method of culture is compared to the gold standard culture protocol in which bovine serum and 3T3 murine fibroblasts are used (GS system). **Methods**: HLEC were cultured in the presence of mitotically active limbal fibroblasts and their putative stem cell characteristics in terms of total colony forming efficiency, cell population doublings and expression of p63alpha and ABCG2 were compared to epithelial cells cultured under GS conditions over several passages. Additionally gene expression differences between the culture systems were explored using a genome level microarray platform. **Results**: The colony forming efficiency decreased with passaging under both culture conditions, however cells cultured

under 3.1SF conditions showed a significant higher colony forming efficiency compared to HLEC cultured under GS conditions at P0 and P1. Immunoreactivity of p63 and ABCG2 was found in both groups until passage 4 and their presence was also confirmed by semi-quantitative RT-PCR. Comparative gene expression array analysis of HLEC cells harvested from 3.1SF versus GS conditions indicated that genes in the MAPK-cascade as well as the Wnt signaling pathways were down-regulated in 3.1SF conditions. Notably, the Wnt signaling pathway genes that were down-regulated in 3.1SF conditions were linked to stem cell differentiation. **Conclusion**: Our data demonstrated that the described serum-free and xenobiotic-free culture system was superior compared to the standard protocol in terms of preserving the progenitor cell characteristics of HLEC. It is therefore proposed to be a useful tool for the in vitro expansion of HLEC for clinical use as well as for the investigation of cell-cell interactions between fibroblasts and epithelial cells in an in-vitro niche model. This work was supported by the Special Trustees of Moorfields Eye Hospital, a research fellowship grant (SCHR 1210/1-1) from the "Deutsche Forschungsgemeinschaft (DFG)", NIHR BMRC for Ophthalmology, Gertrud Kusen Stiftung

POTENTIAL ROLE OF TFF3 IN CORNEAL WOUND HEALING. U. Schulze,[1] L. Contreras Ruiz,[2] A. López,[2] N. Barker,[3] Y. Diebold,[2] F. Paulsen[1],[4]. Department of Anatomy and Cell Biology , Martin Luther University Halle, Germany,[1] IOBA University of Valladolid, Spain,[2] GI Company, Framingham, USA,[3] Department of Anatomy II, Friedrich Alexander University Erlangen, Germany[4]

Purpose. Wound healing is a complex process including cell contact and extracellular matrix (ECM) remodeling as well as epithelial cell migration. Trefoil Factor 3 (TFF3) has been found to act protective and promote wound healing processes. Our study tries to evaluate its proper role in corneal wound healing. **Methods**. After treating corneal epithelial cell line (Araki-Sasaki, HCE) with the proinflammatory cytokine IL- 1b TFF3 expression was analyzed by ELISA to evaluate preconditions. Recombinant human TFF3 (rhTFF3) effect on HCE cell migration was studied by a scratch assay. To evaluate changes in ECM remodelling cells were pretreated with IL-1b and rhTFF3 for 6 and 24h, and MMP1,-9 and -13 expression was analyzed by real time PCR. RhTFF3 effect on tight junction complex proteins ZO1, ZO2, claudins1 and 2, and occludin was studied by real time PCR and Western blot analysis after 5min, 6 and 24h stimulation time. **Results**. TFF3 secretion was induced after IL-1b treatment of HCE cells. rhTFF3 treatment led to enhanced HCE cell migration and changes in MMP and tight junction complex expression pattern. IL-1b induced MMP9 mRNA expression. This was further increased by rhTFF3 after 6 and 24h whereas IL-1b-induced MMP1 and MMP13 expression levels were reduced by additional rhTFF3 treatment. Tight junction protein claudin1 expression decreased after rhTFF3 exposure for 5min, 6 and 24h whereas ZO2 protein expression increased. Other tight junction proteins showed no significant changes on protein level. **Conclusion**. Induction of TFF3 in the presence of IL-1b shows its relevance under inflammatory conditions. TFF3 seems to promote migration of surrounding HCE cells, influence ECM remodelling and the formation of cell-cell contacts and thereby might play an important role in corneal wound healing. Support. DFG grant PA738/9-2;BMBF Roux program grants FKZ 9/18,12/08,13/08;GI Company;Bilateral Research Grant DE2009-0085 and DAAD ID6234017;FEDER-CICYT MAT2007-64626-C02-01

THERAPEUTICAL USE OF A NEW BIODEGRADABLE DRUG DELIVERY SYSTEM FOLLOWING CORNEAL TRANSPLANTATION. J Schwartzkopff[1], A Hyatt[2], L Bredow[1], C Noack[1], P Eberwein[1], K Martin[2], T Reinhard[1] 1University Eye Hospital, Freiburg, Germany 2 Cambridge Centre for Brain Repair, University of Cambridge, United Kingdom

Objective: Allograft rejection is the major cause leading to corneal graft failure. Systemic immunosuppressives improve graft survival. However, toxic side effects often occur. To avoid these, a fibrin based delivery system was loaded with Cyclosporine A (CsA) and analyzed for its efficacy in the rat keratoplasty model. **Method**: CsA release into medium was measured daily by mass spectrometry *in vitro*. Bioactivity of released CsA was verified by analyzing its capacity to inhibit T cells in a standard CFSE-proliferation assay. HE staining was done and the inflammatory reaction following subconjunctival implantation was examined. Keratoplasty was performed between Fisher donor and Lewis recipient rats. A single CsA-fibrin gel was implanted following transplantation. Clinical evaluation was carried out until rejection occurred. An empty delivery system was used for control studies. **Result**: *In vitro*, CsA release was relatively constant over two weeks. Released CsA proved to be bioactive as it inhibited T cells specifically. Following implantation, significant CsA levels were only found in the anterior chamber whereas no CsA was detectable in serum. Gels stayed stable for more than seven days causing only mild mononuclear side reaction. Implantation lead to >60% graft survival following keratoplasty compared to 0% in control treated animals ($p<0.01$). **Conclusion**: Our results demonstrate that a modified fibrin-gel releases bioactive CsA *in vitro. In vivo*, only a mild inflammatory reaction is observed following implantation. Even without relevant CsA serum levels, a single CsA-fibrin-gel implantation is sufficient to promote allograft survival. We therefore propose fibrin as a carrier system for immunosuppressive drugs in the treatment of keratoplasty or other inflammatory diseases of the ocular surface. JS, AH, LB, CN, PE, KM, TR: no commercial relationship. Financial support from the University Eye Hospital Freiburg.

EICOSANOIDS IN THE OCULAR SURFACE AND TEAR FILM. Michal L. Schwartzman. Departments of Pharmacology & Ophthalmology, New York Medical College, Valhalla, New York, USA

Purpose: To maintain the cornea as an optically transparent barrier, a sophisticated self-resolving inflammatory-reparative process must be in place to balance inflammation and immune privilege while promoting wound repair. Such a process must include proas well as anti-inflammatory circuits that work in concert to initiate, mediate and resolve inflammation in a controlled manner so as to allow for the repair process to proceed towards complete restoration of structure and function, i.e., healing and repair. Among these mediators are the arachidonic acid-derived lipid mediators, namely eicosanoids, which are synthesized through three enzymatic pathways: (i) cyclooxygenase (COX) to form prostaglandins (PGs), prostacyclin and thromboxane; (ii) lipoxygenase (LOX) to form hydroxyeicosatetraenoic acids (HETEs) and leukotrienes (LTs) and lipoxins (LXAs); and (iii) cytochrome P450 (CYP) monooxygenases to form epoxyeicosatrienoic acids (EETs) and HETEs. **Methods**: Corneal tissues and tears were processed for LC-NS/MSbased lipidomics along with biochemical and molecular/genetic methods to examine the contribution of each pathway to the pathogenesis of ocular surface diseases. **Results:** The primary enzymatic site of these activities within the cornea is the epithelium. Injury dramatically increases the production of COX-, LOX- and CYP-derived eicosanoids by the corneal epithelium. Moreover, invading inflammatory cells present additional source primarily for COX and LOX activities. Human tear film contains picogram to nanogram amounts of eicosanoids. These eicosanoids have both pro- and anti-inflammatory properties and as such participate in all phases of the inflammatory and reparative response, including initiation, continuation, resolution and ultimately, repair. **Conclusions:** The

specific role of each eicosanoid pathway as well as the implementation of new lipidomics methodology to assess tear film eicosanoid composition and its implication in the pathogenesis of ocular surface disease will be discussed. Supported by NIG grants EY06513 and HL34300 Commercial relationship: None

RAB GTPASES IN REGULATED SECRETION AND DISEASE. Miguel C. Seabra. Molecular Medicine, Imperial College London, London, UK; Faculdade de Ciencias Medicas, Universidade Nova de Lisboa, Portugal; and Instituto Gulbenkian de Ciência, Portugal

One main question in Molecular Cell Biology is focused on understanding how cells achieve their highly sophisticated internal compartmentalisation. How are organelles made, maintained (identified) and how do they communicate with each other? Our work has dealt with this problem and our approach has been to focus on Rab GTPases, key regulators of membrane traffic. We have been studying the role of Rabs in melanosome biology in both skin melanocytes and the retinal pigment epithelium (RPE). We have described that Rab27a regulates melanosome motility by promoting microtubule to actin transitions. This is accomplished via the recruitment of melanophilin and myosin V to mature melanosomes. Mechanistic details about the formation of this complex are still unclear. Griscelli syndrome associates partial albinism with immunodeficiency due to cytotoxic T-lymphocyte killing activity defects and results from loss-of-function mutations in Rab27a. We wish to understand why mutations in Rab27a lead to disease restricted to melanocytes and T-lymphocytes. We suggested that the related protein, Rab27b, which exhibits a more restricted pattern of expression, may compensate for the loss of Rab27a in some cell types. We have recently created a Rab27b knock-out mouse and showed that Rab27b plays an essential role in the formation and secretion of platelet dense granules. Therefore, some cells express preferentially Rab27a and others Rab27b. However, some cells such as mast cells express both. Surprisingly, the loss of each isoform leads to opposite phenotypes, loss of Rab27b leads to hyposecretion whilst Rab27a loss leads to hypersecretion. The molecular basis for these observations and the various roles Rab27 proteins play in regulated secretion will be discussed.

EVALUATION OF METHODS EMPLOYED FOR THE QUANTITAION OF TEAR SECRETION. Michelle Senchyna,[1] Ravaughn Williams,[1] Carolyn Begley,[2] Kelly K. Nichols,[3] Sruthi Srinivasan,[3] Jenny Devenport,1 Michael Brubaker.[1] Alcon Research Ltd,[1] Indiana University School of Optometry, [2] The Ohio State University College of Optometry.[3]

Purpose: Tear secretion is a common measure for the evaluation of dry eye, however an appropriate means to quantify tear secretion has not been well characterized. Three common methods of measuring tear secretion are: (1) the Schirmer 1 Test (ST), (2) the Phenol Red Thread Test (PRT), and (3) Tear Meniscus Height (TMH). Several studies have evaluated these methods however, no study has extensively evaluated the overall performance of the methods in series and with modifications. The objective of this study was to evaluate the reliability, validity, and ability to measure change with each method. **Methods:** This was a three site, 21-day study consisting of 4 visits (V). Subjects were screened at V1 and grouped based on ST results. There were 159 subjects enrolled (53 non dry eye, 57 mild dry eye, 49 moderate-severe dry eye). At V1, half of the subjects were randomized to receive a 25 µL drop of artificial tear (AT) at V3 and V4, 1 minute prior to the conduct of each test, to simulate an increase in tear volume. Two repeats (done 30 minutes apart) of the following tests were done at V2 – V4: (a) unanesthetized ST for 3 min; (b) PRT for 2 min; (c) TMH. **Results:** ST and PRT measurements met test-retest reliability criteria (pearsons r >0.50) at all visits and ST and PRT exhibited moderate reliability between visits in non AT subjects (pearsons r > 0.60). Correlations between tear secretion methods within visits were poor to moderate, suggesting that each method measures different phenomena. While ST and PRT demonstrated an association (r: 0.46 to 0.51), TMH was not associated with ST or PRT. The experimental manipulation of adding volume was successful, yielding statistically significant increases in scores for ST and PRT, which were different from untreated subjects. **Conclusions:** Overall, the results demonstrated that the ST and PRT as performed here are reliable and capable of detecting volume change when present. However, comparison of data demonstrates that the ST and PRT are not interchangeable.

QUANTIFICATION OF TEAR FILM INFLAMMATORY CYTOKINES IN SJOGREN'S DRY EYE. Michelle Senchyna,[1] Ravaughn Williams,[1] Nancy McNamara,[2] Michael Brubaker[1], Pavel Iserovich,[3] Robert Sack.[3] Alcon Research Ltd,[1] U California-San Fransisco,[2] SUNY School of Optometry.[3]

Purpose: As dry eye disease (DED) is recognized to involve ocular surface inflammation, methods capable of quantifying tear cytokines may provide alternative endpoints for diagnosis and / or monitoring treatment efficacy.This study explored the function of an optimized multi-plex array to quantify tear cytokines collected from Sjogren's (SjS) dry eye subjects. **Methods:** SjS subjects were recruited in two independent studies. All SjS subjects met American-European Consensus criteria. Control subjects were age and sex matched non-SjS, non-DED. Tear samples were collected on Schirmer strips. At one site, strips were immediately placed on dry ice while at the second site strips were air dried and maintained at room temperature (RT) for 12 hours. All strips were stored at -80oC until assay. Tears were extracted from the bulb +10 mm of each strip in 75 µL of tear specific buffer. 30µL of each extract was assayed on a custom antibody array (Quansys) capturing complexed and free forms of: IL1a, IL1b, IL2, IL4, IL5, IL6, IL8, IL10, IL12p70, IL13, IL15, IL17, IFNg, TNFa and TNFb using a modified sandwich ELISA protocol optimized to avoid tear matrix effects and to enhance sensitivity via avidin/biotin amplification and chemiluminescent detection. **Results:** With the exception of IL23 all cytokines can be assayed in the low to sub pg/ml range. The cytokine profile of control tears contained measurable levels of IL1b, IL6, IL8, IL15, IL23. SjS samples exhibited a marked increase in the concentration of all cytokines assayed compared to control. For both SjS and control samples, both sets of data were highly correlated suggesting samples can be stored at RT for short periods of time. **Conclusions:** The SjS tear cytokine profile as defined by antigenic reactivity of extracts from Schirmer strips is significantly different from control. Consistent data were obtained from samples collected from two different populations under different conditions. Taken together, quantitation of tear cytokines may represent a reliable, objective endpoint for DED.

ELEVATED TEAR INTERLEUKIN-17 LEVELS IN SJÖGREN'S SYNDROME DRY EYE PATIENTS. Kyoung Yul Seo, Jong-Hyuck Lee, Sang Yep Lee, Sang Min Nam Yonsei university college of medicine, department of ophthalmology

Objective: To determine linterleukin-17 (IL-17) levels in the tear samples of Sjögren's and non-Sjögren's syndrome dry eye patients by comparing with that of control group **Method**: IL-17 and other inflammatory cytokines (IFN-, TNF-, IL-10, IL-6, IL-4, IL-2) were measured by Multiple bead array system in tear samples obtained from 62 eyes of dry eye patients, 16 eyes of control subjects. Of all dry eye patients, we examined patient to confirm Sjögren's syndrome

dry eye patients. According to diagnostic criteria of primary Sjögren's syndrome, we divided dry eye patients to two groups (15 eyes of Sjögren's syndrome patients, 47 eyes of non-Sjögren's syndrome dry eye patients). We compared each dry eye group with control group. Mann-Whitney U test was used to compare cytokines levels. **Result**: The median levels of IL-17 were 19.21 (interquartile range, 5.21 to 35.74) pg/ML in Sjögren's syndrome dry eye group and 5.17 (0 to 9.57) pg/ML in non-Sjögren's syndrome dry eye group. There were significant differences in IL-17, TNF- and IL-6 levels between Sjögren's syndrome group and control group (p=0.002, p=0.006 and p<0.001). However, only TNF- and IL-6 levels, except IL-17, showed significant differences between non-Sjögren's syndrome group and control group (p=0.03 and p<0.001). **Conclusion**: Some inflammatory factors seem to be shared between the Sjögren's syndrome dry eye patient and the non-Sjögren's syndrome dry eye patient. However, IL-17 may play a different role between them.

REFRACTIVE SURGERY ALTERS CONJUNCTIVAL GOBLET CELLS IN PATIENTS WHO DEVELOP DRY EYE. M Shatos[1], D Ryan[2], K Bower[2], C Coe[2], L Peppers[2] E Guilbert[1], J Doherty[1], R Hodges[1], D Dartt[1] [1]Opthal/Harvard Med Sch, Schepens Eye Research Institute, Boston, MA;[2] Walter Reed Army Medical Center, Washington, DC

Objective: To determine if conjunctival goblet cell (GC) profiles differ in persons who develop dry eye (DE) after laser-assisted in situ keratomileusis (LASIK) or photorefractive keratectomy (PRK). **Method**:Tear film status was evaluated by slit lamp biomicroscopy, Schirmer test, tear breakup time and McMonnies DE questionnaire. Patients with clinically significant DE were enrolled in this study. Impression cytology samples (ICS) were taken from superior and temporal conjunctivae pre-, at 1w, 1m and 3m post-op. ICS on membranes were stained with anti cytokeratin-7 (K7) to identify GC, Helix pomatia agglutinin (HPA) for GC secretory product, and DAPI for cell nuclei. Five random fields were counted per sample. Total cell number was determined by counting DAPI stained nuclei; unfilled GC number by K7 positive cells; and filled GC by K7 cells with HPA. Statistics were performed using student's t-test with p<0.05 as significant. **Result:**19 post- DE patients were studied [9 LASIK, (3M, 6 F, average age 33y); 10 PRK (6M, 4F, average age 27 y)]. The %total GC (filled plus unfilled) decreased at 1w in both PRK (27±5) and LASIK (35±7) when compared to their respective pre- levels of 33±4 and 51±7, but recovered at 3m. For LASIK but not PRK, when the total number of GC was set to 100%, the % filled GC significantly increased at 3m post- (69±11) compared to pre- (40±10). LASIK but not PRK, decreased the number of empty GC at 3m (31±11) to pre- values of (60±11). **Conclusion:** In individuals who develop dry eye after refractive surgery, LASIK appears more damaging to the ocular surface than PRK perhaps by destroying corneal sensory nerves, and preventing GC secretion. CR:None; Support: CDMRP W81XWH-04-2-008

THE MECHANISM OF MUCIN SECRETION FROM ISOLATED RABBIT CONJUNCTIVAL TISSUE BY DIQUAFOSOL. Yuko-Takaoka Shichijo, Tadahiro Murakami, Atsuyoshi Dota, Katsuhiko Shinomiya, Osamu Katsuta, Masatsugu Nakamura. Research and Development Division, Santen Pharmaceutical Co., Ltd., Nara-Osaka, Japan.

Purpose. Diquafosol is a widely known P2Y2 receptor agonist that is expected to be a therapeutic medicine for dry eye. We have investigated the effect of diquafosol on the stimulation of mucin secretion, especially MUC5AC, from isolated rabbit conjunctival tissue and the intracellular mechanisms involved. **Methods**. Pieces of rabbit conjunctival tissue (4 mm in diameter) were isolated from the bulbar conjunctiva and cultured in normal bicarbonate Ringer's solution. The concentration of mucin-like glycoproteins and MUC5AC in culture supernatants was measured by enzyme-linked lectin assay with wheat germ agglutinin (WGA) and the enzyme-linked immunoassay with anti-MUC5AC antibody (Clone 45M1), respectively. Intracellular calcium concentration in the conjunctival epithelial cells isolated from rabbit conjunctival tissue was measured using Fura-2 fluorescence. **Results**.Lectin-blot analysis showed that most WGA-binding glycoproteins in culture supernatants of the conjunctival tissue were larger than 200 kDa. Histochemical analysis revealed that mostly mucus (probably MUC5AC) in the goblet cells, and partly the apical part of the conjunctival epithelium showed positive reactivity for staining with WGA. Exposure of the conjunctival tissue to diquafosol resulted in a concentrationdependent increase in the secretion of mucin-like glycoproteins and MUC5AC. Diquafosol increased the intracellular calcium concentration in the conjunctival epithelial cells in a concentration-dependent manner. In addition, this enhanced mucin-like glycoproteins secretion by diquafosol was inhibited by pretreatment with the calcium chelating agent, BAPTA-AM. **Conclusions**. These results suggest that diquafosol stimulates the secretion of mucin-like glycoproteins, especially MUC5AC, from rabbit conjunctival tissue via intracellular calcium pathway after binding to P2Y2 receptors on the conjunctival goblet cells.

OUTCOME OF TRANSPLANTATION OF CULTIVATED ORAL MUCOSAL EPITHELIAL SHEETS PREPARED WITH FIBRIN-COATED CULTURE DISHES. Jun Shimazaki, Masatoshi Hirayama, Takefumi Yamaguchi, Yoshiyuki Satake INSTITUTIONS: Department of Ophthalmology, Tokyo Dental College

Objective: To study surgical outcome of the cultivated oral mucosal epithelial transplantation using substrate-free epithelial sheets prepared with fibrin-coated culture dishes. **Method**: Autologous oral mucosal epithelial cells were harvested on fibrin-coated culture dishes, and a proteinase inhibitor (aprotinin) was added to culture media until the cells reach confluency. Fibrin was dissolved following discontinuation of aprotinin, thus cell sheets without underlying substrates were obtained. We transplanted the cell sheets to 16 eyes of 15 patients (mean age; 60.1±15.4 years) with severe cicatricial keratoconjunctivitis with total limbal deficiency including chemical burns (n=6) , Stevens-Johnson syndrome (n=2), ocular cicatricial pemphigoid (OCP, n=2), and pseudo-OCP (n=6). We retrospectively analyzed the data regarding the efficacy and safety of these patients. **Result**: With a mean follow-up period of 60.6 weeks, 12 eyes (75%) achieved stable ocular surface. Improvements in visual acuity were observed in 10 eyes (63%). As postoperative complications, increased intraocular pressure and persistent epithelial defects were noted in each 3 eyes. There was no rejection or infection after surgery. **Conclusion**: Transplantation of cultivated oral mucosal epithelial sheets prepared using fibrin-coated culture dishes seems to be a promising method for the treatment of severe cicatricial keratoconjunctivitis.

OCULAR SURFACE RECONSTRUCTION. Shigeto Shimmura. Department of Ophthalmology, Keio University School of Medicine

Ocular surface reconstruction (OSR) is a general term used to regenerate the ocular surface of patients with varying degrees of limbal stem cell deficiency. Etiologies include congenital dystrophies such as aniridia, autoimmune disease such as Stevens Johnson syndrome and ocular cicatricial pemphigoid, as well as thermal/ chemical burns. Successful OSR surgery requires the collaboration of stem cell science, up-to-date surgical techniques and adjunctive therapy for dry eye,

Meibomian gland dysfunction and immunological rejection. The past decade has seen significant advances in the field of tissue engineering of stem cells and epithelial sheets. Advances in surgical techniques for deep anterior lamellar keratoplasty (DALK) and keratolimbal allografts (KLAL) have helped improve surgical success in advanced cases. Current investigations include the use of induced pluripotent stem (iPS) cells as a cells source as well as defining the limbal stem cell niche, which is vital in maintaining stem cells long-term. Recent data on the topic will be introduced along with possible breakthroughs in the near future.

IN VIVO IMAGING OF TEAR FILM AND OCULAR SURFACE IN MEIBOMIAN GLAND DYSFUNCTION USING ULTRA HIGH RESOLUTION ANTERIOR SEGMENT OPTICAL COHERENCE TOMOGRAPHY (UHR-OCT).
Mohamed Abou Shousha, Jiahuang Wang and Victor L. Perez
Bascom Palmer Eye Institute, University of Miami, Miller School of Medicine, USA

Objective: Evaluate the use of UHR-OCT in detecting tear film and ocular surface characteristic in patients with meibomian gland dysfunction (MGD). **Methods:** Study included 20 eyes with MGD, 20 with aqueous tear deficiency dry eyes and no MGD and 20 normal eyes. Subjects were imaged using novel, custom-built UHR-OCT. Images were used to describe the characteristics of ocular surface and the tear film. Expression of the meibomian glands of MGD patients was done and UHR-OCT images were captured before and after expression. Custom-made software was used to creat reflectivity profile and measure changes in the peak correlated to tear film and ocular surface. **Results:** UHR-OCT images of MGD eyes depicted thick hyper-reflective particle in the tear film, as well as increased roughness of the ocular epithelial surface. On the other hand, images of dry eyes with no MGD demonstrated increase roughness of the ocular epithelial surface and a tear film that was undetectable and inseparable from the epithelial layer. Normal subjects neither had the tear film thick hyper-reflective particles nor the epithelial irregularity. After expression of the meibomian glands in MGD eyes, UHR-OCT image demonstrated a significant increase in the tear film hyper-reflective thick particles and in the total hyper-reflectivity of the tear film and ocular surface peak on the UHR-OCT reflectivity profile. **Conclusions:** UHR-OCT is capable of detecting diagnostic characteristics of the tear film and ocular surface in MGD and could provide a novel means to objectively and quantitatively diagnose MGD. No financial interest to disclose.

ADHESION OF TRANSFERRIN AND ALBUMIN TO FDA GROUP II OMAFILCON CONTACT LENSES.
Darshan Solanki, Sophia Cuprillnilson, Brooke Liberman, Andrea Janoff, Edward O. Keith Nova Southeastern University

Objective:Tear protein deposits on contact lenses can cause irritation of the conjunctiva and are associated with bacterial infection. The adhesion of transferrin and albumin to Omafilcon contact lenses was investigated. The charges on protein molecules interact with the contact lens hydrogel polymer causing adhesion of the proteins to the lenses. **Methods**: Lenses were incubated for 5 days in a solution (2 mg/mL) of each protein. Bicinchoninic acid colorimetry was performed to determine protein concentration in the vials and protein adhesion to lenses. **Results**: The albumin concentration in the incubation vials remained fairly constant while the concentration of transferrin in the vials fluctuated. Albumin initially adhered to the lenses, declined slightly on subsequent days, and increased again on day five. Transferrin adhered to the lenses consistently, reaching peak deposition after four days of incubation, before declining on day five. The adhesion of transferrin to the Omafilcon lenses was similar to the adhesion of transferrin to Hilafilcon lenses (both FDA Group II: nonionic high water). Albumin adhesion to Omafilcon contact lenses resembled the adhesion of lysozyme to the same lenses. **Conclusion**: Human serum albumin has three domains that allows albumin to bind and release hydrophobic molecules while transferrin has numerous positive charges. Thus, the negatively charged hydrophilic polymer of the Omafilcon lenses facilitated transferrin adhesion but limited albumin adhesion. Supported by a NSU President's Fac

CLINICAL INVESTIGATION OF COMPLICATIONS OF THE SUPEREAGLE ® PLUG
Yukiko Sonomura,[1,2] Norihiko Yokoi,[2] Aoi Komuro,[2] Kayoko Inagaki,[1,2] Shigeru Kinoshita.[2] Yamashiro public Hospital,[1] Department of ophthalmology, Kyoto prefectural university of medecine-[2] Kyoto, Japan

Objective. Punctal occlusion by a silicone plug is effective for severe dry eye. There have been reports about complication of silicone plugs. The purpose of this study is to investigate the complications of the SuperEagle® plug (Eagle Vision, Inc.). **Methods**. 129 puncta in 79 eyes of 61 patients (11 males, 50 females, mean age; 57.9 years) were examined. The intracanalicular migration rate during plug insertion, extrusion rate, granulation tissue formation within the canaliculus, whitish material around the plug suggesting possibility of bacterial biofilm formation on punctal plug and enlargement of punctum size after extrusion were investigated. **Results**. The mean follow up period was 169.9 days. The migration rate was 0 %. The extrusion rate was 41.9%during the follow up period. The average period until the extrusion was 81.8days. Granulation was formed in 20.2%. 25.9% of puncta were completely occluded by granulation after extrusion. The whitish material around the plug was not seen. There was no significant change in the size of the punctum between the size at the initial insertion and at extrusion. **Conclusions**. The insertion of Super Eagle® plug is found to be safe without intracanalicular migration. The extrusion rate was not low, but there was high incidence of complete punctal occlusion by granulation after extrusion.

COMPARATIVE TEAR FLUID PROTEOMIC STUDY OF DRY EYE, BLEPHARITYS AND CONTROLS PATIENTS AS A TOOL FOR DIFERENTIAL DIAGNOSIS AUTHORS.
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Objective: To determine differences in tear protein expression pattern between dry eye (DE), Blepharitis (BL), and Control (CT) patients, and to develop a differential disease prediction system based on individual and combined tear detection of specific biomarkers. **Method:**Tear samples were collected from 16 BL and 16 DE affected patients, and 12 CT using a Merocel sponge (Oasis, 0525). Tear were eluted, centrifugated, and immunodepleted prior 2DE analysis using 40µg of total protein. The analysis of the obtained gels was performed using Progenesis SameSpots software (Nonlinear dynamics). Proteins with higher variation (p-value <0.05) were identified using MALDI-TOF/TOF spectrometer, validated by ELISA assays and analyzed using ROC curves. **Results:** The spot proteins highest differentially expressed were identified as CST4, CST1, S100A6, GSTP1, ANXA1, LGALS7, S100A9 proteins. From these proteins, GSTP1(fold=5.7), S100A4(fold=3.4), S100A6(fold=8.2), ANXA1(fold=3.0) were upregulated in dry eye patients. CST1(fold=-3.6), CST4(fold=-2.0), were downregulated in blepharitis, and LGALS7 were strongly downregulated in both dry eye (fold=-6.2) and blepharitis (fold=-3.37). Functional analysis reveals a great implication of inflammation

and oxidative stress processes, highly related to dry eye disease. Moreover, ROC curves analysis using ELISA results indicate a high accuracy in patient classification. **Conclusion:**A differential protein expression pattern between dry eye, blepharitis and control groups was found according to our 2DE-based proteomics approaches. Our results demonstrate the presence of specific biomarkers able to discriminate between the studied groups providing a powerful tool for specific tear diagnosis of these pathologies. Additionally, to our knowledge Galectin 7 (LGALS7) has not been previously report in tears. This study was supported by grants from Basque Country Government (NET Program Project BIOFTAL IN-2009/0000058). The authors have not commercial relationship

NON-INVASIVE IMAGING OF KEY PLAYERS IN OCULAR SURFACE INFLAMMATION.
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Objective: Ocular surface diseases such as allergy or dry-eye comprise a strong inflammatory component. Although individual inflammatory responses differ markedly, clinical symptoms often overlap and complicate diagnosis and appropriate treatment. Since the characterization of inflammation normally requires tissue probing, a non-invasive imaging technique would enable differentiation of key players in ocular surface inflammation in vivo. This study was set up to evaluate two-photon autofluorescence microscopy for the non-invasive characterization of these key players without the use of artificial dyes. **Methods:**A modified two-photon microscope, equipped for autofluorescence detection and fluorescence lifetime measurements (FLIM) was used. T-cells, B-cells, macrophages, erythrocytes etc. were isolated from BALB/c mice and characterized in vitro. For in vivo studies, conjunctiva-associated lymphoid tissue (CALT) of anesthetized BALB/c-mice was examined. CALT was used as an in vivo model that contains most cellular and non cellular components of inflammation. **Results:**Lymphocytes, macrophages, epithelial cells, goblet cells and erythrocytes were differentiated by unique optical features based on intracellular fluorophores or structural characteristics. Autofluorescence spectra of macrophages differed markedly from erythrocytes and epithelial cells. FLIM enabled further differentiation of non-adhesive vs. adhesive macrophages. Excitation wavelengths of 710-730 nm enabled visualization of elastic fibres whereas imaging at 800-850 nm induces Second Harmonic Generation of collagen fibrils. **Conclusion:**Two-photon autofluorescence microscopy enables characterization of cells and tissue structures involved in inflammatory processes of the ocular surface without the necessity of tissue probing and the use of artificial dyes. This technique has the potential to enable a clinical non-invasive characterization and grading of ocular surface inflammation. [Supported by grants of the University of Luebeck, GlaxoSmithKline and Bausch&Lomb]

ALLERGIC MEDIATORS IN TEAR FROM CHILDREN WITH SEASONAL AND PERENNIAL ALLERGY.
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Objective: To investigate the concentration of allergic mediators in tears of children with seasonal allergy (SA), perennial allergy (PA) and controls, as a tool for the diagnosis of ocular allergic inflammation. **Method**:Twenty children with allergy (17 seasonal allergic, and 3 perennial allergic) and sixteen healthy children were included in this study. Tear samples were collected using a Merocel sponge (Oasis, 0525), and immediately eluted by incubation in elution buffer and subsequent centrifugation at 20000 rpm 30 min at 4ºC. Level concentrations of Histamine (HIS), Tryptase (TPS), Eosinophil Chimiotatic Factor (ECF), Major Basic Protein (MBP), Eosinophil Cationic Protein (ECP), Eosinophil-derived Neurotoxin (EDN), IgE and E-selectin were measured using enzyme - linked immunosorbent assays (ELISA). Data were compared with the U Mann-Whitney test ($p<0.05$), and Multivariate analyses were also performed. **Results**: Tear levels of TPS (P=0.014), MBP (P=0,032), ECP (P=0.0041), IgE (P= 0.014) and EDN (P=0.00077) present significant differences in the allergy group compared to control. HIS, E-selectin, ECF did not show significant differences when are evaluated individually. However, logistic regression analyses including all molecules indicate a higher precision in the classification of patients. **Conclusion**: The simultaneous analyses of allergic mediators in children tears with SA and PA showed a significant elevated concentration in EDN, ECP and MBP in allergic group. Controversially, tear IgE and TPS levels were found to be decreased. Statistical analyses reveal a diagnostic accuracy of 94.4% using the eight molecules panel. Our results suggest that measuring these mediators may facilitate the ocular allergic inflammation diagnosis in tears.

DISTRIBUTION OF AQUEOUS DEFICIENT AND EVAPORATIVE DRY EYE IN A GENERAL PATIENT POPULATION.
Benjamin D. Sullivan[1], Michael A. Lemp[7]. [1]TearLab Corp. [2]Georgetown University Department of Ophthalmology

Objective: A prospective, multi-site clinical study evaluated the distribution of patients that could be classified as having purely aqueous deficient dry eye, evaporative dry eye, or a combination of the etiologies. **Methods:** Schirmer's tests and meibomian gland dysfunction (MGD) grading (Foulks/Bron scoring) were evaluated in both eyes of 224 dry eye subjects (n=174 female, n=50 male, age=49.6±16.4 years) chosen from the general patient population across 11 sites in the EU and US. The more severe measurement between the two eyes was used in analysis. Subjects were considered to have pure aqueous deficient dry eye disease if their Schirmer value was < 7 mm, while their MGD grade was ≤ 2. Patients were classified as purely evaporative dry eye if their MGD grade was > 2 and a Schirmer value of ≥ 7 mm. Finally, subjects were placed into the mixed category if they exhibited both a low Schirmer value < 7 and evidence of meibomian gland dysfunction, with a grade > 2. **Results:** Of the 224 dry eye subjects, 180 fell into one of the three categories. 100 could be classified as having meibomian gland dysfunction while only 14 could be classified as aqueous deficient. The remaining 66 subjects showed evidence of both meibomian gland dysfunction and aqueous deficiency. Across the entire patient population, 74% of the patients demonstrated signs of meibomian gland disease. **Conclusions:** The prevalence of meibomian gland disease far outweighs that of pure aqueous deficient dry eye in a general patient population. Support: TearLab Inc. & Alcon Laboratories. TearLab: BDS (E,I,P), MAL (C,I)

LACK OF CORRELATION OF COMMONLY USED TESTS FOR THE ASSESSMENT OF SEVERITY OF DRY EYE DISEASE.
Benjamin D. Sullivan[1], Anthony J. Bron[2], Christophe Baudouin[3], Gary N. Foulks[4], Kelly K. Nichols[5], Alan Tomlinson[6], Michael S. Berg[1], Michael A. Lemp[7]. [1]TearLab Corp. [2]University of Oxford [3]Quinze-Vingts National Ophthalmology Hospital [4]University of Louisville [5]The Ohio State University [6]Glasgow Caledonian University [7]Georgetown University

Objective: A prospective, multi-site clinical study evaluated the relationship between individual signs and symptoms of dry eye disease. **Methods:** Clinical signs and symptoms were evaluated for 598 eyes in

the general patient population (n=164 Normal, n=434 dry eye), across 11 sites from the EU and US. Tear osmolarity, tear film breakup time (TBUT), Schirmer's test, corneal and conjunctival staining (NEI/Industry), and meibomian gland dysfunction grading (Foulks/Bron scoring) were tested OU, and the OSDI questionnaire was performed (value used for each eye). A full matrix of squared Pearson correlation coefficients (r^2) and an independent components analysis (ICA) mixing matrix were derived from the dataset. **Results:** No correlations above r^2=0.20 were found between any of the signs or symptoms, except for corneal and conjunctival staining, which reported an r^2=0.34 (and measure similar events). The average r^2 for osmolarity (0.04), TBUT (0.12), Schirmer's test (0.08), corneal (0.15) & conjunctival staining (0.16), meibomian grading (0.10) and OSDI (0.11) were consistently low. Similarly, the columns of the ICA mixing matrix exhibited minimal overlap, with each marker clearly identifiable as a unique component. **Conclusions:** The clinical presentation of dry eye disease is multifactorial, with each test contributing different information to the overall disease severity rating. Owing to this independence, it is common to observe patients with conflicting results, *e.g.* low TBUT and no staining, high Schirmer's values but clear symptoms, and low osmolarity with staining. The use of an ICA-based composite index reduces selection bias, more accurately qualifies subjects as normal or dry eye, and allows comparison of individual tests to overall disease severity rating. Support: TearLab Inc. & Alcon Labs. TearLab: BDS (E,I,P), AJB (I) GNF (I), KKN (C,I), AT (I), MSB (E,I), MAL (C,I)

LONGITUDINAL VARIATION IN SIGNS & SYMPTOMS OF DRY EYE DISEASE AS COMPARED TO A COMPOSITE SEVERITY INDEX. Benjamin D. Sullivan[1], Baris Sonmez[2], Ebru Comert[2], Michael S. Berg[1], Michael A. Lemp[3]. [1]TearLab Corp. [2]Ondokuz Mayis Universitesi [3]Georgetown University

Objective: The objective of this study was to evaluate the longitudinal variability of signs and symptoms of dry eye disease. **Methods:** 21 subjects (n=18 female, n=3 male, 47±13 years old) with a history of dry eye symptoms were recruited at a single site. Tear osmolarity, tear film breakup time (TBUT), Schirmer's test, corneal staining (NEI/Industry), meibomian dysfunction assessment (Bron/Foulks scoring), and symptoms (OSDI) were evaluated on three separate days, spaced roughly at time 0, 30 and 90-day timepoints. Longitudinal variability was defined as the ratio of the standard deviation of all measurements to the dynamic range of each clinical test. **Results:** There were no significant differences in the longitudinal variability of each of the clinical signs or symptoms. In particular, osmolarity (11.6% variation over time) was found not to be significantly different than TBUT, Schirmer's, corneal staining, OSDI (13.1%, 11.8%, 11.4%, and 10.2%, with p=0.46, 0.94, 0.89, 0.42 respectively). The maximum change between timepoints was also similar between tests, with osmolarity, TBUT, Schirmer's, corneal staining, and OSDI (62.4%, 100%, 91.4%, 43.8%, 40.8%, respectively) all exhibiting substantial movement over time. Of interest, 71.4% subjects were hyperosmolar (average across all tests > 308 mOsms/L), 48% had breakup times < 5 s, 57% reported Schirmer's values < 10 mm, and 67% showed a corneal staining value > 3/16. **Conclusions:** The longitudinal variation of signs and symptoms is equivalent within mild/moderate dry eye subjects. The best dry eye tests for clinical trials are therefore those that are objective, quantitative, rapid, simple and operator independent. Support: TearLab Inc.: BDS (E,I,P), MSB (E,I), MAL (C,I)

A CASE OF IGG4-RELATED CHRONIC SCLEROSING DACRYOADENITIS. Mi Sun Sung, Joo Hwa Lee Sanggy-Paik Hospital, Inje University, Seoul, Korea

IgG4-related systemic disease (ISD) is recently proposed clinical entity as an autoimmune disorder, which characterize histologically by a dense lymphoplasmacytic infiltration, fibrosis and the presence of IgG4-positive plasma cells. The commonest components of this disease are autoimmune pancreatitis, chronic sclerosing cholantitis and chronic sclerosing sialadenitis (Kuttner's tumor). We report a case of chronic sclerosing dacryoadenitis of the lacrimal gland with abundant IgG4-positive plasma cells. A 51 year-old man presented with both upper eyelids swelling for 1 year. In the ocular examination, hard and non-tender masses were palpated in both upper eyelids but any other ophthalmologic abnormal finding was not noted. The serum IgG concentration was elevated. The patient underwent anterior orbitotomy via lid crease incisions, and the lacrimal gland showed severe lymphoplasmacytic infiltration with lymphoid follicles and sclerosing fibrosis. On immunostaining, the lymphocytes were polyclonal and composed of both B and T cells. And there were numerous aggregates of IgG4-positive plasma cells. Based on these findings, IgG4-related chronic sclerosing dacryoadenitis was diagnosed.

DIFFERENCES IN MEIBOMIAN GLAND PHYSIOLOGY BETWEEN PRE- AND POST- MENOPAUSAL WOMEN. Tomo Suzuki[1,2], NorihikoYokoi[2], Aoi Komuro[2], and Shigeru Kinoshita[2] INSTITUTIONS:[1]Department of Ophthalmology, Kyoto City Hospital, Kyoto, Japan [2]Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan

Objective: We previously reported that meibomian gland physiology changes during the menstrual cycle and has sex differences. In this study, we assessed the meibomian gland physiology in postmenopausal women, and then compared them with those in premenopausal women as well as the influence of serum sex steroid hormones. **Methods:** Six women in their fifties (average age of menopause: 50±4 years-old) and six women in their twenties with a regular 28-day menstrual cycle were recruited for this study, with informed consent being obtained from all subjects prior to enrollment. The volume of meibum by meibometry, fluorescein tear-film breakup time (F-BUT), diameter of the meibomian gland orifice (MGO) using slit-lamp photography with high magnification, meibomian gland morphology by meibography, and the serum concentration of sex steroid hormones were evaluated every 3-5 days for 5 consecutive weeks. **Results:** The MGO diameter and the volume of meibum were significantly higher in postmenopausal women than in premenopausal women (average ± S.E.: 0.210±0.9 mm, 0.175±0.003 mm, respectively; 0.155±0.006 ODU, 0.073±0.015 ODU, respectively; p<0.001). However, the F-BUT showed no significant differences in postmenopausal and premenopausal women (5.6±0.24 sec, 6.1±0.92 sec, respectively). In premenopausal women, the MGO diameter was negatively correlated with the serum concentration of estradiol (p<0.005), but in postmenopausal women it was positively correlated with the serum concentration of testosterone and progesterone (p<0.005). **Conclusions:**Significant differences in the meibomian gland physiology were seen in pre- and post-menopausal women. Serum levels of sex steroid hormones play an influence on meibomian gland physiology and may exert an impact on evaporative dry eye.

CORNEAL SENSATION AND LACRIMAL SECRETION BEFORE AND AFTER DESCEMET STRIPPING AUTOMATED ENDOTHELIAL KERATOPLASTY. Yumiko Tamari,Yukari Imai,Takefumi Yamaguchi,Kenji Konomi,Seika Den,Yoshiyuki Satake,Jun Shimazaki Department of Ophthalmology,Tokyo Dental Collage Ichikawa General Hospital

Objective:To examine the corneal sensation and lacrimal secretion before and after Descemet stripping automated endothelial keratoplasty(DSAEK). **Method**:Retrospective study involving consecutive 17 eyes of 15 patients who underwent DSAEK. We measured corneal sensation using Cochet-Bonnet esthesiometer and

lacrimal secretion using Schirmer test and tear clearance test in early postoperative period (2 to 18 months). Exclusion criteria included the history of herpetic keratitis and penetrating keratoplasty, and use of topical -blockers and nonsteroidal anti-inflammatory agents.DSAEK were performed through 5.0 mm temporal corneoscleral incisions in all eyes. **Result**:DSAEK procedure was uneventful and the corneal clarity was obtained in all eyes. We found no statistically significant difference in corneal sensation comparing before and after DSAEK(14.22±1.80 and 3.02±1.68 g/mm^2,P=0.23). There were no significant differences in Schirmer test(8.94±4.71 vs z.32±8.70mm,P=0.76) and tear clearance test (3.18±0.73 vs 3.35±1.17, P=0.55) between before and after DSAEK. **Conclusion**:The results of this study indicate that the relative preservation of corneal sensation and tear function contributes to the early recovery of visual function after DSAEK, which may be one of the advantages over penetrating keratoplasty.

SPECIFIC SITE-DIRECTED MUTATIONS IN THE *STREPTOCOCCUS PNEUMONIAE* CORNEAL VIRULENCE FACTOR PNEUMOLYSIN ABROGATE LYTIC ACTIVITY AND CORNEAL EROSIONS. Sidney Taylor, Justin Thornton, Larry S. McDaniel, Melissa E. Sanders, Mary E. Marquart. Department of Microbiology, University of Mississippi Medical Center, Jackson, MS, USA

Purpose: This study was designed to compare the hemolytic activity of wild-type pneumolysin (Ply), a cholesterol-dependent cytolysin of *Streptococcus pneumoniae* and corneal virulence factor, to three single amino acid substitution Ply mutants: PLYA370E, PLYL460E, and PLYW433F. These proteins were assessed for their ability to lyse sheep red blood cells and to cause corneal erosions in rabbits. **Methods:** Recombinant wild-type and mutant forms of Ply were expressed in *E. coli* using the pET101 expression vector. Mutant Ply expression plasmids were constructed by site-directed mutagenesis of the wild-type vector. Relative activity of wild-type and each mutant form of Ply was determined by hemolysis of sheep red blood cells. Corneal erosion formation was analyzed as follows: Intrastromal injections of 1µg of Ply in 10µl of PBS were carried out in New Zealand white rabbits. Pictures were taken at various time points between 0 and 24 hours, and corneal erosion diameters were measured. **Results:** Wild-type Ply exhibited 100% hemolysis of 2% sheep red blood cells at a minimum concentration of 5pg/µl. PLYW433F and PLYA370E were able to reach 100% lysis, but only at concentrations of 12.5ng/µl and 37.5ng/µl respectively. PLYL460E was not able to reach 100% hemolysis. These result show that wild-type Ply has at least 2500 times the hemolytic activity of mutant variants. Moreover, wild-type Ply produced a corneal erosion of approximately 5 mm in diameter 6 hours after injection, whereas each mutant produced a pinpoint erosion of less than 1 mm. **Conclusion:** Ply is known to bind cholesterol in the target cell membrane in order to form a multimeric pore-forming complex, and has been shown to be a key virulence factor in pneumococcal keratitis. The three mutation sites, amino acid positions 370, 433, and 460, are all necessarily involved in the lytic mechanism of action and corneal erosion formation. Support: Public Health Services Grant R01EY016195, National Institutes of Health, USA

THE EFFECT OF EYE DROP WHICH COMBINES SODIUM HYALURONATE AND CARBOXY METHYL CELLULOSE IN TREATING DRY EYE Hungwon Tchah, Jae Yong Kim, Myoung Joon Kim, Jae Hyung Kim, Jooen Lee. Department of Ophthalmology, University of Ulsan, Asan Medical Center, Seoul, Korea

Objective: To evaluate the efficacy and safety of sodium hyaluronate (SH) and carboxy methyl cellulose (CMC) combination eye drop and to decide optimum concentration of CMC in treating dry eye. **Method:** 54 dry eye patients randomly divided into 3 groups (18 patients for each group), SH 0.1% +CMC 0.3% for group A, SH 0.1% +CMC 0.5% for group B and normal saline for group C were instillated 5 times a day for 8 weeks, respectively. Inclusion criteria for dry eye were Ocular Surface Disease Index (OSDI) >10, Tear Break Up Time (TBUT) <7sec, Fluorescein staining (FS) ≥ 4/15, Schirmer test with anesthesia ≤ 7 /5min. Visual acuity (VA), TBUT, FS, Schirmer Test, OSDI, and VAS (Visual Analog Scale) of adverse reactions (itching and burning sensation) were evaluated before and after instillation of test eye drops. **Result:** Patients' age was 41+/-14.1 years. TBUT , FS and Shirmer test score was improved in group A compared to group B and C . There was no difference in OSDI and VAS among groups. **Conclusion:** Combination eye drop of SH 0.1% +CMC 0.3% seemed to be more effective than that of SH 0.1% +CMC 0.5% and no definite adverse reactions. This combination eye drop might be more useful .than using SH or CMC separately. This study was supported by DHP Korea

MEASURING THE OPTICAL EFFECTS OF TEAR FILM INSTABILITY. Larry N. Thibos, Indiana University School of Optometry, Bloomington, IN, USA

A variety of technologies may be used to measure the optical effects of tear film instability, including videokeratoscopy, interferometry, and wavefront aberrometry. Dynamic videokeratoscopy and interferometry isolate rapid changes in the anterior surface of the tear film between blinks, which are major contributors to the deterioration of the optical quality of the whole eye. However, to understand and to account for the visual disturbances associated with tear film instability and breakup requires optical measurement of the eye's entire optical system responsible for forming the retinal image. Dynamic wavefront aberrometry between blinks is the method of choice for that application because it provides a spatial map of changes in the whole eye's refractive power across the pupil, including the component due to non-uniform thinning of the tear film. These spatial maps, in turn, may be used to calculate the changes in retinal image quality that lead to symptoms of blurry vision. Dynamic aberration maps may also be useful for diagnosis of dry eye, for improved retinal imaging using adaptive optics, and for monitoring the efficacy of dry eye treatments and therapy. Future extension of classical wavefront aberrometry to include an analysis of light scatter caused by exposure of the rough corneal surface in areas of tear breakup will add value to current approaches.

MEASURING LIGHT SCATTER DURING TEAR BREAK-UP WITH SHACK-HARTMANN WAVEFRONT ABERROMETER. Larry N. Thibos, Jayoung Nam, Nikole Himebaugh, Haixia Liu, Arthur Bradley. School of Optometry, Indiana University, Bloomington, IN, USA

Purpose. When the tear film disrupts locally, tear film thickness becomes non-uniform and the irregular epithelial surface may be exposed. Light scatter by this rough refracting surface reduces the optical quality of the eye that leads to reduced visual performance. Gross wavefront aberrations of the tear film account for some, but not all, of this phenomenon. Our goal was to extend wavefront analysis to include effects of light scatter by quantifying the amount of blur in spot images produced by individual lenslets in the Shack-Hartmann wavefront aberrometer (SHWA). **Methods.** We developed a metric called "radial variance" to quantify the size of spot images captured by individual lenslets in the SHWA. For this metric, the amount of scatter present over each lenslet is equal to the size of the SHWA spot minus the size of the retinal beacon produced by the

probe beam. Because the SHWA is a double-pass instrument, we lack independent knowledge of the size of the retinal beacon. However, sufficient information is available to establish lower bound and upper bounds. The method was validated with computer modeling of a theoretical test case and then applied to data from human eyes. **Results.** The radial variance of SHWA spots increased significantly in the region of tear break-up, indicating increased light scatter. The regions of local tear break-up over the pupil matched topographically the regions of increased light scatter. According to optical theory, increased light scatter is proportional to the amount of local phase variation in the wavefront aberration map on a microscopic scale. **Conclusions.** The spatially resolved map of light scatter using spot quality analysis of the SHWA data images is a valid method of quantifying retinal image degradation during tear break-up in the eye. [Supported by NIH/NEI grant R01EY05109 to LNT.]

CHARACTERISATION OF MEIBUM LIPIDS IN ASIANS WITH AND WITHOUT DRY EYE. Louis Tong,[1,2,3] Sin-Man Lam,[4] Shyam S Chaurasia,[1] Siew-Sian Yong,[1] Guanghou Shui,[4] Markus R Wenk[4] [1]Singapore Eye Research Institute, [2]Singapore National Eye Center, [3]Duke-NUS Graduate Medical School, [4]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore

Purpose: In Asia, there is a high prevalence of dry eye and Meibomian gland disease (MGD), conditions with significant morbidity. Previous studies have demonstrated that the human Meibum comprises a complex mixture of lipids, and MGD has been linked to abnormalities of Meibum lipids. Here, we investigated the lipid profiles of human Meibum and determine their relationship to dry eye. **Methods**: A standard dry eye questionnaire, tear break up time, Schirmer's test and corneal fluorescein dye staining were obtained from 41 dry eye patients(mean age 59.6 years) and 14 controls(mean age 33.1 years), with more women than men in both groups. Meibum was collected, lipids extracted and analysed using HPLC and the 3200 and 4000 Q-trap® LC/MS/MS systems. **Results**: There was no significant difference in the overall representation of most of the lipid classes studied (triacylglycerides (TAG), phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl glycerol (PG), sphingomyelin (SM) and ceramides (CER)) in patients compared to controls, except a higher proportion of phosphatidylinositol (PI) in patients compared to controls. Specific lipid species in seven classes of lipids were significantly upregulated in dry eye patients: TAG (3 species), SM (2 species), PI (5 species), PE (4 species), lysoPC (2 species), PC (13 species), CER (2 species), glucoceramides and dihexylceramides (4 species). Lipids that showed significant differences include PC 40:7, PC 40:6, PI 38:4, PI 38:3, PI 40:4 and SM 18:0/16:0. At least 3 symptoms of tear dysfunction were correlated with PC 40:7, PC 40:6 and PI 38:3 levels. PI 38:4 was also inversely correlated (r=- 0.40) to Schirmer's test values. **Conclusions**: The Meibum lipids described in this study established a signature for Asian population. Determination of specific lipids may allow clinicians to classify the subtype of tear and ocular surface dysfunction, and this has implications for treatment of patients as well as sub-grouping in the conduct of clinical trials in ocular surface disease. [No commercial relations, Grant support: NMRC/1206/2009 and NMRC/CSA/013/2009]

ANTI-AGING APPROACH FOR THE TREATMENT OF DRY EYE. Kazuo Tsubota[1], Motoko Kawashima[1], Takaaki Inaba[1], Murat Dogru[1], Yoko Ogawa[1], Shigeru Nakamura[1], Ken Shinmura[2], Akihiro Higuchi[1], Tetsuya Kawakita.[1] Department of Ophthalmology[1], Department of Internal Medicine[2], Keio University School of Medicine, Tokyo, Japan

Purpose: To introduce the current knowledge about the basic science of aging and how we can apply this for the prevention and treatment of dry eye. **Methods:** Calorie restriction and oxidative stress control were studied on the dry eye animal models. Beneficial effects of these two major interventions will be shown. At the same time, I report my personal experience as a dry eye patient, which has been alleviated by the anti-aging approach. **Results:** Tear production and opacity decrease with age; however, calorie restriction as well as oxidative stress control could increase tear production. The mechanism of this observation remains unclear, but age-related cytokines as well as inflammatory cytokines seem to be related. **Conclusion:** Recent advances in the understanding of aging have paved a new way of thinking about intervening with the aging process. Commercial Relationships: none Grant Support: none

TOLL-LIKE RECEPTOR 2 IS INVOLVED IN CORNEAL DISEASE SEVERITY OF *STREPTOCOCCUS PNEUMONIAE* KERATITIS IN THE ABSENCE OF PNEUMOLYSIN. Nathan Tullos, Erin Norcross, Sid Taylor, Quincy Moore, Melissa Sanders, Mary E. Marquart. Department of Microbiology, University of Mississippi Medical Center, Jackson, MS, USA

Purpose: Previous findings suggested that TLR 2 was not involved in corneal disease severity caused by *Streptococcus pneumoniae*, and that TLR 4 was highly involved, likely due to stimulation by pneumolysin. The purpose of this study was to elucidate the effect of TLR 2 in the absence of pneumolysin. **Methods:** C57BL/6 and TLR 2-/- mouse corneas were inoculated with 108 colony forming units (CFU) of *S. pneumoniae* clinical strain K1263 and a pneumolysin deficient isogenic mutant (ΔPLY K1263). Clinical examinations were performed once daily for 21 days. CFU were enumerated 1 and 3 days post-infection (p.i.). **Results:** There was a significant difference in bacterial log10 CFU recovered from wild type mice compared to TLR 2 -/- mice (n = 8 each) 1 day following infection with strain K1263 (1.27 ± 0.64 for wild type; 3.77 ± 0.38 for TLR 2 -/-; P = 0.005), but not following infection with ΔPLY K1263 (TLR 2-/- :1.738 ± 0.71 and C57BL/6: 0.749 ± 0.676; n = 10 each; P = 0.33). By day 3 most eyes were sterile. Clinical scores were not significantly different between mouse strains at any time when they were infected with K1263 (P > 0.06). Scores for ΔPLY K1263, however, were initially high for both strains of mice 1 day p.i., but throughout the remainder of the experiment the TLR 2 -/- mice had significantly lower clinical scores (≤ 1.19; n = 47 at day 1) than the C57BL/6 mice (≥ 2.25; n = 44 at day 1; P ≤ 0.018). **Conclusions:** Lack of host TLR 2 in pneumolysin deficient pneumococcal keratitis caused decreased disease severity, suggesting that TLR 2 activation may induce harmful immune stimulation. In the absence of pneumolysin, other virulence factors appear to be causing corneal rosions.[Support: Public Health Services Grant R01EY016195, National Institutes of Health, USA]

DOES OCULAR IMPRESSION TAKING CAUSE DISTORTION OF THE OCULAR SURFACE? Jennifer Turner, Matthew Dobson, Paul J Murphy, Christine Purslow School of Optometry & Vision Sciences, Cardiff University, United Kingdom.

Objective: Ocular impression taking remains a valuable method of obtaining information about ocular surface contour but the amount of transient deformation is unknown. This study aimed to evaluate this in two ways: using a scanning-slit videokeratoscope (Orbscan IIz) pre and post-impression procedure, and comparing the impression cast to the ocular surface *in-vivo* using anterior segment optical coherence tomography (AS-OCT Visante™). **Method**: Topography measurements of the right eye of 105 healthy subjects (35M, 68F; age 24.65±6.74 yrs) were taken before and after an ocular impression procedure. Radii of curvature were serially sampled in the principal meridians and compared using paired t-testing. AS-OCT scans where taken of the

right eye and the resultant cast in 12 healthy subjects (5M, 7F; age 27.31±4.47 yrs) and radii of curvature sampled. Areas under the curve (AUC) were compared using paired t-testing. **Result**: Mean post-impression shape changes assessed by topography across the central 9mm (diameter) of the ocular surface indicated small, but insignificant increases in the radii of curvature (+0.019±0.26mm horizontally and +0.008±0.11mm vertically; 0.209 <0.809). Comparing the eye surface *in-vivo* to that of the cast, there was no significant difference in AUC for the horizontal (cast 32.12±1.11mm2 vs eye 31.86±1.18mm2; p=0.138) or vertical meridian (cast 6.42±0.32mm2 vs eye 6.42±0.32mm2; p=0.911). **Conclusion**: Modern ocular impression taking has no significant effect on corneal topography up to 9mm horizontally and 5mm vertically. When comparing the eye *in-vivo* with its resultant cast the areas under the curve in both principal meridians indicated good reproducibility of eye contour, although this is limited to more central comparison of contours. Commercial relationship: Menicon Co.Ltd, Japan.

IN VITRO CYTOTOXICITY OF HYDROGEN PEROXIDE TO CORNEAL EPITHELIAL CELLS JL Ubels, DS Mlnarik, BJ Konynenbelt Department of Biology, Calvin College, Grand Rapids, MI, USA

Purpose: Peroxide based systems are used as biocides some in contact lens solutions and lubricant eye drops. A battery of in vitro tests was used to determine whether residual H_2O_2 in neutralized ophthalmic formulations adversely affects structure and function of human corneal epithelial cells. **Methods:** HCLE cells, grown as monolayers or stratified constructs on microporous membranes, were exposed to formulations containing H_2O_2 (0-0.01%) at pH 7.0 or 7.9 for 10-60 min. This was followed by microscopy and measurement of viability, fluorescein permeability and transepithelial resistance (TER). Cells were also exposed to 0.1–0.4% unneutralized H_2O_2 in culture medium with FBS. **Results:** Formulations had reversible adverse effects on morphology (swelling, junction separation) and caused a slight, significant decrease in viability of monolayer cells. These changes were more pronounced at pH 7.9, but were unrelated to the presence of H_2O_2. The formulations had no adverse effects on morphology, viability or barrier function of stratified cells. Stratified cells were exposed to unneutralized H_2O_2 in culture medium to determine ability of the in vitro test methods to detect damage by H_2O_2. Fluorescein permeability of stratified cells was increased after exposure to H_2O_2 at 0.2% or above for 60 min in culture medium and continued to increase after cells were returned to medium without H_2O_2 for 4 hr. Exposure to H_2O_2 in culture medium for 60 min at concentrations of 0.3% or above caused a significant decrease in TER, with a continued decrease in barrier function when cells were returned to control medium. **Conclusions:** While stratified HCLE cells can be damaged by high levels of H_2O_2, the low, residual levels of peroxide present after neutralization of the test formulations had no adverse effects in vitro, suggesting that H_2O_2, used appropriately, is a safe biocide. Comparison of effects of formulations on monolayer vs. stratified cultures shows that monolayer cultures are more easily damaged and that data obtained using stratified cells may be more predictive of effects of formulations on intact corneas. [Supported by Alcon Research, Ltd., Fort Worth TX)

INHIBITION OF UVB ACTIVATION OF SEK1/MKK4 AND JNK1 IN CORNEAL EPITHELIAL CELLS BY ELEVATED EXTRACELLULAR K^+ JL Ubels, MP Schotanus, LR Koetje, JL Louters Department of Biology, Calvin College, Grand Rapids, MI, USA

Purpose: JNK, which is activated by SEK1/MKK4 in the MAP kinase pathway, can activate several apoptotic pathways, including direct effects on transcription of pro-apoptotic genes and activation of caspases-8 and -9 via the intrinsic mitochondrial pathway. We have shown that UVB causes apoptosis of human corneal limbal epithelial cells by activation of K^+ channels, leading to activation of caspases-8, -9 and -3. UVB-induced activation of these caspases and DNA degradation can be inhibited by elevated extracellular $[K^+]$ (Exp Eye Res. 81:140,2009). The purpose of this study was to determine whether UVB activates JNK and SEK1 in HCLE cells, whether high $[K^+]_o$ inhibits this activation and to demonstrate that high $[K^+]_o$ prevents loss of intracellular K^+ when channels are activated by UVB. **Methods:** HCLE cells were exposed to 100-200 mJ/cm^2 UVB and incubated in culture medium with 5.5-100 mM K^+. Intracellular K^+ was measured by lysing cells in DI water and measuring K^+ in lysates by ion chromatography. Activation of JNK and SEK1 was detected using antibodies to the phosphorylated proteins and western blotting. **Results:** $[K^+]_i$ decreases by 30-50% within 10 min after UVB exposure. This loss of K^+ is inhibited by incubation of the cells in elevated $[K^+]_o$. SEK1 and JNK are phosphorylated within 15 min after UVB exposure and elevated $[K^+]_o$ inhibits activation of these signaling molecules. **Conclusions:** The data suggest that activation of SEK1 and JNK are early events in UVB-induced apoptosis and support the concept that loss of $[K^+]_i$ due to UVB-induced activation of K^+ channels is involved in the initiation of apoptosis. The inhibitory effect of elevated $[K^+]_o$ on these events supports our overall hypothesis that the relatively high $[K^+]$ in tears (25 mM) contributes to the protection of the corneal epithelium from ambient UVB by reducing UV-induced loss of K^+ from epithelial cells. (supported by NIH grant EY018100)

A NEW MOUSE MODEL OF DRY EYE DISEASE (*Tet-mev-1* Mice) : OXIDATIVE STRESS AFFECT FUNCTIONAL DECLINE IN LACRIMAL GLAND. Yuichi Uchino,[1,2,3] Tetsuya Kawakita,[2] Masaki. Miyazawa,[3] Takamasa Ishii,[3] Hiromi Onouchi,[3] Kayo Yasuda,[3] Shigeto Shimmura,[2] Naoaki Ishii[3,] Kazuo Tsubota[2]. Ophthalmology, Tokyo Electric Power Company Hospital[1], Ophthalmology, Keio University School of Medicine,[2] Tokyo, Japan, Molecular Life Science, Tokai University School of Medicine,[3] Kanagawa, Japan

Purpose: A mutation in SDHC subunit constituting mitochondrial complex II caused superoxide anion overproduction leading to excessive apoptosis, precocious aging or tumorigenesis in both nematode *C. elegans mev-1* and mouse fibroblast cells SDHC E69. We have constructed conditional transgenic mice (*Tet-mev-1*) with the mutation in SDHC gene coding SDHC V69E mutation using our unique tetracycline (Tet On/Off) system. *Tet-mev-1* homozygous (*Tet-mev-1* 37 *Tg/Tg*) mice increased O2- levels in the mitochondria and apoptosis induction of several tissues. The purpose of our study was to determine the histopathological and biochemical alternations in the lacrimal gland of *Tet-mev-1* mice as a dry eye model. **Methods**: Tear function test (tear quantity measured with 0.5 µl microcapillary) and were performed on *Tetmev- 1* mice (n=5) aged 3 months and wild type mice (n=5). At 3 months age, these mice are sacrificed and the lacrimal glands were collected for measurement of superoxide anion (O2-) and oxidative protein to evaluate the oxidative damage. As histopathological analyses, HE, Azan stainings and 8-OHdG immunostaining were performed. **Results**: Tear quantity values in *Tet-mev-1* mice were lower compared to the wild type mice. The lacrimal gland of *Tet-mev-1* mice overproduced superoxide anion (O2-) and oxidative protein compared to the wild type mice. Histopathological analyses showed the hallmarks of lacrimal gland inflammation by presence of intense mononuclear leukocytic infiltration and fibrosis around acinar cell staining by 8-OHdG antibody in the lacrimal gland of *Tet-mev-1* mice. **Conclusions**: *Tet-mev-1* mice revealed decreased tear production with the morphological changes. These findings strongly suggest that oxidative stress can be a causative factor for the development of dry eye disease.

IMPLEMENTATION OF A NEW QUESTIONNAIRE INTO RECENTLY REVISED JAPANESE DRY EYE DIAGNOSTIC CRITERIA. Miki Uchino[1,2], Murat Dogru[2], Yuichi Uchino[2], Samantha Ward[2], Tais Wakamatsu[2], Yoko Ogawa[2], Norihiko Yokoi[3], Kazuo Tsubota[2] [1]Ryogoku Eye Clinic, [2]Keio University School of Medicine, Tokyo, Japan, [3] Kyoto Prefectural University of Medicine, Kyoto,Japan

Purpose: To evaluate the implementation of a new symptom questionnaire (OSDI modified into Japanese) into the revised Japan Dry Eye Diagnostic Criteria and to investigate the changes in the dry eye diagnosis. **Methods:** 239 Subjects(75 male, 164 female, average age 39.7 years) seen for general and dry eye examination filled out a questionnaire (modified OSDI) with questions pertaining to alternations in visual function, ocular symptoms, and environmental triggers often associated with dry eye disease. A dry eye severity score (DESS) was given based on the numbers of questions and the total scores. From the results of Schirmer I test (ST), tear break up time(BUT), and fluorescein(F) and Rose Bengal (RB) staining, patients were diagnosed with Define Dry Eye (DDE), Probable Dry Eye (PDE), or No Dry Eye (nonDE), using both of the old and revised diagnostic criteria. **Results:** All 44 subjects diagnosed as non DE in old criteria were diagnosed as non DE in new diagnostic criteria. 57 subjects diagnosed as PDE in old criteria were diagnosed as non DE (18 subjects) and PDE (39 subjects) in new diagnostic criteria. 138 diagnosed as PDE in old criteria were diagnosed as PDE (58 subjects) and DDE (80 subjects) in new diagnostic criteria. The average DESS score in new diagnostic criteria was 29.8 in non DE, 33.2 in PDE, and 41.2 in DDE. **Conclusion:** The DESS obtained from new symptom questionnaire was found to correspond well to the revised dry eye diagnosis. The new diagnosis criteria expected to diagnose severe dry eye subjects only.

THE EXPRESSION AND FUNCTION OF RIG-I AND MDA-5 IN HUMAN OCULAR SURFACE EPITHELIUM. Mayumi Ueta[a,b], Norihiko Yokoi[a], Satoshi Uematsu[c], Taro Kawai[c], Shizuo Akira [c], and Shigeru Kinoshita[a] [a]Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan [b]Research Center for Inflammation and Regenerative Medicine, Faculty of Life and Medical Sciences, Doshisha University, Kyoto, Japan [c]Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan

Objective:To examine the expression and function ofRIG-I and MDA5, two new receptors that recognize double-stranded RNA (dsRNA) such as polyinosinic:polycytidylic acid (polyI:C). **Methods**:The presence of RIG-I and MDA5mRNAs in ocular surface epithelium was examined by reverse-transcription polymerase chain reaction (RT-PCR) and up-regulation of those mRNAs by polyI:C stimulation in human conjunctival epithelial cells were confirmed by quantitative RT-PCR and immunoblot analysis. The functions of RIG-I and MDA5 in conjunctival epithelial cells were examined using knock-out (KO) mice of IPS-1, a common adapter molecule of RIG-I and MDA5. **Results**:The mRNA of RIG-I and MDA5were detected in human conjunctival and corneal epithelium.Quantitative RT-PCR showed that RIG-I and MDA5 mRNA were significantly up-regulated upon polyI:C stimulation, and immunoblot analysis confirmed that the expression levels of these proteins were increased in polyI:C-stimulated primary human conjunctival epithelial cells (PHCjECs). In the conjunctival epithelium of IPS-1 KO mice, mRNA expression of several genes in polyI:C induced genes significantly down-regulated compared with wild-type mice. **Conclusions**:The results on this study showed that RIG-I and MDA5 are expressed in ocular surface epithelial cells and contribute to the induction of many transcripts by dsRNA such as polyI:C.

DRY EYE AND SECONDARY SJÖGREN'S SYNDROME IN MIXED CONNECTIVE TISSUE DISEASE (MCTD). Fany S. Usuba [1], Priscila Novaes[1], Milton R. Alves [1] [1] Division of Ophthalmology, School of Medicine of the University of São Paulo, São Paulo, Brazil.

Objective: To determine the prevalence and severity of dry eye, sicca symptoms and secondary Sjögren's syndrome (SS) in MCTD patients using the American-European Consensus Group (AECG) criteria for SS. **Methods**: 44 consecutive MCTD patients (Kasukawa's criteria) were included. They answered a SS symptoms questionnaire, and underwent dry eye diagnostic tests: Schirmer's I, tear break-up time (TBUT), fluorescein and Rose Bengal staining for the cornea and conjunctiva. Forty-one age and gender-matched healthy controls were evaluated for dry eye. Dry eye was classified as definite (presence of one positive tear dynamic test - Schirmer's test or TBUT - and one positive ocular surface diagnostic test - fluorescein or Rose Bengal staining) or probable (presence of only one abnormal dry eye test). A modified classification of 4 grades of dry eye severity according to Behrens *et al* was also applied. Salivary scintigraphy was performed to investigate concentration and secretion abnormalities. Exclusion criteria were previous or current use of xerogenic drugs or smoking habit. **Results**: The mean age of the patients was 44.7±12.4 years and the mean disease duration was 10.8±7.3 years. Definite dry eye was present in 22% of patients and this number increased to 86% when considering probable dry eye. These values were similar to the severity gradingclassification: 16% of patients had severe dry eye while 70.4% had mild to moderate dry eye. Definite and probable dry eye as well as severe, moderate and mild dry eye presented statistically significant dry eye diagnostic test values when compared to the control group (p=0.001). Sicca symptoms were present in 38.6% of patients and 31.8% of patients had also positive salivary glands scintigraphy leading to the diagnosis of SS. **Conclusions**: Mild to moderate dry eye are a common feature in MCTD. At least one third of patients presented sicca symptoms and had secondary SS as defined by the AECG criteria. Periodical ocular investigation should be done to achieve an early diagnosis and treatment in patients with MCTD.

UNCHANGED GOBLET CELL COUNTS AND EPITHELIAL METAPLASIA IN SEASONAL ALLERGIC CONJUNCTIVITIS OUTSIDE THE POLLEN SEASON. Amarilla Veres, Krisztina Kosina-Hagyó, János Németh INSTITUTIONS:Semmelweis University, Dept. of Ophthalmology

Objective: allergic inflammation disturbes the ocular surface milieu and is a possible causative factor of tear dysfunction. We investigated the possible consequences of the repeated inflammation shubs outside the pollen season to understand the core mechanism of developing dry eye. **Method**: We collected temporal bulbar conjunctival impression citology specimens (0,4 µm Millicell) of 8 seasonal allergical patients (mean±SD: 28.0±2.1 years) outside the pollen season and 16 normal non-allergic controls (23.38±8.9 years). We determined the density of goblet cells on haematoxilin-eosin stained specimens and graded conjunctival epithelial squamous (CES) metaplasia after Nelson. We measured the non-invasive tearfilm break-up time (NIBUT) with Tearscope and measured the C3a complement level in collected tear samples with ELISA kit (Quidel). Mann-Whitney test was applied. **Results**: The seasonal allergical patients outside the pollen season and the normal non-allergic controls did not differ significantly in the goblet cell count (median [range]: 131.25 [56.25–1250] vs. 78.13 [18.75–256.25] cells/mm^2; P=0.32) and C3a level (median [range]: 9.80 [0.78-122.78] vs. 6.43 [2.08-33.60] ng/ml; P=0.57) in the examined groups. Any or mild CES metaplasia was detected in most of the speciments of the allergic and the non-allergic groups (6/8 vs. 14/16 cases, P=0.97). Moderate CES metaplasia occurance was 2/8 vs

2/16 cases respectively. The difference of NIBUT was also not significant between the allergic and the non-allergic patients (mean±SD: 26.16±11.34 vs. 17.03±10.08 sec; P=0.07). **Conclusion**: temporal alteration of the signaling molecules on the ocular surface in the pollen season does not predestinate goblet cell count alteration or conjunctival epithelial metaplasia outside the season.

HYPEROSMOLAR STRESS ENHANCES HLA-DR EXPRESSION IN HUMAN CONJUNCTIVA. P. Versura, V Profazio, C Coslovi, L Foroni, C. Schiavi, E C Campos Ophthalmology Unit, Alma Mater Studiorum University of Bologna, Italy

Purpose The expression and distribution of HLA-DR were evaluated in conjunctival epithelium to verify whether initiation of the immune response by the conjunctival epithelium can occur after hyperosmolar stress. **Methods** Subconfluent primary human conjunctival epithelial cells (pHCECs) and human conjunctival organ cultures (hCOCs) cultured in iso-osmolar medium (305 mOsm/L) were exposed for 24 hrs to media with progressively higher osmolarity with or without TRP channel inhibitor (ruthenium red). Tear osmolarity was measured with TearLab Osmolarity System (OcuSense) in 15 normal subjects and 25 dry eye (DE) patients; conjunctival imprint cytology samples were obtained at the nasal bulbar area. HLA-DR expression was evaluated by immunocytochemistry on imprints from control subjects and DE patients, on pHCECs, on formalin fixed-paraffin embedded hCOCs, and by RT-PCR. Statistical evaluation was performed by applying the paired Student's t-test, the Spearman's rho and the Pearson's r correlation coefficients (significance p<0,05). **Results** HLA-DR expressed differently in DE vs control subjects (% mean±SD respectively 46,16±7,2 vs. 7,48±1,14, p< 0,0001) and exhibited significantly high correlations vs tear osmolarity value (r 0,614, p<0,0001). In vitro experiments showed a progressive increase in HLA-DR expression as the media's osmolarity was increased, from 6,75±1,16 (% mean±SD) in cells cultured in normal osmolarity to 9,96±1,37 and 12,94±4,04 in cells cultured in 350 and 400 mOsM/L media, respectively (p<0,05). A stepwise progressive increase was also found in hCOCs. Results were confirmed by RT-PCR evaluations. TRP channel inhibitor administration reduced significantly HLA-DR expression in hyperosmolar cultured cells **Conclusions** Data suggest that HLA-DR overexpression in human conjunctiva is in direct relationship with tear hyperosmolarity in DE patients and in *in vitro* models. Study supported in part by a grant from Fondazione Cassa di Risparmio in Bologna to ECC

PERFORMANCE OF MEIBOMETRY IN ASSESSING MEIBOMIAN GLAND DYSFUNCTION. P. Versura, A. Bron*, V. Profazio, M. Ortolani, C. Coslovi, Ec Campos Ophthalmology Unit, University of Bologna, Italy and *Nuffield Laboratory of Ophthalmology, Oxford University, UK

Purpose – To evaluate the diagnostic performance of Meibometry in Meibomian gland dysfunction (MGD). **Methods** - Ninety-six subjects (138 eyes, 62 women, 34 men, median age 49.5 and 52.7 yrs respectively) and 30 normal control subjects (55 eyes) were enrolled. Eighty six eyes were classified as high delivery (HD)-MGD, 52 as low delivery (LD)-MGD. Direct Meibometry(DM) measurements were made with an MB550 Meibometer (Courage-Khazaka GmbH). Standard curves were constructed relating arbitrary Meibometer optical density units (AU) to lipid equivalent values (µl). Integrated Meibometry (IM) was performed on scanned images of the lipid blot. Subjective symptoms were scored by OSDI, and Schirmer test I, Break Up Time (BUT), tear osmolarity (Tearlab, Ocusense), conjunctival scraping cytology were performed. Statistical analysis used SPSS 14.0 and MedCalc 5.0 software **Results.** AU values plotted on a log scale correlated highly with the lipid equivalent values (R^2= 0.913). Significant differences were found between control subjects vs all MGD patients and between HD vs LD-MGD patients for all the parameters evaluated (p <0.05 for all comparisons). Findings were: controls: 300±121 AU (0.04±0.015 ml), LD-MGD: 218±122 AU (0.03±0.015 ml) and HD-MGD: 564±115 AU (0.07±0.015 ml) (median±SD). DM and IM were significantly correlated (r=0.691, p<0.0001) and DM was correlated with BUT, OSDI score, scraping score and tear osmolarity, especially in LD-MGD patients. The selected DM diagnostic cut-off for LD-MGD was≤275 AU and for HD-MGD was ≥ 450 AU, (PPV 91). **Conclusions** Meibometry distinguished normal subjects from MGD subgroups with a good degree of accuracy.

IN VIVO CONFOCAL MICROSCOPY OF MEIBOMIAN GLANDS IN SJOGREN'S SYNDROME. Edoardo Villani, Michela De Capitani, Silvia Beretta, Daniela Galimberti, Francesco Viola, Roberto Ratiglia. Clinica Oculistica Università degli Studi di Milano. Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.

Purpose: To evaluate the morphological changes of the meibomian glands (MGs) and the status of periglandular inflammation in patients with primary (SSI) and secondary (SSII) Sjogren's syndrome by in vivo laser confocal microscopy (LSCM) and to investigate the correlations between clinical and confocal findings. **Methods**: Twenty patients with SSI, 25 with SSII, 20 with meibomian gland disease (MGD) and 20 age– and gender–matched control subjects were consecutively enrolled. Each partecipant completed an Ocular Surface Disease Index questionnaire and underwent a full eye exam (including BUT, fluorescein and lissamine green staining, and Schirmer test) and LSCM examination of the MGs (to study acinar unit density and diameter, meibum secretion reflectivity, atrophic and fibrotic changes and inflammatory cells density in basal epithelium, interstitium and glandular epithelium). **Results**: All the clinical and confocal parameters showed statistical significant differences among the groups (P<0.001, Kruskal Wallis test). Confocal microscopy showed no differences between SSI and SSII (Mann-Whitney U test). Compared to control subjects, SS MGs showed higher periglandular inflammation and secretion reflectivity and more evident atrophic and fibrotic changes (P<0.001, Mann-Whitney U test). Compared to MGD, SS MGs had higher acinar density, smaller diameter, higher density of periglandular inflammatory cells and lower secretion reflectivity (P<0.001, Mann-Whitney U test). In SS patients, the 3 considered confocal signs of inflammation were significantly interrelated and correlated with corneal fluorescein staining (P≤0.01, Spearman). Acinar density and diameter were strongly correlated between them (P<0.001) and with BUT (P<0.05). **Conclusions**: LSCM is capable of effectively revealing morphological and inflammatory changes in MGs. We found a prevalence of inflammatory signs–easily distinguishable from MGD obstructive changes–in SS patients. Commercial relationships: none. Financial support: none.

EVALUATION OF LIPID OXIDATIVE STRESS STATUS IN DRY EYE DISEASE. Tais H. Wakamatsu[1AB],Murat Dogru[1A,2],Yukihiro Matsumoto[1AB],Takashi Kojima[1AB],Minako Kaido[1AB],Osama M.A. Ibrahim[1AB],Ayako Igarashi[2],Enrique A. Sato[1AB],Yoshiyuki Ichihashi[1B],Jun Shimazaki[2]and Kazuo Tsubota[1B] [A]J&J Ocular Surface and Visual Optics, [B]Ophthalmology, [1]Keio University School of Medicine, Tokyo, Japan;[2]Ophthalmology, Tokyo Dental College, Chiba, Japan

Purpose: The purpose of this study was to evaluate the levels of lipid oxidative stress marker and inflammatory cells from tears and

conjunctiva of patients with Sjögren Syndrome (SS) and normal subjects. **Methods**: Twenty-six eyes of 16 patients (16 females) with SS and 7 eyes of 6 (2 males and 4 females) normal healthy controls were examined in this prospective study. All subjects underwent Schirmer test, tear film break up time, fluorescein / Rose Bengal stainings, confocal laser scan microscopy of the nasal bulbar conjunctiva, tear collection for HEL (hexanoyl-lysine) ELISA and brush cytology from the nasal and temporal conjunctiva. The brush cytology samples underwent immunohistochemistry (IHC) staining with HEL and 4HNE (4-hydroxy-2-nonenal) to study lipid oxidation. Haematoxylin-Eosin and IHC staining with HEL, 4HNE were also performed on conjunctival samples of SS patients and controls. **Results**: The tear stability and vital staining scores were significantly worse in eyes with SS patients ($p<0.01$) compared to the controls. The density of conjunctival inflammatory cells was higher in SS subjects (mean: 448.7 ± 183.1 cells/mm2) compared to normal controls (mean: 45.1 ± 30.6 cells/mm2). The numbers of conjunctival cells positively stained for HEL and 4HNE were significantly higher in patients with SS compared with controls. The tear HEL concentrations tended to be higher in SS patients compared to controls and correlated significantly with Rose Bengal staining scores and inflammatory cell density in in vivo confocal microscopy. Conjunctival specimens also revealed considerably higher numbers of cells positively stained for inflammations markers as well as HEL and 4HNE in the IHC stainings. Positive correlations between reactive oxidative stress (ROS) markers, conjunctival inflammation and ocular surface epithelial damage were observed. **Conclusion**: Increase of the oxidative stress status in the conjunctiva of SS patients seems to play an important role in the pathogenesis of the dry eye disease. A close relationship may exist between ROS production, lipid peroxidation related membrane damage and inflammatory processes in dry eye.

IN VIVO VISUALIZATION OF PRE-CORNEAL TEAR FILM IN DRY EYE PATIENTS. Jianhua Wang, MD, PhD,[1,2] Lele Cui, MD[1,3] Victor L. Perez, MD, Meixiao Shen, MSc,[1] Michael R. Wang, PhD[2] [1]Bascom Palmer Eye Institute, University of Miami Miller School of Medicine [2]Electrical and Computer Engineering, University of Miami [3]School of Ophthalmology and Optometry, Wenzhou Medical College

Purpose: To visualize pre-corneal tear film (PCTF) in dry eye patients using ultra-high resolution optical coherence tomography (UHR-OCT). **Methods:**A custom built UHR-OCT with ultra-high resolution (~3 µm) was used to image the PCTF and tear meniscus at the vertical meridian. Thirty eyes of total 15 previously diagnosed dry eye patients (7 males and 8 females, age 51.7 ± 20.5 yrs) with aqueous tear deficiency were studied. Images were taken during normal and delayed blinking. PCTF was visualized. The PCTF thickness and tear meniscus volumes (TMV) were obtained. **Results:** During normal blinking, the PCTF was visualized on 3 of 30 eyes with averaged PCTF of 3.3µm (SD 3.8µm) from all 30 eyes. The TMV was 0.9µl (SD 0.5). During delayed blinking, PCTF was visualized on 23 eyes with significantly increased thickness of 5.8µm (SD 7.4µm, $P<0.05$). The TMV was 1.6µl (SD 1.3µl), which was significantly greater than that during normal blinking ($P<0.05$). During delayed blinking, PCTF was 3.1µm (SD 1.7) on the eyes without the visualized PCTF and the TMV was 0.9µl. On those eyes with the PCTF being visualized, the PCTF was 6.6µm (SD 8.2) and the TMV was 1.9µl (SD 1.3), which were significantly greater than that on those eyes which had the PCTF not being visualized ($P<0.05$). **Conclusions:** This was the first study for in vivo visualization of the PCTF on dry eye patients using UHR-OCT. The PCTF could be directly visualized on some eyes, especially during delayed blinking. (Supported by a research grant from Allergan, Inc)

TEAR MITOGEN LACRITIN RAPIDLY COUNTERS INFLAMMATORY STRESS IN HUMAN CORNEAL EPITHELIAL CELLS. Ningning Wang, Gordon W. Laurie Cell Biology, University of Virginia

Objective: The rapid stress response of interferon-gamma-sensitized human corneal epithelial cells to TNF includes the self-catabolic process known as autophagy. Autophagy is both a regulator and effector of innate and adaptive immunity. The human prosecretory mitogen lacritin, normally present in human tears, counters autophagy in a biphasic dose-dependent manner within 1 - 5 min. Here we present new studies addressing the mechanism.**Method:** Human corneal epithelial (HCE; Riken) cells were sensitized with INFG and then treated with TNF in the presence of 10 nM lacritin or inactive C-25 lacritin for 0, 1, 5 or 15 min or at 0, 0.1, 1, 10, 100 or 1000 nM lacritin or C-25. Lysates were collected and immunoprecipitates of autophagosome marker LC3 were blotted for mediators of autophagosome formation and turnover including ATG4B, ATG3, ATG7, ATG12, ULK1 and p62. **Result:**A) Lacritin, but not C-25, reduced the quantity of lipidated autophagosome marker LC3-II within 1 - 5 min via a biphasic dose response that is optimal at 10 nM. B) Ubiquitin-binding and NFkB pathway activator p62 (SQSTM1) binds LC3 via a biphasic lacritin, but not C-25 dose response, that is optimal at 10 nM. C) LC3 binding to LC3 activating protease ATG4B, to LC3 activating E1-like protein ATG7, to ATG12 and to autophagic serine/threonine kinase ULK1 was absent at 1 min in lacritin, but not C-25, treated cells. **Conclusion:** Lacritin signaling rapidly blocks the cellular machinery needed for autophagosome formation when human corneal epithelial cells are subjected to stress from inflammatory cytokines. Supported by EY018222 (to GWL). N. Wang, UVa Patent Fdn. G.W. Laurie, EyeRx, C; UVa Patent Fdn, P.

EFFICACY OF SURGERY FOR CONJUNCTIVOCHALASIS WITH SUBJECTIVE AS WELL AS OBJECTIVE SYMPTOMS. Hitoshi Watanabe[1,2]Sizuka Koh[2], Yuichi Hori[1] Kansai Rosai Hospital[1], Osaka University Medical School[2]

Objective: Conjunctivochalasis often causes poor formation of tear meniscus leading to poor spread of the tear film over the ocular surface at blinking. This sometimes induces the epithelial damage of the cornea, and, in this sense, conjunctivochalasis is one of the risk factors to dry eye. In those eyes, the corneal damage often can't be relieved with usual eye drop and surgery for conjunctivochalasis is necessary to relieve subjective and objective symptoms. In this study, the efficacy of the conjunctivochalasis surgery is investigated. **Methods:** Sixty eyes of 45 conjunctivochalasis patients with dry eye receiving the designed conjunctivochalasis surgery were enrolled in this study. Prominent conjunctivochalasis along the lower lid was observed in all the eyes before surgery and their objective and subjective symptoms were not sufficiently controlled by the usual eyedrop. Subjective symptoms of the patients were assessed before and after surgery by questionnaires. Scores of corneal fluorescein staining ranged from 0 to 15 were also evaluated before and after surgery. **Results:** Irritation, the most chief subjective symptom, before surgery was recognized in 93.3 % of the patients was improved to 8.3% after surgery. Corneal fluorescein staining scores were significantly improved from 2.6±1.1 before surgery ±to 0.9±0.7after surgery ($P<0.01$). **Conclusion:** Conjunctivochalasis causes objective and subjective symptoms and the designed surgery is very effective in resolving corneal damage as well as patient complaints.

THE PROTEINS AND THEIR INTERACTIONS IN HUMAN AND RABBIT TEARS: IMPLICATION ON TEAR FILM STABILITY. Eric Xiaojia Wei,[1,2] Zhenjun Zhao[1,2] and Mark DP Willcox.[1,2] Brien Holden Vision Institute, Sydney, Australia,[1] The School of Optometry and Vision Science, University of New South Wales, Sydney, Australia.[2]

Purpose. Protein-protein interactions may play a role in tear film stability due to their effect on tear film viscosity.Salt concentrations in solution have an effect on protein-protein interactions and thus tear film stability. Rabbits have a more stable tear film than humans. Thus, we sought to examine the tears of rabbits and humans for proteinprotein interactions, osmolality and metal types and amounts. **Methods**. Tears were collected from normal adult humans (n=10) and rabbits (n=6) using microcapillary tubes and pooled for each study group. Protein components were analyzed using one- (1D) and two-dimensional (2D) gel electrophoresis (GE) and identified using mass spectrometry. Potential protein-protein interactions were studied using Blue Native-GE and Native 2D-GE and Co-immuno-precipitation. Tear osmolality was measured by vapor pressure osmometery and metals analyzed using inductively coupled plasma (ICP) mass spectrometry or ICP atomic emission spectroscopy. **Results**. The protein concentration in the tears of rabbits was 2-fold higher than in the humans. In rabbit tears, the most abundant proteins were lipophilins whereas in human tears lysozyme, lactoferrin and lipocalin were predominant. Several potential protein-protein interactions were identified in both human (DMBT1-lactoferrinlysozyme and lactoferrin-lipocalin) and rabbit tears (interactions of lipophilin subclasses). Major metals in tears did not differ between species, however, rabbit tears had much higher levels of Mg2+and Ca2+. Notably, rabbit tears had double the osmolality of human tears. **Conclusions**. The high abundance of lipophilins and their possible interactions in rabbit tears may contribute to the stability of their tear film. The higher concentration of Mg2+and Ca2+ in tears may help maintain the protein-protein interactions and consequently tear film stability.

EXOCYTIC MACHINERY REVEALED BY HIGH RESOLUTION INTRAVITAL MICROSCOPY IN LIVE ANIMALS. Roberto Weigert Intracellular membrane Trafficking Unit, Oral and Pharyngeal Cancer Branch National Institute for Dental and Craniofacial Research National Institute of Health, Bethesda, MD USA

The physiology of the major secretory organs is regulated by a complex equilibrium of sorting and trafficking events at the level of both the secretory and the endocytic pathways. Our major effort has been directed towards the understanding of the machinery controlling regulated protein secretion. A full understanding of these processes has been hampered by the lack of reliable in vitro systems that recapitulate the complexity of these organs, and by the fact that most in vivo studies have relied on measurements of the levels of secreted proteins as an indirect estimate of the secretory granule exocytosis. To this aim, we set up an experimental system to image and track the secretory granules in the salivary glands of live animals, which is based on the use of high-resolution intravital microscopy performed on a series of transgenic mouse models expressing various fluorescently labeled molecules. We have shown that the dynamics of the secretory granules in a live animal is regulated differently than what previously reported in *in vitro* systems. Furthermore, we have shown that the actin-myosin complex that is recruited on the secretory granule during secretion serves multiple functions: 1) to prevent the homotypic fusion between the granules, 2) to provide a scaffold to prevent either the osmotic stress or hydrostatic imbalances from disrupting the collapse of the granules, and 3) to drive the collapse of the SCGs to the APM through the recruitment of the myosin II. In conclusion, this novel approach has enabled us to dynamically dissect sub-cellular processes under physiological conditions where the proper three-dimensional architecture, signaling networks and metabolic pathways are maintained and not altered as occurs in *in vitro* or *ex-vivo* models. We envision that this approach will be extended to address fundamental biological issues not only in the context of exocytosis or membrane trafficking but also in all the other areas of cell biology.

IS INFLAMMATION INVOLVED IN THE "TIRED EYE" RESPONSE? Mark DP Willcox, Percy Lazon de la Jara, Eric Papas, Jennie Diec, Zhenjun Zhao Brien Holden Vision Institute, Sydney, Australia

Purpose: Many people complain of "tired eyes" towards the end of the day, with complaints usually including descriptions of discomfort, dryness and irritation. The purpose of the current research was to examine tears at the beginning and end of the day for the amount and type of inflammatory mediators. **Methods**:45 people were asked to rate their ocular comfort, in the morning (after 2 hours of awaking) and in the evening prior to sleep, on a scale of 1-100; 100 being perfectly comfortable, 1 being extremely uncomfortable. After rating their comfort, each person collected tears using a microcapillary tube. Tears were then stored at 5°C and transported to the laboratory where they were transferred to a -80°C freezer until use. Tears were then examined for the a amount of various inflammatory mediators using either ELISA or microbead assay. Pro- and anti-inflammatory mediators examined included histamine, bradykinin, cortisol, and cytokines and chemokines. **Results**:Comfort of the population reduced from the morning to the evening, with an average reduction of 3 points (84.3 ± 13.4 AM to 81.3 ± 15.5 PM; p=0.021). Only bradykinin, cortisol, IP-10 and LTB_4 were significantly increased in tears in the morning compared to the evening ($p \leq 0.026$). A subset of cytokines including IFN and IL-6 were all significantly increased in tears in the evening compared to the morning ($p \leq 0.045$). For the population, there was no significant difference in amounts of histamine or 20 other cytokines including IL-12p70, IL-8, VEGF, IL-1. However, for a subset of individuals who had a >10 point drop in comfort over the day there was a small increase in the concentration of histamine in tears in the evening (19.5 ± 2.3ng/ml) compared to those people who had <10 point comfort drop (11.7 ± 2.9ng/ml). **Conclusions**:The "tired eyes" that people complain of in the evening may be the result of the increased production of a subset of inflammatory mediators and these increase in the tears of subjects during the day.

EVALUATION OF CORNEAL STAINING IN A HEALTHY, NON-DRY EYE POPULATION. Ravaughn Williams,[1] Judy Vittitoe,[1] Michael Brubaker,[1] Michelle Senchyna,[1] Gary Foulks.[2] Alcon Research Ltd,[1] Fort Worth, TX, USA; University of Louisville,[2] Louisville, KY, USA.

Purpose. Fluorescein corneal staining (FCS) is an important indicator of corneal epithelial integrity and is used as a key diagnostic tool in the clinical assessment of dry eye disease. In order to establish FCS endpoints that can be used to assess clinically meaningful changes in corneal health, it is important to understand the dynamics in healthy, non-dry eye subjects.. Though there have been numerous studies where FCS in non-dry eye subjects has been described, interpretation of the data is difficult due to the marked differences in the methods utilized in those studies.. Therefore, the primary objective of this study was to quantify the levels of FCS in a healthy, non-dry eye population. **Methods.** One hundred and twenty-two (122) subjects ≥18 years of age, who did not have dry eye disease were enrolled across three US sites. Equal numbers of females and males were enrolled in each of four age categories. The subjects who successfully met the inclusion and exclusion criteria were seen at

Day 0 (screening visit, V1), Day 14 ± 5 days (V2) and Day 28 ± 5 days (V3) at approximately the same time of day after 1pm. At each study visit, FCS was assessed at 2.5 to 3 minutes after instillation of 5ul of 2% preservative free sodium fluorescein. FCS was assessed using a 0 to 4 scale and in 5 regions of the cornea per the NEI grid. Other dry eye clinical assessments were made during the study visits. **Results.** The percent of healthy non-dry eye subjects with a composite FCS score of zero at Visit 1, Visit 2 and Visit 3 were 54.1%, 67.5% and 71.7%, respectively (mean scores were 0.79, 0.60 and 0.58, respectively). The correlation coefficients between Visit 1 and Visit 2, Visit 1 and Visit 3, and Visit 2 and Visit 3 were 0.30, 0.43 and 0.47, respectively. **Conclusions.** FCS is a normal finding in the healthy, non-dry eye population which is not representative of dry eye disease but of a common process occurring on the corneal surface. These findings need to be considered when establishing FCS endpoints within clinical studies evaluating the efficacy of dry eye treatment strategies.

A UNIQUE OCULAR SURFACE INTERFEROMETER (OSI) TO MEASURE DYNAMIC LIPID LAYER THICKNESS (LLT).
T.Willis[1], S.M. Grenon[1], D.R. Korb[1,2], C.A. Blackie[1,2], W. Weber[3], R. Chinnock[3]. [1]TearScience, Morrisville, NC; [2]Korb Associates, Boston, MA; [3]Optimum Technologies, Southbridge, MA.

Objective: To provide an ophthalmic imaging device intended for use on dry eye patients to capture, archive, manipulate and store digital images of specular (interferometric) tear film observations. **Methods:**The LipiView® Ocular Surface Interferometer (OSI) acquires the specularly reflected tear film colors. A spatially modulated light source allows removal of unwanted background images and stray light. Blinks and artifacts are removed automatically. Statistical data are compared to a master look-up table (LUT). The LUT is calculated using optical physics, commercial and custom modeling tools, precise calibrations of the white light source and camera spectral responses, along with published refractive index and dispersion values for lipid and aqueous layers. This LUT has a spiral locus in RGB space. The processed output, expressed as Interference Color Units (ICUs), correlates with Lipid Layer Thickness (LLT), using a kinemetric distribution. **Results:**The processed output matches well to the expected LUT RGB spiral, demonstrating agreement with theory. In addition a previous study on an early prototype OSI indicates the clinical suitability and applicability of this imaging technology. **Conclusion:**The LipiView® OSI is a viable objective tool for live evaluation of LLT. It is likely to significantly impact the clinical diagnosis

DIFFERENTIAL EFFECT OF INDIVIDUAL ELECTROLYTES ON CORNEAL EPITHELIAL BARRIER FUNCTION DURING HYPEROSMOTIC STRESS.
Ashley Woodward,[1] Michelle Senchyna,[3] Pablo Argüeso.[1,2] Schepens Eye Research Institute,[1] Harvard Medical School,[2] Boston, MA, USA, Alcon Research, Ltd.,[3] Fort Worth, TX, USA.

Purpose. Hyperosmolarity is one of the defining characteristics of dry eye disease. The purpose of this study was to determine the contribution of individual electrolytes to barrier function and cell viability in human corneal limbal epithelial (HCLE) cells under hyperosmotic stress. **Methods.** Stratified cultures of HCLE cells were exposed to short- and long-term hyperosmotic stress. For short-term stress, HCLE cells were incubated in 8-well slides with 0-500 µM $CaCl_2$, $MgCl_2$, KCl and NaCl for 5 minutes. For long-term stress, HCLE cells were stratified in transwell inserts in the presence of 300-550 mOsm hyperosmotic solutions for 7 days. Barrier function was assessed using rose bengal dye uptake, to determine alterations in glycocalyx integrity, and transepithelial electrical resistance (TER), an indicator of tight junction permeability. Cell viability was determined using the colorimetric MTT assay. **Results.** Individual electrolytes produced differential responses in barrier function and cell viability during hyperosmotic stress. Short-term exposure of stratified HCLE cells to calcium, magnesium, and potassium resulted in a 40-50% increase in rose bengal uptake, whereas sodium did not significantly alter barrier function. In these experiments, the increase in dye uptake after calcium exposure was abrogated ($p<0.05$) by the metal chelator EDTA, suggesting that barrier dysfunction in this model is not dependent on osmotic pressure. Long-term exposure to hyperosmotic solutions did not affect TER or cell viability when osmolarity values were below 400 mOsm. Above 400 mOsm, calcium severely disrupted TER and cell viability, by 95 and 99%, respectively, whereas sodium produced a more moderate effect, by 47 and 70%, respectively. **Conclusions.** Data from this study indicates that individual electrolytes differentially induce damage to stratified cultures of corneal epithelial cells during hyperosmotic stress. Targeting specific ions could represent a new alternative for the diagnosis and treatment of dry eye. Support. Alcon Research, Ltd., Fort Worth, TX, USA

VARIABILITY OF THE SCHIRMER TEST RESULTS.
Hiroko Yamagami, Ayumi Ota, Nozomi Kinoshita, Fumihiko Toyoda and Akihiro Kakehashi. Department of Ophthalmology Jichi medical university, Saitama Medical Center, Saitama, Saitama, Japan.

Propose. The Schirmer Test is the most commonly applied clinical test for lacrimal secretary function. Although the date is notoriously inaccurate, it remains the mainstay in the clinical diagnose of dry eye. We investigated the variability of the Schirmer test of normal eyes. **Methods.** Five healthy volunteers (4 women and 1 man) were included. The Schirmer 1a test (S-T) was performed once a week and twice a day in the morning (AM) and in the afternoon (PM), more than four days. The measurements were conducted in the same room in which the temperature was maintained at 23 to 26°C and the humidity was 35 to 70%. **Results.** The average S-T (mean±SD mm, AM/PM) of case 1 (26-year-old woman) was 29±5.6/29.7±7.5 on the right eye, 28.8±7.1/33.5±2.5 on the left. For case 2 (25-year-old woman), it was 28.5±7.6/31.3±4.5 on the right, 19.8±12.1/20.8±8.5 on the left. Case 3 (39-year-old woman), it was 17±3.6/20.3±9.0 on the right, 19.7±3.1/19.3±7.3 on the left. Case 4 (50-year-old woman), it was 13.6±5.9/10.3±4.6 on the right and 13.5±4.5/11.3±4.5 on the left. Case 5 (22-year-old man), it was 10.2±6.2/15.7±8.3 on the right and 8.8±3.7/14.6±7.0 on the left. There were no significant differences between the S-T in AM and PM. The low S-Ts (<10 mm) were measured in Case 4 and 5 several times. **Conclusions.** It seems that, there is variability of the Schirmer test results, because of the large deviations. The reexamination of S-T is necessary for diagnoses of dry eye.

DONOR-RELATED CANDIDA KERATITIS AFTER DESCEMET STRIPPING AUTOMATED ENDOTHELIAL KERATOPLASTY.
Katsuya Yamazoe, Seika Den, Yoichi Tanaka, Kazuki Hotta, Jun Shimazaki 1)Kameda Medical Center 2)Tokyo Dental College

Purpose: To report a case of donor-to-host transmission of *Candida albicans* after Descemet stripping automated endothelial keratoplasty (DSAEK). **Methods**: A 74-year-old man with bullous keratopathy and cataract (BCVA=20/500) underwent uneventful DSAEK combined withcataract surgery on his left eye using donor corneas obtained from an United States eye bank. On postoperative day 20, the graft was clear and vision recovered to 20/30. Five weeks after surgery, the patient complained of ocular pain and blurred vision. Dense white infiltrates were found in the host-graft interface and the incision site associated with hypopyon. Because the culture test in donor corneoscleral rim showed positive growth of *Candida albicans* ,

we started antifugal therapy. **Results**: Despite intensive topical and systemic antifungal therapy, the corneal infiltrates were increased, therefore we performed removal of the DSAEK graft combined with extensive anterior chamber irrigation and intravitreous antifungal agents injection. The DSAEK graft and anterior chamber fluid were positive for *Candida albicans*. With continuous medical therapy including corneal scraping and topical/systemic antifungal therapy, corneal infiltration gradually decreased leading to scarring in approximately two months. Since the visual acuity decreased to hand motion, we performed penetrating keratoplasty (PKP). The visual acuity increased to 20/40 three weeks after PKP. **Conclusion**: Although donor-related Candida keratitis after DSAEK has been previously reported, this is the first case in Asia using an overseas donor tissue. The infection developed posterior to the host cornea, and it was resistant to medical therapy. Removal of the graft seemed to be effective in our case.

EFFECT OF PUNCTAL OCCLUSION ON LIPID-LAYER SPREAD AND TEAR FILM STABILITY IN AQUEOUS-DEFICIENT DRY EYE. Norihiko Yokoi,[1] Rieko Sakai,[1] Anthony J. Bron,[2] John M. Tiffany,[2] Georgi As. Georgiev,[3] and Shigeru Kinoshita.[1] Kyoto Prefectural University of Medicine,[1] Kyoto, Japan; University of Oxford,[2] Oxford, UK; University of Sofia,[3] Sofia, Bulgaria

Objective: This study investigated the effect of upper and lower punctal occlusion (PO) on the initial velocity of tear-film lipid-layer spread [H'(0)] and the stability of the precorneal tear film [non-invasive breakup time (NIBUT)]. **Methods:** The study involved 8 females with aqueous-deficient dry eye (ADDE), without MGD [mean age: 53.5±16.3 (SD)]. We measured the following before and after PO: i. meniscus radius (R, mm) by meniscometry, ii. H'(0) (mm/sec., calculated by the cross-correlation method and the Voigt rheological model) and iii. non-invasive BUT (NIBUT, sec.) (both by video-interferometery), iv. fluorescein BUT (FBUT), v. ocular surface epithelial damage (OSED) assessed by fluorescein staining, and vi. Schirmer 1 test (ST1, mm/5 min.). **Results:** Measured values [mean±SD; before PO and after PO, respectively)] were all significantly improved ($p<0.01$) after PO [R (0.14±0.06; 0.52±0.18); H'(0) (1.03±1.31; 8.19±3.80); NIBUT (1.6±2.4; 9.3±1.3); FBUT (0.8±0.9; 6.0±3.2); OSED score (7.3±1.6; 1.8±1.5); ST1 (2.3±1.8; 11.8±6.0)]. **Conclusions:** Significant improvements of H'(0) and NIBUT occurred after PO, in ADDE. Since H'(0) is an index of tear volume, the improvements were considered due to the increase of aqueous tear volume and the decrease of OSED. [Supported by Grant-in-Aid No. 21659402 for Scientific Research from the Ministry of Education, Science, Culture, and Sports of Japan.]

MAINTENANCE EFFECT OF EXPERIMENTAL DRY EYE AFTER DEPRIVATION OF DESICCATING STRESS IN C57BL/6 MICE. Kyung-Chul Yoon Department of Ophthalmology, Chonnam National University Medical School and Hospital

Objective: To investigate the duration of maintenance of experimental dry eye after deprivation of desiccating stress in C57BL/6 mice **Method**: Experimental dry eye was induced in 6- to 8-week-old C57BL/6 mice, by subcutaneous injection of scopolamine with exposure to an air draft for 10 days. Tear volume and corneal smoothness were measured at baseline, 5 and 10 days after desiccating stress, and 3, 7, 10, 14, and 21 days after deprivation of desiccating stress (n=4). PAS staining and immunohistochemisty were performed to evaluate the density of conjunctival goblet cells and CD4+ T cells in each group. **Result**: Tear volume decreased from 1,230±415 μm at baseline to 360±185 μm ($p<0.01$) at 5 days after desiccating stress, which improved to 887±363 μm ($p=0.37$ compared with baseline) at 3 days after deprivation of desiccating stress. Corneal smoothness was deteriorated from 0.38 ±0.52 at baseline to 2.38±1.19 ($p<0.01$) at 5 days after desiccating stress, which improved to 0.50±0.53 ($p=0.63$ compared with baseline) at 7 days after deprivation of desiccating stress. Density of conjunctival goblet cells and CD4+ T cells which decreased at 5 days after desiccating stress was recovered at 10 days after deprivation of desiccating stress. **Conclusion**: After deprivation of dessicating stress, tear production and corneal smoothness improved on day 3 and day 7, respectively, and conjunctival goblet cells and CD4 T cells were returned to baseline levels on day 10. Experimental dry eye can be reversed after desiccating environment is improved in unsusceptible mice, in contrast to autoimmune mice or adoptively transferred mice. Commercial interests or grant support: none

ISOLATION AND PROPAGATION OF MESENCHYMAL STEM CELLS FROM THE LACRIMAL GLAND. Samantha You, Claire Kublin and Driss Zoukhri Tufts University School of Dental Medicine and Departments of Neuroscience, Tufts University School of Medicine, Boston, MA

Purpose: In previous studies, we reported that murine lacrimal gland is capable of repair following experimentally induced injury and that the repair involved mobilization of stem/progenitor cells. The aim of the present study was to determine if stem/progenitor cells can be isolated from the lacrimal gland and propagated in vitro. **Methods:** Lacrimal gland injury was induced by injection of recombinant human interleukin-1 (IL-1) whereas injection of saline (vehicle for IL-1) served as control. Two and half days following injection, the lacrimal glands were removed and used to prepare explants for tissue culture. One piece of the lacrimal gland was processed for histopathology and immunohistochemistry. Cells derived from the explants were grown in DMEM supplemented with 10% fetal bovine serum. Cells were stained for several stem cell markers including nestin, vimentin, ABCG2, Pax-6 and proliferation was measured using an antibody against Ki67. The adipogenic capability of these cells was also tested in vitro. **Results:** Our results show that stem/progenitor cells can be easily isolated from IL-1 injected, but not saline injected lacrimal glands. The cells stained positive for all markers of stem cells tested and some were also positive for alpha-smooth muscle actin, a marker of myoepithelial cells. Lacrimal gland stem cells proliferate in vitro and can be induced to form adipocytes attesting for their mesenchymal stem cell property. When these cells were grown in collagen scaffolds, they formed acinar- and ductal-like structures. **Conclusion:** We conclude that murine lacrimal glands contain mesenchymal stem cells that participate in tissue repair. These cells can be easily isolated and propagated in vitro. Future studies will test the effect of other scaffolds and growth factors/cytokines known to regulate lacrimal gland functions. Commercial interest: None. Supported by RO1 EY12383.

ALTERATIONS IN TEAR SECRETION, CORNEAL SENSITIVITY AND WOUND HEALING IN DIABETIC RATS. Fu-Shin Yu, Jia Yin and Keping Xu Kresge Eye Institute, Wayne State University School of Medicine

Purpose: This study examines changes in tears secretion, corneal sensitivity, and wound healing in rat type 1 and type 2 diabetes models. **Methods**: Type I DM was induced with streptozotocin (STZ) in male Sprague–Dawley rats and the Goto-Kakizaki (GK) rats, a spontaneous model of type 2 diabetes, bred in house. The ocular surface of diabetic and control rats was examined by fluorescein/Rose Bengal staining; corneal sensitivity by the Cochet-Bonnet method, tear volume by the cotton thread test, and wound healing by epithelial scratch wound. **Results**: Diabetic rats, both type

1 and 2, displayed an increase in Rose Bengal and a great decrease in tear secretion. Corneal epithelial wound closure was delayed in DM rats. The impairment in wound healing appeared associated with an increase in epithelial apoptosis and a decrease in EGFR signaling. Wounding resulted in the loss of corneal sensitivity in both diabetic and normal rats; however regain of corneal sensitivity post injury was much slower in diabetic rats. Treating the diabetic corneas with HB-EGF and TGF- eye drop two weeks prior wounding attenuated the effects of hyperglycemia on rate of epithelial wound closure. **Conclusion**: DM has profound effects on corneal functions including delayed wound healing, reduced tear secretion, and attenuated sensitivity after injury. Impairment of EGFR signaling in epithelial cells and defect in corneal innervations might be the underlying mechanisms of diabetic corneal disorders.

ADVANCED GLYCATION END PRODUCT (AGE) MODIFIED PROTEINS IN TEARS OF DIABETIC PATIENTS.

Zhenjun Zhao,[1,3] Jingfang Liu,[1,2] Bingyin Shi,[2] Shuixiang He,[2] Xiaoli Yao,[2] and Mark D.P. Willcox[1,3], Brien Holden Vision Institute,[1] Sydney, Australia; First Hospital Affiliated to Medical College, Xi'an Jiaotong University,[2] Xi'an, China; The School of Optometry and Vision Science, University of New South Wales,[3] Sydney, Australia.

Purpose.High glucose level in tears of diabetic patients may lead to advanced glycation end product (AGE) modified proteins. This study investigated AGE modified tear proteins and compare their levels in diabetic patients (DM) with non-diabetic controls (CTL). **Methods**. Basal tears were collected from DM with (DR) or without (DNR) retinopathy and CTL. Total AGE modified tear proteins were detected quantitatively by a dot immunobinding assay. The AGE modified tear proteins were separated in 1D and 2D SDS gels and detected by western-blotting. The individual AGE modified proteins were also compared between groups using densitometry. **Results**. Compared with the CTL group, tear concentrations of AGE modified proteins were significantly elevated in DR and DNR groups. The concentration of AGE modified proteins in diabetic tears were positively correlated with HbA1c and postprandial blood glucose level (PBG). Western blotting of AGE modified proteins from 1D SDS gels showed several bands, the major one at around 80-kDa. The intensities of AGE modified protein bands were higher in DM tears than in CTL tears. Western blotting from 2D SDS gels showed a strongly stained horizontal strip, which corresponded to the major band in 1D gels. Most of the other AGE modified protein species were within molecular weight of 40-80-kDa, PI 5.2-7.0. Densitometry analysis demonstrated several AGE modified tear proteins were elevated in DR or DNR tears.**Conclusions**.Total and some individual AGE modified tear proteins were elevated in DM tears. AGE modified tear proteins may be used as biomarkers to diagnose diabetes and/or diabetic retinopathy. This work was supported in part by Brien Holden Vision Institute, and a grant LP0669178 from the Australian Research Council (ARC).

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