5th International Conference on the Tear Film & Ocular Surface: Basic Science and Clinical Relevance

Conference Program & Abstract Book

Taormina, Sicily, Italy
September 5-8, 2007

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Preface

During the past several decades, a significant, international research effort has been directed towards understanding the composition 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 5th International Conference on the Tear Film & Ocular Surface: Basic Science and Clinical Relevance will be held at the Palazzo dei Congressi in Taormina, Sicily, Italy, from September 5 to 8, 2007. 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, over 340 scientists, physicians and industry representatives from numerous countries, including Argentina, Australia, Austria, Belgium, Brazil, Canada, Denmark, Finland, France, Germany, Greece, Italy, Japan, Mexico, Northern Ireland, Norway, Poland, Romania, Serbia, Singapore, South Korea, Spain, Sweden, Switzerland, Tanzania, Thailand, Turkey, 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 Julie Karimi, Roberta Giammorcaro, Eleonora Fumi and Jaka Congressi, Haydée Marangoni and h.design, Sabrina Zappia and CITYNet, Salvino Benanti, Giuseppe Benanti, Comune di Taormina, Taormina Arte, Palazzo dei Congressi, Fabiano Group, Pitto Tefiletti, Salvatore Cilona, San Domenico Palace Hotel, Venuto Viaggi, Alfa Service, Catering Piliere, CST Ciccarelli, La Botte, La Giara, Déjà Vu, Villa Antonio, Rocca Gullotta, Isabella Bambara, Dott. Villari della Sopraintendenza di Messina and Antonio Cutino for their help with the organization of this Conference.

Recognition

The Directors congratulate the following individuals, who were the recipients of the Conference Travel Awards: Amanda Ackerman, Jeffrey Bair, Linda Banbury, Lilian Chiang, Fabian Garreis, Kari Green-Church, Vinodh Kakkassery, Flavio Mantelli, Cuong Nguyen, Rachel Redfern, Shivaram Selvam, Phillip Steven, Padmaja Thomas, Tais Wakamatsu and Ai Yamada.

Thursday, September 6, 2007

OPENING REMARKS

8:00 *Giuseppe Benanti (Italy)*

CLAES H. DOHLMAN CONFERENCE ADDRESS

Chairperson – Claes H. Dohlman (USA)

8:05 Epidemiology Of Dry Eye Disease. Debra A. Schaumberg, ScD, OD, MPH, Brigham and Women's Hospital, Harvard Medical School, Boston MA USA.

SESSION I: TEAR FILM

Mucins: Origin, Properties, Regulation & Function

Chairpersons - Kermit Carraway (USA), Ilene K. Gipson (USA), Friedrich Paulsen (Germany)

8:30 **Keynote Address:** Mucin Structure And Function – State Of The Art. Gunnar C. Hansson, M.D., Ph.D. Department of Medical Biochemistry, Göteborg University, Gothenburg, Sweden.

8:50 **Keynote Address:** O-Glycosylation Of Mucins. Pablo Argüeso Schepens Eye Research Institute and Department of Ophthalmology, Harvard Medical School, Boston, MA, USA

9:10 Comparison of Membrane-Associated Mucins Expression in the Human Ocular Surface and Oral Mucosal Epithelium. Yuichi Hori[1], Kohji Nishida[2], Hiroaki Sugiyama[1], Takeshi Soma[1], Shizuka Koh[1], Tomoyuki Inoue[1], Naoyuki Maeda[1], and Yasuo Tano.[1] Department of Ophthalmology, Osaka University Medical School, Suita, Osaka,[1] Department of Ophthalmology, Tohoku University Medical School, Sendai, Miyagi, Japan[2]

9:20 Identification And Mapping Of Oligosaccharide Moieties On Individual Secreted Mucins. Terence McMaster[1], Sarah Baos[1, 2], Debra Brayshaw[1], Peter Heard[1, 3], Monica Berry[2]. H.H.

Wills Physics Laboratory,[1] Academic Unit of Ophthalmology,[2] and Interface Analysis Centre, [3] University of Bristol, Bristol, U.K.

9:30 TGF-Beta Regulation Of MUC4 In Corneal Epithelial Cells. Kermit L. Carraway, Joseph Lomako, Wieslawa M. Lomako, Coralie A. Carothers Carraway. University of Miami School of Medicine, Miami, FL, USA

9:40 Morphology Of The Lid Wiper Region Of The Human Lid Margin In Histology And In-Vivo Confocal Microscopy. Erich Knop[1], Nadja Knop[2], Andrey Zhivov[3], Donald Korb[4], Jack V. Greiner[5], Rudolf Guthoff[3]. [1]Research Lab. of the Eye Clinic CVK, Charite – Universitätsmedizin Berlin; [2]Dept. for Cell Biology in Anatomy, Hannover Medical School; [3]University Eye Hospital Rostock; [4]Korb Associates, Boston; [5]The Schepens Eye Research Institute and Dept. Ophthalmology, Harvard Medical School, Boston, MA, USA

9:50 Discussion

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

Salt, Water & Protein: the Tear Film Aqueous Layer

Chairpersons - Darlene A. Dartt (USA), Austin K. Mircheff (USA), James W. Putney, Jr. (USA)

10:50 **Keynote Address:** Store-Operated Calcium Influx In Epithelial Cells: Recent Advances. Anant B. Parekh. Department of Physiology, University of Oxford, Oxford, UK

11:10 **Keynote Address:** Mechanisms Of Compound Exocytosis. J. Michael Edwardson. Department of Pharmacology, University of Cambridge, United Kingdom.

11:30 Calcium Entry Mechanisms In Epithelial Cells. James W. Putney, Jr. National Institute of Environmental Health Sciences-NIH, Research Triangle Park, NC, USA.

11:40 The Role Of Rab27b In The Regulation Of Intracellular Trafficking In The Lacrimal Gland. Lilian Chiang,[1] Kaijin Wu,[1] Francie Yarber,[1] Serhan Karver,[2] Sarah F. Hamm-Alvarez.[1,2] School of Pharmacy,[1] Keck School of Medicine,[2] University of Southern California, Los Angeles CA, USA.

11:50 Acinar Cell Paracrine Mediators Enforce Self-Tolerance In Rat Lacrimal Gland. A.K. Mircheff, T. Nakamura, M. de Saint Jean, Y. Wang, University of Southern California, Los Angeles, CA, USA

12:00 Myoepithelial Cells Originate From Nestin-Positive Precursors In The Lacrimal Gland. Marie A. Shatos, Linda Jonsson, Robin R. Hodges, Laura M. Tarko and Darlene A. Dartt. Schepens Eye Research Institute and Harvard Medical School, Boston, MA, USA

12:10 Discussion

12:25 Poster Viewing & Lunch

Poster Discussion I

Chairpersons - Esen K. Akpek (USA), Margarita Calonge (Spain), Robert A. Sack (USA)

1:55 EGF Activates Protein Kinase C*a* and -E and ERK1/2 To Stimulate Cultured Rat And Human Conjunctival Goblet Cell Proliferation. Darlene A. Dartt, Robin. R. Hodges, and Marie A. Shatos. Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School, Boston, MA.

2:00 Calcium And Cyclic AMP Alterations In Purinergic Regulation Of Rabbit Lacrimal Gland Acinar Cell Secretion. Stina K. Carlsson[1], Maria C. Edman[1], Sarah Hamm-Alvarez[2], J. Peter Gierow[1]. University of Kalmar, Kalmar, Sweden[1] and University of Southern California, Los Angeles, CA, USA[2].

2:05 Detection Of Surfactant Proteins A, B, C And D In The Human Lacrimal System And In Tear Fluid. Lars Bräuer,[1] Christian Kindler,[1] Kristin Jäger,[1] Saadettin Sel,[2] Bernhard Nölle,[3] Uwe Pleyer,[4] Matthias Ochs,[5] Friedrich P. Paulsen[1], [1]Department of Anatomy and Cell Biology and [2]Department of Ophthalmology, Martin Luther University of Halle-Wittenberg, Germany; [3]Department of Ophthalmology, Christian Albrecht University Kiel, Germany, [4]Department of Ophthalmology, Charite, Berlin, [5]Department of Anatomy, University of Bern, Switzerland.

2:10 Low abundance protein in tears of vernal keratoconjunctivitis (VKC) patients. Andrea Leonardi,[1] Massimo Bortolotti,[1] Sonal Sathe[2] and Robert Sack.[2] [1] Ophthalmology Unit, Department of Neuroscience, University of Padua, Italy. [2]SUNY College of Optometry, New York, NY, USA.

The Lipid Layer: Biogenesis & Functional Properties

Chairpersons - Ben J. Glasgow (USA), James P. McCulley (USA), John Tiffany (UK)

2:15 **Introduction:** Thomas J. Millar (Australia)

2:20 **Keynote Address:** Sebum, The Lipid Skin Layer: Biogenesis And Functional Properties. Christos C. Zouboulis. Departments of Dermatology, Venereology, Allergology and Immunology, Dessau Medical Center, Dessau, and Laboratory for Biogerontology, Dermato-Pharmacology and Dermato-Endocrinology, Institute of Clinical Pharmacology and Toxicology, Charité Universitaetsmedizin Berlin, Campus Benjamin Franklin, Berlin, Germany.

2:35 **Keynote Address:** Surface Tension Reducing And Host Defense Functions Of Lung Surfactant. Sam Hawgood, University of California San Francisco, San Francisco, California, USA.

2:50 **Keynote Address:** Functional Role Of The Tear Film Lipid Layer. Thomas J Millar. School of Natural Sciences, University of Western Sydney, Australia.

3:05 Evaporation Through Defined Surface Lipid Layers. John Tiffany, Naomi Lizinde. Nuffield Laboratory of Ophthalmology, University of Oxford, Oxford UK.

3:15 Role Of Evaporation On Aqueous Tear Loss And Potential Strategies For Treatment. McCulley JP, Uchiyama E, Di Pascuale MA, Butovich IA. Ophthalmology, University of Texas Southwestern Medical Center, Dallas, TX, USA.

3:25 Loss Of BMP Signaling Results In Meibomian Gland Dysplasia And An Altered Palpebral Conunctival Epithelium. David G. Ryan and Robert M. Lavker; Dept. of Dermatology, Northwestern University Medical School, Chicago, IL.

3:35 Discussion

3:50 Poster Session I (with Coffee & Tea)

Dynamics of the Tear Film: Biomechanics to Biomarkers

Chairpersons – Monica Berry (UK), Franz Grus (Germany), P. Ewen King-Smith (USA)

4:35 **Keynote Address:** Rheological Effects On Tear Film Rupture. Madhu S.R.Gorla[1] and Rama S.R.Gorla.[2] Rush University Medical Center, Chicago, IL, USA[1], Cleveland State University, Cleveland, OH, USA[2]

4:50 **Keynote Address:** Development And Application Of Tear Lipidomics In Mass Spectrometry. Bryan M. Ham Pacific Northwest National Laboratory, Richland, WA USA.

5:05 **Keynote Address:** Tear "Omics": Application To Clinical Problems. Roger W. Beuerman,[1,2,3] Lei Zhou, [1,2] Singapore Eye Research Institute,[1] Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore,[2] School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore.[3]

5:20 Contributions Of Evaporation And "Tangential Flow" To The Mean Thinning Rate Of The Tear Film. P. Ewen King-Smith,[1] Jason J. Nichols,[1] Richard J. Braun,[2] College of Optometry, Ohio State University, Columbus, Ohio,[1] Department of Mathematical Sciences, University of Delaware, Newark, Delaware[2]

5:30 Human Tear Fluid Phospholipid Transfer Protein (Pltp) Interacts With Lysozyme. Timo Tervo[1], Niko L. Setälä[1,2], Matti Jauhiainen[2] and Juha M. Holopainen[1]. [1]Department of Ophthalmology, University of Helsinki, Finland, [2]Department of Molecular Medicine, National Public Health Institute, Biomedicum, Helsinki, Finland.

5:40 Proteomics Study Of The Influence Of Contact-Lens Cleaning Solutions On The Protein Profiles In Tear Film. F. Grus, S Beyer, N Bozkurt, S. Haeder, N. Pfeiffer. Experimental Ophthalmology, Dept. of Ophthalmology, University of Mainz, Germany.

5:50 Discussion

Poster Session I

Chairpersons - Esen K. Akpek (USA), Margarita Calonge (Spain), Robert A. Sack (USA)

1. Functions Of The Membrane-Associated Mucin MUC16 At The Corneal Surface. Ilene K. Gipson, Timothy D. Blalock. Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School, Boston, MA, USA.

2. Expression Of Soluble And Membrane Bound MUC16 In Dry Eyed Postmenopausal Women. Sruthi Srinivasan[1], Elizabeth Joyce[1], Miriam L. Heynen[1] Lyndon Jones[1], Trefford Simpson[1], Daniel A Gamache[2], Michelle Senchyna[2]. [1]Center for Contact Lens Research, School of Optometry, University of Waterloo, Ontario, Canada, [2]Alcon Research Ltd, Fort Worth, Texas, USA.

3. Assay Of MUC16 In Conjunctiva And Tears Of Postmenopausal Women With And Without Dry Eye. Sandra Spurr-Michaud[1], Michelle Senchyna[2], Sruthi Srinivasan[3], Robert Ritter III[2], Pablo Argueso[1], Elizabeth Joyce[3], Miriam Heynen[3], Lyndon Jones[3], Daniel A. Gamache[2], Ilene K. Gipson[1] Schepens Eye Research Institute and Department of Ophthalmology, Harvard Medical School[1], Boston MA, Alcon Research Ltd[2], Ft. Worth Texas, and Center for Contact Lens Research, School of Optometry, University of Waterloo[3], Ontario, Canada.

4. Tear Flow And MUC16 Expression In Sjögren's Syndrome, Kcs And Normals. Barbary Caffery[1], Elizabeth Joyce[1], Miriam L Heynen[1], Robert Ritter III[2], Lyndon Jones[1], Trefford Simpson[1], Allan Slomovic[3], Daniel A. Gamache[2], Michelle Senchyna[2]. [1]Center for Contact Lens Research, School of Optometry, University of Waterloo, Ontario, Canada, [2]Alcon Research Ltd, Fort Worth, Texas, USA. [3]Toronto Western Hospital, Toronto, Ontario, Canada.

5. Membrane-Associated Mucins Are Affected By Inflammatory Mediators On Ocular Surface Epithelial Cells. Vinodh Kakkassery, Sandra Spurr-Michaud, Beatrice Perez, Timothy Blalock, and Ilene K. Gipson. Schepens Eye Research Institute, Harvard Medical School, Boston, MA, USA.

6. MUC1 Gene Polymorphism In Dry Eye Patients. Yoannis Imbert, Gary N. Foulks, Mark D. Brennan, Marcia M. Jumblatt, George John, Hassan A. Shah, Catherine Newton, William W. Young, Jr. Schools of Dentistry and Medicine, University of Louisville, Louisville, KY, USA.

7. Alteration Of Mucin Expression In The Ocular Surface Epithelium In Patients With Dry Eye. Seika Den[1], Murat Dogru[1,2], Jun Shimazaki[1], [2]Department of Ophthalmology, Tokyo Dental College, Chiba, Japan, [1] Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan [2]

8. Immunohistochemical Investigation Of The Filament In Filamentary Keratitis. Hidetoshi Tanioka, Norihiko Yokoi, Aoi Komuro, Takasumi Shimamoto, Satoshi Kawasaki, Akira Matsuda, Shigeru Kinoshita. Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan.

9. Inhibition Of Galectin-3 Association With Cell Surface O-Glycans Results In Corneal Epithelial Barrier Dysfunction. Flavio Mantelli[1], Zhiyi Cao[2], Noorjahan Panjwani[2], Pablo Argüeso[1]. [1]Schepens Eye Research Institute & Dept. Ophthalmology, Harvard Medical School and [2]Tufts University School of Medicine, Boston, MA, USA.

10. 3-D Visualization Of Mucin Release By Laser Scanning Microscopy. Assumpta Peral[1,3], Jesús Pintor[2,3]. Department of Optics II (Optometry and Vision)[1], Department of Bioquemistry and Molecular Biology IV[2], School of Optics, University Complutense of Madrid[3].

11. Mucins In Symptomatic And Asymptomatic Contact Lens Wearers. Monica Berry[1], Heiko Pult[2], Christine Purslow[2], Jeff Nyman[3], Paul J Murphy[2]. [1]Academic Unit of Ophthalmology, Bristol, UK; [2]School of Optometry and Vision Sciences, Cardiff, UK; [3]Pennsylvania College of Optometry, Philadelphia, USA.

12. Conjunctival Morphology With Daily Wear Of Silicone-Hydrogel Lenses. Chantal M-L Coles, Noel A Brennan, Heather RM Connor, Robert G McIlroy. Brennan Consultants, Melbourne, Australia.

13. **Discussion:** EGF Activates Protein Kinase C*a* and -E and ERK1/2 To Stimulate Cultured Rat And Human Conjunctival Goblet Cell Proliferation. Darlene A. Dartt, Robin. R. Hodges, and Marie A. Shatos. Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School, Boston, MA.

14. Effect Of Protein Kinase Ca And P42/P44 Mapk On Egf-Stimulated Rat Cultured Conjunctival Goblet Cell Proliferation. Jeffrey A. Bair, Marie A. Shatos, Robin R. Hodges and Darlene A. Dartt. Schepens Eye Research Institue, Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School. Boston, MA.

15. Quantitative Analsyis Of Conjunctival Goblet Cells After Exposure To Latanoprost With 0.02% Benzalkonium Chloride, Travoprost With Sofzia, Or Preservative Free Artificial Tears. Malik Y. Kahook MD[1], Robert J. Noecker MD MBA[2]. University of Colorado Health Sciences Center[1], University of Pittsburgh Medical Center[2]

16. Quantitative Analsyis Of Corneal Epithelial Desmosomes After Exposure To Latanoprost With 0.02% Benzalkonium Chloride, Travoprost With Sofzia, Or Preservative Free Artificial Tears. Malik Y. Kahook MD[1], Robert J. Noecker MD MBA[2]. University of Colorado Health Sciences Center[1], University of Pittsburgh Medical Center[2]

17. Biopsy Of The Bulbar Conjunctiva In Contact Lens Wearers With Conjunctival Flaps. Maria Markoulli[1], Ian C. Francis[2,3], Jim Yong[4], Eric Papas[1,5]. Institute for Eye Research[1], Prince of Wales Hospital[2], University of New South Wales[3], South Western Sydney Area Pathology Service[4], Vision Cooperative Research Centre[5], Sydney, Australia.

18. Expression Of The Carnitine Transporter Octn2 In Ocular Epithelium. Qian Garrett[1], Shunjiang Xu[1], Peter Simmons [2], Joseph Vehige[2], Mark Willcox.[1] Institute for Eye Research, Sydney, Australia,[1] Allergan Inc, Irvine, USA.[2]

19. **Discussion:** Calcium And Cyclic AMP Alterations In Purinergic Regulation Of Rabbit Lacrimal Gland Acinar Cell Secretion. Stina K. Carlsson[1], Maria C. Edman[1], Sarah Hamm-Alvarez[2], J. Peter Gierow[1]. University of Kalmar, Kalmar, Sweden[1] and University of Southern California, Los Angeles, CA, USA[2].

20. $P2x_7$ Purinergic Receptors Active P42/P44 Mapk And Stimulate Protein Secretion From Rat Lacrimal Glands. Robin R. Hodges, Marie A. Shatos, Joanna Vrouvlianis, and Darlene A Dartt. Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School. Boston, MA.

21. Intralobular Duct Cells Of Rabbit Lacrimal Gland Are Actively Involved In Lacrimal Fluid And Electrolyte Production. Chuanqing Ding[1], Janos Petri-Peterdi[2], Austin K. Mircheff[2], Joel

E. Schechter[1]. Cell & Neurobiology1, Physiology & Biophysics[2], University of Southern California, Los Angeles, CA, USA.

22. Ion Fluxes Across Rabbit Acinar Cell Monolayers On Polyester Membrane Scaffolds. Shivaram Selvam[1,2], Padmaja B. Thomas[1], Hovhannes J. Gukasyan[3], Douglas Stevenson[1], Alan S. Yu[4A], Melvin D. Trousdale[1,4B], Joel E. Schechter[4B,4C], Austin K. Mircheff[4B,4D], Ronald E. Smith[1,4B], Samuel C. Yiu[1,4B]. [1]Ocular Surface Center, Doheny Eye Institute, Los Angeles, CA; [2]Mork Family Department of Chemical Engineering and Materials Science, Depts of [A]Medicine, [B]Ophthalmology, [C]Cell and Neurobiology, [D]Physiology and Biophysics, [4]Keck School of Medicine, USC, Los Angeles, CA; [3]La Jolla Laboratories, Pfizer Inc., San Diego, CA, USA.

23. Cultivation Of Lacrimal Gland Acinar Cells In A Microgravity Environment. S. Schrader*, C. Kremling*, M. Klinger**, H. Laqua*, G. Geerling***, *Department of Ophthalmology, University of Luebeck, Germany, **Department of Anatomy, University of Luebeck, Germany, ***Department of Ophthalmology, Julius-Maximilian-University Wuerzburg, Germany.

24. Establishment Of Primary Acinar Culture Cells Of Lacrimal Gland. Angélica Gobbi Jorge, Ana Carolina Dias, Leticia P. Roma, Carolina Maria Módulo, Rubens Bertazolli Filho, Eduardo M. Rocha. Departamento de Oftalmologia, Otorrinolaringologia e Cirurgia de Cabeça e Pescoço e Departamento de Clínica Médica da Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brazil.

25. Mechanism Of Secretion In Lacrimal Gland Of Diabetic Rats. Leticia P. Roma[1], Daniel A. Cunha[1], Ana Carolina Dias[2], Carolina Maria Módulo[2], Angélica Gobbi Jorge[2], Alexandre Martins Braz[2], Eduardo Melani Rocha[2].[1]Department of Physiology, Institute of Biology, Unicamp, Campinas, SP, [2]Department of Ophthalmology, Faculty of Medicine of Ribeirão Preto, USP, Ribeirão Preto, SP Brazil.

26. Influence Of Insulin Treatment On Lacrimal Gland And Ocular Surface Of Diabetic Rats. Ana Carolina Dias, Carolina Maria Módulo, Angélica Gobbi Jorge, Alexandre Martins Braz, Rubens Bertazolli Filho, Jayter Silva de Paula, Alceu A. Jordão Jr., J. Sérgio Marchini, Eduardo M. Rocha. Departamento de Oftalmologia, Otorrinolaringologia e Cirurgia de Cabeça e Pescoço e Departamento de Clínica Médica da Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brazil.

27. Influence Of Aspirin Treatment On Lacrimal Gland And Ocular Surface Of Diabetic Rats. Carolina Maria Módulo, Ana Carolina Dias, Angélica Gobbi Jorge, Alexandre Martins Braz, Rubens Bertazolli Filho, Jayter Silva de Paula, Alceu A. Jordão Jr, Eduardo M. Rocha. Departamento de Oftalmologia, Otorrinolaringologia e Cirurgia de Cabeça e Pescoço e Departamento de Clínica Médica da Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo.

28. Tear Prosecretory Mitogen Lacritin: Pondr Predicted Ordered And Disordered Domains, And Conservation Of Predicted Structure In Putative Orthologues. Gordon W. Laurie[1], Ningning Wang[1], Ronald W. Raab[2], Robert L. McKown[2]. [1]Dept. of Cell Biology, University of Virginia, Charlottesville VA, USA. [2]Dept. of Integrated Science and Technology, James Madison University, Harrisonburg, VA, USA.

29. Relaxin, Relaxin-Like Factor And Relaxin-Like Receptors LGR7 And LGR8 At The Ocular Surface. Ulrike Hampel[1], Thomas Klonisch[2], Friedrich Paulsen[1]. [1]Department of Anatomy and Cell Biology, Martin Luther University Halle-Wittenberg, Germany, [2]Department of Human Anatomy and Cell Science, University of Manitoba, Winnipeg, Manitoba, Canada.

30. Steroid Hormones In The Tear Film. Linda Banbury[1], Carol Lakkis[2], Carol Morris[1], [1]Centre for Phytochemistry and Pharmacology Southern Cross University, Lismore, NSW, Australia, [2]Clinical Vision Research Australia, University of Melbourne, Melbourne VIC Australia.

31. REM Sleep and Tear Secretion. A Hypothesis. Juan Murube,1 Joaquin Carbonell.[2] University of Alcalá-Madrid,[1] San Francisco Hospital. Madrid.[2]

32. Surfactant Protein Gene Expression In Ocular Surface Tissues: Sex And Hormonal Influence. Stephen M. Richards, David A. Sullivan, Schepens Eye Research Institute & Harvard Medical School, Boston, MA, USA.

33. **Discussion:** Detection Of Surfactant Proteins A, B, C And D In The Human Lacrimal System And In Tear Fluid. Lars Bräuer,[1] Christian Kindler,[1] Kristin Jäger,[1] Saadettin Sel,[2] Bernhard Nölle,[3] Uwe Pleyer,[4] Matthias Ochs,[5] Friedrich P. Paulsen[1], [1]Department of Anatomy and Cell Biology and [2]Department of Ophthalmology, Martin Luther University of Halle-Wittenberg, Germany; [3]Department of Ophthalmology, Christian Albrecht University Kiel, Germany, [4]Department of Ophthalmology, Charite, Berlin, [5]Department of Anatomy, University of Bern, Switzerland.

34. Androgens Regulate Autophagy In The Mouse Meibomian Gland. Frank Schirra, Berthold Seitz. Eye Infirmary, Saarland University Hospital, Homburg/Saar, Germany.

35. The Effect Of Menstrual Cycle On The Meibomian Gland Physiology. T.Suzuki[1,2], N.Yokoi[2], A.Komuro[2], S.Kinoshita[2]. Department of Ophthalmology, Kyoto City Hospital[1] and Department of Ophthalmology, Kyoto Prefectural University of Medicine,[2] Kyoto, Japan.

36. Management Of Symptomatic Evaporative Dry Eye Secondary To Meibomian Gland Dysfunction. K.G. Boboridis, D. Mikropoulos, A.G.P. Konstas, N.S. Georgiadis. 1st Ophthalmology Department, Aristotle University of Thessaloniki, Greece.

37. Changes In Visual Acuity Following Meibomian Gland HEAT Therapy. E.Ian Pearce[1], M. Anne Pentland,[1] Samina Shabbir,[1] Erin S. McDonald,[1] Khameran Ahmed,[1] Glyn Walsh, [1] Niall

C. Strang[1] & Rob J. Fuller[2] Vision Sciences, Glasgow Caledonian University,[1] Glasgow, Royal Eye Infirmary,[2] Plymouth, UK.

38. The Impact Of A Near Task On Tear Stability. Meredith Jansen, Monica Bedroya, Carolyn Begley, Robin Chalmers. Indiana University.

39. Non-Invasive In Vivo Investigation Of The Tear Film Stabilization Process On Cornea And Soft Contact Lenses Using Interferometry. Dorota Szczesna[1], Henryk Kasprzak[1], Ulf Stenevi[2], Institute of Physics, Wroclaw University of Technology, Wroclaw, Poland[1], Department of Ophthalmology Sahlgren's University Hospital, Mölndal, Sweden[2].

40. Contributions Of Evaporation And Dry Eye Status To Pre-Lens Tear Film Thinning. Jason J. Nichols, Elisa Skadahl, Ewen King-Smith. College of Optometry, Ohio State University, Columbus, Ohio.

41. Does The Water Permeability Of The Corneal Epithelium Help Prevent Excessive Evaporative Thinning Of The Tear Film? P. Ewen King-Smith,[1] Jason J. Nichols,[1] Kelly K. Nichols,[1] Barbara A. Fink,[1] Kari B. Green-Church,[2] Richard J. Braun,[3] College of Optometry,[1] Mass Spectrometry and Proteomics Facility,[2] Ohio State University, Columbus, Ohio; Mathematical Sciences Dept., University of Delaware, Newark, Delaware[3]

42. Direct Evaporation Measurements On Cornea And Bulbar Conjunctiva. John Tiffany, Aravinthan Varatharaj. Nuffield Laboratory of Ophthalmology, University of Oxford, Oxford UK.

43. Biophysical Properties Of Lipids Spread At The Pre Ocular Tear Film. Fausto Miano. SIFI S.p.A., Catania, Italy.

44. Differential Tear Film Characteristics Of Dry Eye Patients. Cécile Maissa, Michel Guillon OTG Research & Consultancy, London, UK.

45. Differential Tear Film Characteristics Of Dry Eye Contact Lens Wearers. Cécile Maissa, Michel Guillon OTG Research & Consultancy, London, UK.

46. Modelling Tear Volume And Osmolarity In The Normal And The Dry Eye. Eamonn Gaffney,[1] John M. Tiffany[2], Anthony J. Bron[2], Mathematical Institute[1] and Nuffield Laboratory of Ophthalmology, [2] University of Oxford, UK.

47. The Thickness Of The Tear Film As A Function Of Space And Time. P. Ewen King-Smith,[1] Barbara A. Fink,[1] Jason J. Nichols,[1] Kelly K. Nichols,[1] Kim L. Boyer,[2] College of Optometry,[1] Electrical Engineering Dept.,[2] Ohio State University, Columbus, Ohio.

48. Computing Tear Film Dynamics: Blinkcycles, Evaporation, Reflex Tearing And Dewetting. R.J. Braun,[1] K.L. Maki,[1] T.A. Driscoll,[1] L.P. Cook,[1] And P.E. King-Smith.[2] Department Of Mathematical Sciences, University Of Delaware, Newark, DE.[1] College Of Optometry, The Ohio State University, Columbus, OH, USA.[2]

49. Modeling Tear Film Evolution During Multiple Blink Cycles And Partial Blinks. R.J. Braun,[1] A. Heryudono,[1] T.A. Driscoll,[1] K.L. Maki,[1] L.P. Cook,[1] and P.E. King-Smith.[2] Department of Mathematical Sciences, University of Delaware, Newark, DE.[1] College of Optometry, The Ohio State University, Columbus, OH, USA.[2]

50. The Relation Between Tear Break-Up And Blinking. Carolyn G. Begley, Nikole L. Himebaugh, Indiana University School of Optometry, Bloomington, IN, USA

51. Tear Film Dynamics During Contact Lens Wear. Michel Guillon, Cécile Maissa. OTG Research & Consultancy, London, UK.

52. Tear Function And Lipid Layer Alterations In Chronic Graft-Versus-Host Disease. Yumiko Ban,[1] Yoko Ogawa,[1] Eiki Goto,[1,2] Miki Uchino,[1] Naoki Terauchi,[1] Maiko Seki,[1] Mika Nakaya,[1] Megumi Saeki,[1] Murat Dogru,[1] Kazuo Tsubota.[1] Department of Ophthalmology, School of Medicine, Keio University, Tokyo, Japan,[1] Department of Ophthalmology, School of Dental Medicine, Tsurumi University, Kanagawa, Japan.[2]

53. Tear Distribution On Ocular Surface Imaged With Ultra-High Resolution Optical Coherence Tomography. Jianhua Wang, Shuliang Jiao, Jayachandra Palakuru. Bascom Palmer Eye Institute, University of Miami, Miami, FL, USA.

54. Lipids Of Human Meibum Revisited – Historical Survey And Recent Developments. Igor A. Butovich and James P. McCulley. UTSouthwestern Medical Center, Dallas, TX, USA.

55. Quantitative Detection Of Cholesterol In Tears By A Simple And Sensitive Mass Sprctrometry Method. Zhenjun Zhao[1],[2,] John Korth[3], Yulina Aliwarga[1], Todd Mitchell[3], Stephen Blanksby[3] and Mark Willcox[1],[2]. 1 The Institute for Eye Research, Sydney, Australia and the Vision Cooperative Research Centre, Sydney, Australia, 2 The School of Optometry and Vision Science, University of New South Wales, Sydney, Australia and 3 The Department of Chemistry, University of Wollongong, Wollongong, Australia.

56. Effect Of A Liposomal Spray On The Preocular Tear Film. Jennifer P. Craig[1], Christine Purslow[2], Paul J. Murphy[2], James S. Wolffsohn[1]. Ophthalmic Research Group, Aston University, Birmingham[1] and School of Optometry and Vision Sciences, University of Cardiff[2], UK.

57. Influencing Factors Of Tear Film Deposition On Silicone Hydrogel Lenses: Clinical Relevance. F.P. Carney, W.L. Nash, C Amos, C.H. Wang, KB Sentell. CIBA Vision Corporation, Duluth, Georgia, USA.

58. Protein And Lipid Deposits On Lenses Are Affected By Lens Material And Solutions, And Associated With Clinical Performance. M. Willcox, T. Naduvilath, N. Carnt, Z. Zhao. Institute for Eye Research, University of New South Wales, Sydney, Australia.

59. Evidence For Two Binding Sites In Tear Lipocalin. Ben J. Glasgow,[1,2] Oktay K. Gasymov,[2] Adil R. Abduragimov,[1] Jules Stein Eye Institute,[1] Departments of Ophthalmology,[1] and Pathology and Laboratory Medicine,[2] UCLA School of Medicine, Los Angeles, CA.

60. Proteomic Analysis Of Conjucntival Swab By Mass Spectrometry. Vicky McGilligan,[1] Joanna E. Graham,[1] Jonathan E Moore,[1,2] Geoff McMullan,[1] Robert LJ Graham,[1] Raymond O Beirne,[1] Stephen C. Downes,[1] Tara CB. Moore[1]. Centre for Molecular Biosciences, University of Ulster, Northern Ireland,[1] Royal Group Hospitals, Belfast, Northern Ireland.[2]

61. The Human Tear Film Proteome And Its Potential Application To Disease Biomarker Discovery. Kari B. Green-Church[§], Kelly K. Nichols, Paul Eichenseer[§], Richard Sessler[§], Nan M. Kleinholz[§], Jason J. Nichols. [§]Mass Spectrometry and Proteomics Facility, College of Optometry, The Ohio State University, Columbus, OH, USA.

62. Identification Of Biomarkers For Conjunctivochalasis Diagnosis In Tear By 2d-Based Proteomics Approach. Arantxa Acera,[1] Tatiana Suárez,[1] Ignacio Rodríguez-Agirretxe,[2] Elena Vecino,[1] Juan A. Durán.[1,2] Basque Country University[1] and ICQO,[2] Bilbao, Spain.

63. **Discussion:** Low abundance protein in tears of vernal keratoconjunctivitis (VKC) patients. Andrea Leonardi, Massimo Bortolotti, Sonal Sathe and Robert Sack. 1 Ophthalmology Unit, Department of Neuroscience, University of Padua, Italy. 2 SUNY College of Optometry, New York, NY, USA.

64. Chitinase Levels In The Tears Of Subjects With Ocular Allergic Diseases. Pasquale Aragona[1], Maria Musumeci[2], Adriana Maltese[3], Claudio Bucolo[3], Laura Rania[1], Filippo Drago[3], Salvatore Musumeci[4]. [1]Department of Ophthalmology, University of Messina, Messina, Italy. [2]Department of Hematology, Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome, Italy. [3]Department of Experimental and Clinical Pharmacology, School of Medicine, University of Catania, Catania, Italy. [4]Department of Pharmacology, Gynecology and Obstetrics, Pediatrics, University of Sassari and Institute of Biomolecular Chemistry, National Research Council (CNR), Li Punti (SS), Italy.

Friday, September 7, 2007

SESSION II: OCULAR SURFACE

Repair & Regeneration: Are Stem Cells the Answer?

Chairpersons - James L. Funderburgh (USA), Winston W. Y. Kao (USA), Robert M. Lavker (USA)

8:00 **Keynote Address:** Ocular Surface Repair And Regeneration. M. Elizabeth Fini. Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, FL, USA

8:20 **Keynote Address:** Current And Future Perspectives For Ocular Stem Cells: Biology And Therapeutic Potential. Julie T. Daniels. Cells for Sight Transplantation & Research Programme, UCL Institute of Ophthalmology, London, UK.

8:40 Self-Renewal Or Aging In Ocular Epithelial Cells. Graziella Pellegrini[1,2] , Vanessa Barbaro[1], Anna Testa[2], Enzo Di Iorio[1], Fulvio Mavilio[2], and Michele De Luca[1,2], [1]Epithelial Stem Cell Research Center, The Veneto Eye Bank Foundation, H. SS Giovanni and Paolo, Venice, Italy, [2]Department of Biomedical Sciences, University of Modena and Reggio Emilia, Modena, Italy.

8:50 Stem Cells Of Epithelium And Stroma: Proximity Is Not Identity. J Funderburgh, Y Du, S Harvey, M Funderburgh. Department of Ophthalmology, University of Pittsburgh, Pittsburgh, PA.

9:00 Bone Marrow Cells Can Differentiate And Assume Keratocyte Characteristics Of Keratocan Expression In Mouse Corneas. Winston W.-Y. Kao[1,2], Hongshan Liu[1], Yasuhito Hayashi[1], Chia-yang Liu[1], Eric Carlson[3], Ophthalmology[1] and Cell and Cancer Biology[2], University of Cincinnati, Cincinnati Ohio, Ophthalmology[3], Case Western Reserve University, Cleveland, Ohio.

9:10 EMMPRIN/CD147 Promotes Myofibroblasts Differentiation By Inducing Asma Expression And Collagen Gel Contraction: Implications In Tissue Remodelling. Eric E Gabison[1,5], Eric Huet[1], Benoit Vallée[1], Dominika Szul[1], Franck Verrecchia[2], Samia Mourah[3], James V Jester[4], Than Hoang-Xuan[5], Suzanne Menashi[1]. [1]CRRET laboratory, CNRS UMR 7149, University Paris XII, 94010 Créteil, France. [2]INSERM U 697, Hôpital Saint-Louis, Paris, France. [3]INSERM U716, Laboratoire de Pharmacologie, Hôpital Saint-Louis, Paris, France. [4]Department of Ophthalmology, University of California at Irvine, Irvine, California. [5]Department of Ophthalmology at Fondation Ophtalmologique A. de Rothschild and Bichat Hospital, Paris, France.

9:20 Discussion

9:35 Poster Session II (with Coffee & Tea)

Innate & Adaptive Immunity: For Whom the Bell Tolls

Chairpersons - Virginia Calder (UK), Uwe Pleyer (Germany), Manfred Zierhut (Germany)

10:20 **Keynote Address:** An Essential Role For MYD88 And IL-1R1, But Not TLR2 Or TLR4, In A Murine Model Of Fusarium Solani Keratitis. Bakir Tarabishy, Mahmoud Ghannoum and Eric Pearlman. Case Western Reserve University, Cleveland, Ohio.

10:35 **Keynote Address:** Role Of Antimicrobial Peptides At The Ocular Surface. Alison M McDermott. University of Houston, College of Optometry, Houston, TX, USA.

10:50 **Keynote Address:** Pathogenesis Of Allergic Conjunctivitis. Santa Jeremy Ono. Emory Eye Center, Emory University School of Medicine, Woodruff Health Sciences Center, Emory University, Atlanta, GA, USA.

11:05 Human Mast Cell Chemokine Production: Effects Of Anti-Allergic Drugs. Grazyna Galatowicz[1], Samantha W.-Y.Chan[1], Michael E. Stern[2] & Virginia L. Calder[1]. [1]UCL Institute Of Ophthalmology, London, UK; [2]Allergan Inc., CA.

11:15 Ocular Surface Inflammation And Corneal Changes In Patients With Cicatricial Pemphigoid. Stefano Barabino,[1] Cristina Mingari,[2] Marina Papadia, [1,3] Marina Bertolotto,[1] Paola Vacca,[2] Federico Solignani,[1] Cristiana Valente,[1] Maurizio Rolando.[1,3] [1]Ocular Surface Research Center, Department of Neurosciences, Ophthalmology, and Genetics, University of Genoa, [2]DIMES, University of Genoa, [3] IS.PRE Oftalmica, Genoa, Italy.

11:25 Intravital Real-Time Imaging Of Conjunctiva-Associated Lymphoid Tissue. Philipp Steven[1,2], Gereon Huettmann[3], Norbert Koop[3], Andreas Gebert[2] Eye Hospital, UK-SH, Campus Luebeck[1], Institute of Anatomy[2] and Institute of Biomedical Optics[3], University of Luebeck, Germany.

11:35 Discussion

11:50 Poster Viewing & Lunch

Poster Discussion II

Chairpersons - Jose M. Benitez del Castillo (Spain), Erich Knop (Germany), Andrea A. Leonardi (Italy)

1:20 Application Of Bone Marrow Cells And Cd117+ Stem Cells Promotes Corneal Ulcer Healing. Saadettin Sel,[1] Martin Schilling,1 Kathrin Friebel,[1] Elke Vetter,[1] Andreas Simm,[3] Norbert Nass,[3] Hassan Nakhai,[4] Thomas Kalinski,[5] Gernot Duncker,[1] Friedrich Paulsen[6] [1]Department of Ophthalmology, Martin Luther University Halle-Wittenberg, [2]Department of Ophthalmology Vogtland-Klinikum Plauen, [3]Department of Cardio-thoracic Surgery, Martin Luther University Halle-Wittenberg, [4]Department of Internal Medicine II, Klinikum Rechts der Isar, Technical University of Munich, [5]Department of Pathology, Otto-von-Guericke-University, Magdeburg, [6]Department of Anatomy and Cell Biology, Martin Luther University Halle-Wittenberg

1:25 T_H17 Cells In Exocrine Gland Tissues Of An Animal Model For Sjögren's Syndrome (SjS). Cuong Nguyen[1], Minnie Hu[1], Carol Stewart [2] & Ammon Peck[1] Departments of Oral Biology[1] & Oral Medicine[2], College of Dentistry, University of Florida, Gainesville, FL USA.

1:30 Pathogen Or Commensal: A PCR Based Study Of Ocular Surface Bacterial Flora In Normal And Dry Eyes. Joanna E. Graham[1], Jonathan E. Moore[1,2], Xu Jiru[3], John E. Moore[1,2], Edward Goodall[1], James Dooley[1], Darlene A. Dartt[3], Stephen C. Downes[1], Tara CB. Moore[1]. Centre for Molecular Biosciences, University of Ulster, Northern Ireland[1], Royal Group Hospitals, Belfast, Northern Ireland[2], Schepens Eye Research Institute, Boston, USA[4].

1:35 Histopathological Alterations In Senescent Cu, Zn–Superoxide Dismutase-1 (SOD–1)–Knock-Out Mice: A New Model For Dry Eye. Tais Hitomi Wakamatsu[1], M. Dogru[1,2], Y. Sasaki[1], S. Ward[1], Y. Imamura[1], Y. Ogawa[1], A. Igarashi[2], T. Shimizu[3], T. Shirasawa[3], J. Shimazaki[2], K. Tsubota[1]. Ophthalmology Department, Keio University, Tokyo, Japan[1], Ophthalmology Department, Tokyo Dental College, Ichikawa, Japan[2], Gerontology, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan[3].

Old Bugs & New: Classical and Emerging Pathogens

Chairpersons - Michelle C. Callegan (USA), James Chodosh (USA), Denise de Freitas (Brazil)

1:40 **Keynote Address:** Old Bugs And New: Classical And Emerging Pathogens. Michael S. Gilmore[1,2], Susan Heimer[1,2], Ai Yamada[1,2], Irmgard Behlau[1], and Keeta S. Gilmore[1]. Schepens Eye Research Institute[1], Harvard Medical School[2], Boston, MA, USA.

1:55 **Keynote Address:** What's Wrong With Immunization As An Approach To Preventing Infection? Gerald B. Pier, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA.

2:10 **Keynote Address:** Contact Lens Associated Fungal Keratitis: What We Know And What We Need To Find Out. Eduardo C.Alfonso, MD., Edward WD Norton Professor of Ophthalmology, Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, FL, USA

2:25 Corneal Epithelial Cell Killing Of Internalized Bacteria. Amanda D. Ackerman, Annette A. Angus, Suzanne Fleiszig, University of California, Berkeley, CA USA

2:35 Corneal Stroma And The Innate Immune Response To Adenovirus Infection. James Chodosh, Ashish V. Chintakuntlawar. Molecular Pathogenesis of Eye Infection Research Center, Dean McGee Eye Institute, Departments of Ophthalmology, Cell Biology, Microbiology & Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA.

2:45 Antimicrobial Peptides Are Lytic To *Acanthamoeba Castellanii.* Sacramento R.S.[1]; Freitas D.[1]; Martins R.M.[2]; Foronda A.[1]; Dobroff A.S.[2]; Miranda A.[2]; Mortara R[2] Schenkman S.[2] Departments of [1]Ophthalmology and [2]Microbiology, Immunology and Parasitology UNIFESP/EPM São Paulo, Brazil.

2:55 Discussion

3:10 Poster Session II (with Coffee & Tea)

The Challenge of Aging

Chairpersons - Gary N. Foulks (USA), Juan Murube (Spain), Graziella Pellegrini (Italy)

3:55 **Keynote Address:** Physiological Mechanisms And Clinical Impact Of Aging. Andreas Simm. Department of Cardiothoracic Surgery, Martin-Luther University Halle-Wittenberg, Halle, Germany.

4:15 **Keynote Address:** The Influence Of Aging On The Tear Film And Ocular Surface. Eduardo M. Rocha. Department of Ophthalmology, Otorrinolaringology and Head & Neck Surgery, Faculty of Medicine of Ribeirão Preto, São Paulo University, Brazil.

4:35 Reactive Oxygen Species Can Be Controlled By The Secretory Glycoprotein, Clusterin, From Side Population Cells In The Lacrimal Gland: A New Intervention For Age-Related Dry Eye Disorders. Kazuo Tsubota,[1] Kenji Mishima,[2] Kumi Obara, [2] Hiroyuki Yamada,[2] Hiroko Inoue, [2] Ichiro Saito.[2] Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan[1] Department of Pathology, Tsurumi University School of Dental Medicine, Yokohama, Japan[2]

4:45 Lowering The Graft's Age Turns The Heavy Rejection After Keratoplasty In Baby Rats To A Low Risk Situation. Johannes Schwartzkopff, Florian Birnbaum, Thomas Reinhard. Eye Hospital, University of Freiburg, Germany.

4:55 Characterization Of Human Meibum In Relation To Age And Dry Eye Using Infrared Spectroscopy. Douglas Borchman, Gary N Foulks, Donghai V. Ho, Jonathan Mathews, Eric M. Schwietz. Department of Ophthalmology and Visual Science, University of Louisville, Louisville, KY, USA

5:05 Dry Eye In Congenital Aniridia. Gonzalo Carracedo [1], Assumpta Peral [1] Jesús Pintor [2]. Dept. Optica II (Optometría y Visión) [1], Dept. Bioquímica y Biología Molecular IV [2], E.U. Optica (Universidad Complutense), Madrid, Spain.

5:15 Discussion

Poster Session II

Chairpersons - Jose M. Benitez del Castillo (Spain), Erich Knop (Germany), Andrea A. Leonardi (Italy)

1. Expression Of Semaphorin And VEGF Ligands And Receptors Following Corneal Injury. M.I Rosenblatt, C. Yu, D. Eliason, E. Graue, M. Zhang, Department of Ophthalmology and Vision Science, University of California, Davis, CA, USA

2. Enhanced Wound Healing Properties Of Serum Diluted With Platelet Releasate. Karsten Kasper,[1] Dirk Hartwig,[2] Thilo Wedel,[3] Stefan Schrader,[2] Tim Menke,[2] Gerd Geerling.[1] University Wuerzburg,[1] University Luebeck,[2] University Kiel,[3] Germany.

3. **Discussion:** Application Of Bone Marrow Cells And Cd117+ Stem Cells Promotes Corneal Ulcer Healing. Saadettin Sel,[1] Martin Schilling, [1] Kathrin Friebel,[1] Elke Vetter,[1] Andreas Simm,[3] Norbert Nass,[3] Hassan Nakhai,[4] Thomas Kalinski,[5] Gernot Duncker,[1] Friedrich Paulsen[6] [1]Department of Ophthalmology, Martin Luther University Halle-Wittenberg,

[2]Department of Ophthalmology Vogtland-Klinikum Plauen, [3]Department of Cardio-thoracic Surgery, Martin Luther University Halle-Wittenberg, [4]Department of Internal Medicine II, Klinikum Rechts der Isar, Technical University of Munich, [5]Department of Pathology, Otto-von-Guericke-University, Magdeburg, [6]Department of Anatomy and Cell Biology, Martin Luther University Halle-Wittenberg.

4. Trefoil Factor Family Peptide 3 Promotes Re-Epithelialization Of Corneal Wounds. Friedrich Paulsen,[1,] Anne Jansen,[2] Chee-Wai Woon,[3] Fabian Garreis,[1] Kristin Jäger,[1] Deike Varoga,[2] Daniel Podolsky,[4] Nicolas Barker,[3] Saadettin Sel[5], [1]Department of Anatomy and Cell Biology and [5]Department of Ophthalmology, Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany; [2]Institute of Anatomy, Christian-Albrecht-University of Kiel, Kiel, Germany; [3]The GI Company, Framingham, MA, USA; [4]Gastrointestinal Unit, Massachusetts General Hospital, Boston, MA, USA.

5. Long-Term Follow-Up Of Autologous Cultured Limbal Stem Cell Transplantation. Paolo Rama[1], Stanislav Matuska[1], Giorgio Paganoni[1], Alessandra Spinelli[1], Maurizia Viganò[1], Chiara Insacco[1], Graziella Pellegrini[2], Michele De Luca [2], [1]Ophthalmology, San Raffaele Hospital, Milano, Italy, [2]Epithelial Stem Cell Lab, Veneto Eye Bank Foundation, Venice, Italy.

6. Long-Term Outcome Of Limbal Epithelial Cells *In Vivo* Cultivated On Amniotic Membrane (Livam) Transplantation. Woo Chan Park, Dong Jun Lee, Ji Hyun Rho, Hyun Chul Cheon. Dept. of Ophthalmology, Dong-A University, Busan, Korea.

7. Expression Of Tight Junction-Related Proteins In Cultivated Oral Mucosal And Limbal Epithelial Sheets. Jun Shimazaki, Kazunari Higa, Fumito Morito, Yoshiyuki Satake. Department of Ophthalmology, Tokyo Dental College.

8. TOLL-Like Receptor Expression And Dry Eye. R.L. Redfern, J.A. Baxter, R.Y. Reins, A.M. McDermott. College of Optometry, University of Houston, Houston, TX.

9. Ocular Surface Expression And Regulation Of β-Defensins. Fabian Garreis[1], Thomas Schlorf[1], Deike Varoga[2], Friedrich P. Paulsen[1]. [1]Department of anatomy und cell biology, Martin-Luther-Universität Halle-Wittenberg, Germany, [2]Department of anatomy, Christian-Albrechts-Universität Kiel, Germany.

10. Expression Of Metalloproteinase 9 And Transglutaminase 2 In Conjunctival Cells Of Dry Eye Patients. Pasquale Aragona[1], M'hammed Aguennouz[2], Rosaria Spinella[1], Mariagrazia De Pasquale[2], Laura Rania[1], Rossana Di Pietro[1], Sebastiano Giuffrida[3]. [1]Dept. of Ophthalmology and [2]Dept of Neuroscience, University of Messina (Italy) and [3]Bausch and Lomb IOM, Catania (Italy)

11. Tear Cytokine Profiles In Dry Eye And Effect Of Cyclosporine Emulsion Therapy. Cintia S. De Paiva[1]; Lauren S. Blieden[1], Helen Y. Lam[1], William J. Farley[1], Frank Bucci[2], Michael E. Stern[3], Stephen C. Pflugfelder[1], [1]Ocular Surface Center, Cullen Eye Institute, Baylor College of Medicine, Houston, TX, [2] Bucci Laser Vision Institute, Wilkes-Barre, PA, [3]Allergan, Irvine, CA.

12. Comparison Of Cytokine Levels In The Tears And Saliva. Carol Morris,[1] Paul Connellan,[1] Linda Banbury,[1] Shelly Ames,[2] Carol Lakkis.[2] Centre for Phytochemistry and Pharmacology, Southern Cross University, Lismore, Australia,[1] Clinical Vision Research Australia, The University of Melbourne, Melbourne, Australia.[2]

13. Assessment Of Cytokine Levels In The Tears Of Contact Lens Wearers And Non-Contact Lens Wearers. Carol Lakkis,[1] Shelly Ames,[1] Paul Connellan,[2] Linda Banbury,[2] Carol Morris.[2] Clinical Vision Research Australia, The University of Melbourne, Melbourne, Australia,[1] Centre for Phytochemistry and Pharmacology, Southern Cross University, Lismore, Australia. [2]

14. Micro-Well Plate Array Characterization Of Inflammatory Mediators In Normal And Dry Eye Tears. Robert Sack[1], Sonal Sathe[1], Ann Beaton[1], Trinka Vijmesi[2] and Nancy McNamara.[2] [1]SUNY Opt, [2]UCSFMS.

15. Protein Array Characterization Of The Secretion Of Inflammatory, Immune And Angiogenic Modulators By Immortalized Human Cornea Epithelium In Response To Bacterial Stimulation. Robert Sack[1], Sonal Sathe[1], Ann Beaton[1], Nancy McNamara[2], Minjian Ni[3], Suzanne Fleiszig[3]. [1]SUNY Opt, [2]UCSFMS, Proctor Foundation, [3]UC Berkeley Opt.

16. Comparison Of MMP-9 Level In Tear Of MGD, NSDE And Normal Population. [1]Elsa L.C. Mai, [2]Yi-Yun Chou, [3]Chia-Che Chang. [1] Far Eastern Memorial Hospital, Taiwan, [2,3] Institute of Biomedical Sciences, National Chung-Hsing University. Taiwan.

17. Automated Identification And Quantification Of Tear Proteins From Sjogren's Syndrome By LC/MS On A Hybrid Linear Ion Trap Mass Spectrometer. Kazuko Kitagawa[1], Naohisa Tomosugi[2], Hideyuki Tuchida[2], Hiroshi Sasaki[1]. 1: Department of Ophthalmology. 2: Division of Advanced Medicine, Medical Research Institute, Kanazawa Medical University, Ishikawa, Japan.

18. Biomarkers For Sjögren's Syndrome Detected In Saliva Using High-Resolution Mass Spectrometry And Bioinformatics. Driss Zoukhri[1], Mabi Singh[1], Claire Kublin[1], Athena Papas[1], Ian Rawe[2], Kevin Dawson[3], William F. Haddon[3], Earl White[3], Kathy Hanley[3], Daniel Tusé[3], and Wasyl Malyj[3]. Department of General Dentistry, Tufts University School of Dental Medicine, Boston, MA[1]. Schepens Eye Research Institute and Department of Ophthalmology Harvard Medical School, Boston, MA[2]. Predictive Diagnostics, Inc., Vacaville, CA[3].

19. Murine Lacrimal Gland Is Capable Of Repair Following Experimentally Induced Inflammation. Claire L. Kublin[1], Liz Macari[1], and Driss Zoukhri[1,2]. Department of General Dentistry, Tufts University School of Dental Medicine[1] and Department of Neuroscience, Tufts University School of Medicine, Boston, MA[2].

20. Role Of Thrombospondin In Lacrimal Gland Inflammation. Sharmila Masli[12], Bruce Turpie[1], J. David Rios[1] and Darlene Dartt[1,2]. Schepens Eye Research Institute[1], Harvard Medical School[2], Boston, MA.

21. Increased Expression Of The Autoimmune-Related Genes, FGG And PADI2, In NOD Mouse Lacrimal Glands Relative To BALB/C Mouse Lacrimal Glands Characterized By CDNA Microarray And Real-Time PCR. Kaijin Wu[1], Xiaodong Li[1], Michelle MacVeigh[2], Sarah F. Hamm-Alvarez[1]; [1]Department of Pharmacology and Pharmaceutical Sciences, and [2]Center for Liver Disease, University of Southern California, Los Angeles CA, USA.

22. Do Genetic Alterations In Sex Steroid Receptors Contribute To Lacrimal Gland Disease In Sjögren's Syndrome? Stephen M. Richards, David A. Sullivan. Schepens Eye Research Institute and Harvard Medical School, Boston, MA, USA.

23. **Discussion:** T_H17 Cells In Exocrine Gland Tissues Of An Animal Model For Sjögren's Syndrome (SjS). Cuong Nguyen[1], Minnie Hu[1], Carol Stewart [2] & Ammon Peck[1] Departments of Oral Biology[1] & Oral Medicine[2], College of Dentistry, University of Florida, Gainesville, FL USA.

24. Autoimmune Dacryoadenitis Induced By Auto-Adoptive Transfer Of $CD4^+$ T Cells. Padmaja.B. Thomas[1], D. M Samant[1], S. Selvam[1, 5], D. Stevenson[1], J.D Gray[4], A.K. Mircheff1, [3], J.E. Schechter[1, 2], M.D. Trousdale[1]; [1]Doheny Eye Institute, Depts. of [2]Cell & Neurobiology, [3]Physiology & Biophysics, [4] Division of Rheumatology & Immunology, and [5]Mork Family Dept. of Chemical Engineering and Materials Science, Keck School of Medicine, University of Southern California, Los Angeles, CA.

25. Prolactin-Induced Dacryoadenitis In Rabbit. A.K. Mircheff, Y. Wang, P.B. Thomas, S. Song, M.D. Trousdale, J.E. Schechter, University of Southern California, Los Angeles, CA, USA.

26. Collagen Secretion From Hsp 47-Expressing Lacrimal Gland Myoepithelia In Dry Eye Associated With Chronic Graft Versus Host Disease. Yoko Ogawa [1, 2], Mohammed S. Razzaque[5], Kaori Kameyama[3], Go Hasegawa[2] Shigeto Shimmura[1], Masataka Kawai[1], Kazuto Yamazaki[3], Shinichiro Okamoto[4], Yasuo Ikeda[4], Kazuo Tsubota[1],Yutaka Kawakamai[2] and Masataka Kuwana.[4] From the [1]Department of Ophthalmology, the [2]Division of Cellular Signaling, Institute for Advanced Medical Research, the [3] Department of Diagnostic Pathology, the [4]Department of Internal Medicine, Keio University, School of Medicine, Tokyo, Japan; the [5]Department of Developmental Biology, Harvard School of Dental Medicine, Boston, MA, USA.

27. Dry Eye After Hematopoieic Stem Cell Transplantation. Miki Uchino[1], Yoko Ogawa[1], Yuichi Uchino[1], Takehiko Mori[2], Shinichiro Okamoto[2], Kazuo Tsubota.[1] Department of Ophthalmology[1]Keio Bone Marrow Transplant Program, Division of Hematology, Department of Internal Medicine[2], Keio University, School of Medicine, Tokyo, Japan.

28. A Case Of Severe Meibomitis During The Toxic Epidermal Necrolysis, Stevens-Johnson Syndrome. Hiroko Yamagami,[1] Akihiro Kakehashi,[1] Kozue Ishizaki,[1] Fumihiko Toyoda,[1] Chiho Mameuda,[1] Maki Kakurai,[2] Toshio Demitsu,[2] [1]Department of Ophthalmology, Jichi Medical University, Omiya Medical Center, [2]Department of Dermatology, Jichi Medical University, Omiya Medical Center, Omiya, Saitama, Japan.

29. Ocular Cicatrizing Pemphigoid: Is It Still One Of The Worst Disorders Of The Ocular Surface? Manfred Zierhut[1]. Department of Ophthalmology, University of Tuebingen, Germany.

30. Corneal Involvement In Rheumatoid Arthritis: An *In Vivo* Confocal Study. Edoardo Villani, Daniela Galimberti, Francesco Viola, Chiara Mapelli, Roberto Ratiglia. Eye Clinic University of Milan; Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Fondazione IRCCS, Milan, Italy.

31. The Inhibitory Effects Of Corneal Inflammation By Green Tea Polyphenol. J. C. Kim[1] and Y. H. Ryu[1]. [1]Department of Ophthalmology, Chung-Ang University Medical Center.

32. Anatomical And Immunological Changes Of The Cornea In Patients With Pterygium. Marina Papadia[1], Stefano Barabino[1], Cristiana Valente[1], Sebastiano Giuffrida[2], Maurizio Rolando[1]. [1] Ocular Surface Research Center, Department of Neurosciences, Ophthalmology, and Genetics, University of Genoa, Genoa, Italy, [2] Bausch & Lomb IOM, Catania, Italy, [3] IS.PRE Oftalmica, Genoa, Italy.

33. Transduced dendritic cells: A tool to modulate the immune response? Pleyer U, Lie X*** , Schlieckeiser S, Sawitzki B**, Ritter T**** Department of Ophthalmology, and Institute of Immunology*, Charité – University Medicine Berlin, Germany, Department of Ophthalmology, Union Hospital, Tongji Medical College***, Huazhong University of Science and Technology, China, Regenerative Medicine Institute****, National University of Ireland, Galway, Ireland

34. Organized Conjunctival Associated Lymphoid Tissue In The Rabbit. Thomas E. Phillips, Charlette Cain and Carisa Petris. University of Missouri, Columbia, MO, USA.

35. Eye-Associated Lymphoid Tissue (EALT) Of The Rabbit Ocular Surface Contains M-Cells In CALT And LDALT. Nadja Knop[1] and Erich Knop[2]. [1] Dept. for Cell Biology in Anatomy, Hannover Medical School, Germany; [2] Research Laboratory of the Eye Clinic CVK, Charité – Universitätsmedizin Berlin, Germany.

36. Eye-Associated Lymphoid Tissue (EALT) - Are We Using The Right Animal Models For Inflammatory Ocular Surface Disease? Erich Knop[1] and Nadja Knop[2.]. [1]Research Lab of the Eye Clinic CVK, Charite – Universitätsmedizin Berlin; [2]Dept. for Cell Biology in Anatomy, Hannover Medical School.

37. Ocular Trust 2: Longitudinal Nationwide Surveillance Of Antimicrobial Susceptibilities In Ocular Isolates. Penny A. Asbell,[1] Daniel F. Sahm[2] for the Ocular TRUST Study Group. [1]Mount Sinai School of Medicine, New York, NY, USA and [2]Eurofins Medinet Inc., Anti-Infective Services, Herndon, VA, USA

38. Conjunctival Bacterial Flora in Contact Lens Wearers. Jasmina Stojsic[1], Dragan Stojsic[1], Vladislava Masulovic[2], Milos Vejnovic[1], Novkovic Mile[3]; Dept.of Ophthalmology, [1]General Hospital, Sombor, Serbia; Dept.of Microbiology [2]Sombor, Serbia; General Hospital, [3]Vrbas, Serbia.

39. **Discussion:** Pathogen Or Commensal: A PCR Based Study Of Ocular Surface Bacterial Flora In Normal And Dry Eyes. Joanna E. Graham[1], Jonathan E. Moore[1,2], Xu Jiru[3], John E. Moore[1,2], Edward Goodall[1], James Dooley[1], Darlene A. Dartt[3], Stephen C. Downes[1], Tara CB. Moore[1]. Centre for Molecular Biosciences, University of Ulster, Northern Ireland[1], Royal Group Hospitals, Belfast, Northern Ireland[2], Schepens Eye Research Institute, Boston, USA[3].

40. Time-Kill Assay Results For A Linalool-Based Eyelid Cleanser. Jeffrey P. Gilbard, MD, Department of Ophthalmology, Harvard Medical School, Advanced Vision Research, Woburn, Massachusetts, USA.

41. The Proinflammatory Response Of Human Corneal Epithelial Cells To Toxigenic *Staphylococcus Aureus*. Ai Yamada, Susan R. Heimer, Michael S. Gilmore. Schepens Eye Research Institute, Harvard Medical School, Boston, MA, USA.

42. Corneal And Intraocular Inflammatory Responses To Endophthalmitis In Toll-Like Receptor-Deficient Mice. M.C. Callegan[1-3], B.D. Novosad[2], R.T. Ramadan[3]. Departments of Ophthalmology[1] and Microbiology/Immunology[2], and Oklahoma Center for Neuroscience[3], University of Oklahoma Health Sciences Center and Dean A. McGee Eye Institute, Oklahoma City OK, USA

43. Herpes-Like Keratitis Associated With Acute Febrile Neutrophilic Dermatosis. (Sweet's Syndrome). Min Hee Suh ,[1 2] Joon Young Hyon,[1,3] Won Ryang Wee,[1,2] Jin Hak Lee.[1,3] Seoul Artificial Eye Center, Seoul National University Hospital Clinical Research Institute,[1] Seoul National University Bundang Hospital,[2] Department of Ophthalmology, Seoul National University College of Medicine,[3] Seoul, Korea.

44. **Discussion:** Histopathological Alterations In Senescent Cu, Zn–Superoxide Dismutase-1 (SOD–1)–Knock-Out Mice: A New Model For Dry Eye. Tais Hitomi Wakamatsu[1], M.

Dogru[1,2], Y. Sasaki[1], S. Ward[1], Y. Imamura[1], Y. Ogawa[1], A. Igarashi[2], T. Shimizu[3], T. Shirasawa[3], J. Shimazaki[2], K. Tsubota[1]. Ophthalmology Department, Keio University, Tokyo, Japan[1], Ophthalmology Department, Tokyo Dental College, Ichikawa, Japan[2], Gerontology, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan[3].

45. Endoscopy-Guided Vitreoretinal Surgery Following Penetrating Corneal Injury. Motoko Kawashima, M.D.[1], Shinichi Kawashima, M.D.[2], Jun Shimazaki, M.D[1]. From [1] Department of Ophthalmology, Tokyo Dental College, Chiba, Japan and [2] Department of Ophthalmology, International University of Health and Welfare, Tokyo, Japan.

46. Clinical Experience Of Managing Ectasia After Lasik Surgery: Taiwanese Experiences. David Chaokai Chang MD PhD, Taiwan Nobel Eye Institute, Taipei, Taiwan

47. Brunescent Cataract Extraction In A Case Of Severe Rheumatoid Arthritis. Dilek D. Altinors[1], Yonca A. Akova[1], Baskent University, Department of Ophthalmology , Ankara, Turkey.

48. Current Applications of Autologous Serum Drops in Ocular Surface Reconstruction. Dilek D. Altinors[1], Yonca A. Akova[1], Baskent University, Department of Ophthalmology , Ankara, Turkey.

49. Tear Function And Ocular Surface After Muller Muscle- Conjunctival Resection. Suat Hayri Ugurbas[1,] Atilla Alpay[1], Burak Bahadır[2], Zonguldak Karaelmas University, Faculty of Medicine, Department of Ophthalmology1 and Department of Pathology[2], Zonguldak, Turkey.

50. The Improved Surgical Technique For Conjunctivochalasis. Yukiko Sonomura, Norihiko Yokoi, Aoi Komuro, Masakazu Nishii, Kayoko Inagaki, Hidemi Chihara, Shigeru Kinoshita. Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan.

51. Long-Term Results Of Surperior Conjunctivochalasis Operation For Treating Superior Limbic Keratoconjunctivitis. Aoi Komuro[1,2], Norihik0 Yokoi[2], Kazuichi Maruyama[2], Shigeru Kinoshita[2]. Department of Ophthalmology, Nishijin Hospital[1] and Department of Ophthalmology, Kyoto Prefectural University of Medicine[2], Kyoto, Japan.

52. A New Surgical Punctal Occlusion Using Fibrous Tissue Under Lacrimal Caruncle. Norihiko Yokoi, Hidemi Chihara, Masakazu Nishii, Aoi Komuro, Takasumi Shimamoto, Shigeru Kinoshita. Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan.

53. Transplantation Of Labial Salivary Glands To Conjunctiva In Cases Of Severe Dry Eyes. Peter Raus, Miró, Center for Eyelid Surgery and Aesthetic Medicine of the Face, Mol, Belgium.

54. Evaluation Of Patients With Dry Eye Syndromes For Associated Medical Conditions. Esen Karamursel Akpek[1], Alena Klimava,[1]; Jennifer E. Thorne,[2] Don Martin,[3] Kaevalin

Lekhanont,[1]Ann Ostrovsky[1]. [1]The Ocular Surface Diseases and Dry Eye Clinic, and [2]Division of Ocular Immunology, Wilmer Eye Institute, and [3]Rheumatology Division, Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD.

Saturday, September 8, 2007

SESSION III: TEAR FILM & OCULAR SURFACE INTERACTIONS

The Impact of Environmental & Physical Stresses

Chairpersons - Penny A. Asbell (USA), Cintia de Paiva (USA), Mark Willcox (Australia)

8:00 **Keynote Address:** Mechanotransduction Of Hydration Of Pulmonary Surfaces Mediated By Extracellular ATP. Richard C. Boucher, M.D., Cystic Fibrosis/Pulmonary Research and Treatment Center The University of North Carolina at Chapel Hill, Chapel Hill, NC

8:15 **Keynote Address:** A Mouse Model Of Lacrimal Keratoconjunctivitis: Evidence That Some Forms Of Dry Eye Disease Are Immune-Mediated. Jerry Y. Niederkorn[1], Michael Stern[2], Stephen C. Pflugfelder[3], Karyn F. Siemasko[2], Jianping Gao[2], Virginia L. Calder[4], and Margarita Calonge[5]. U.T. Southwestern Medical Center, Dallas, Texas[1], Allergan, Irvine, California[2], Baylor College of Medicine, Houston, Texas[3], Univ. College London, London, UK[4], and IOBA, Univ. Valladolid, Spain[5].

8:30 **Keynote Address:** Effects Of Contact Lens Wear On The Tear Film And Ocular Surface: Future Directions. Fiona Stapleton, Institute for Eye Research, School of Optometry and Vision Science and Vision Cooperative Research Centre, University of New South Wales, Sydney, Australia.

8:45 **Keynote Address:** Impact Of Refractive Surgery On The Tear Film And The Ocular Surface. Dimitri Azar, MD. University of Illinois at Chicago, Chicago, IL, USA

9:00 **Keynote Address:** Do Socio-Cultural Factors Have An Impact On Vision? Paul Courtright, Kilimanjaro Centre for Community Ophthalmology, Tumaini University, Moshi, Tanzania.

9:15 Discussion

9:30 Poster Session III (with Coffee & Tea)

The Sensation Problem: the Biology & Psychology of Pain & Irritation

Chairpersons - Jutta Horwath-Winter (Austria), Kelly K. Nichols (USA), Timo Tervo (Finland)

10:15 **Keynote Address:** The Perception Of Pain. Linda M. Bartoshuk. Center for Smell and Taste, University of Florida, Gainesville, FL, USA.

10:30 **Keynote Address:** The Neural Basis Of Sensation In Intact And Injured Corneas. Carlos Belmonte. Insituto de Neurociencias de Alicante. Universidad Miguel Hernandez-CSIC, San Juan de Alicante, Spain.

10:45 **Keynote Address:** The Sensation Problem: The Biology And Psychology Of Pain And Irritation. Carolyn G. Begley, Indiana University School of Optometry, Bloomington, IN, USA

11:00 Measurement Of Ocular Surface Irritation On A Linear Interval Scale With The Ocular Comfort Index (OCI). Michael E Johnson,[1,2] Paul J Murphy.[1] School of Optometry and Vision Sciences, Cardiff University, UK;[1] Bristol Eye Hospital, UK.[2]

11:10 Ocular Surface Sensitivity and Symptoms in Contact Lens Wear. Blanka Golebiowski,[1] Eric Papas,[1] Carolyn Begley,[2] Fiona Stapleton.[1] [1]Vision Cooperative Research Centre, Institute for Eye Research and School of Optometry and Vision Science, University of New South Wales, Sydney, Australia. [2]School of Optometry, Indiana University, Bloomington, IN, USA.

11:20 Quality Of Life In Postmenopausal Women With Dry Eye. Kelly K. Nichols and Lisa A. Jones The Ohio State University College of Optometry, Columbus, OH, USA.

11:30 Discussion

11:45 Poster Viewing & Lunch

Poster Discussion III

Chairpersons - Murat Dogru (Japan/Turkey), Jason J. Nichols (USA), Teruo Nishida (Japan)

1:15 Implementation Of A New Questionnaire Into The Recently Revised Japanese Dry Eye Diagnostic Criteria. Samantha Ward,[1] M. Dogru, [1] T. Wakamatsu, [1,2] O. Ebrahim, [1] M. Kaido,[2] Y. Matsumoto, [2] N. Yokoi, [3] M. Ueda, [4] A. Tsuyama, [4] K. Tsubota[2] Keio Univ, J&J OSVO,[1]

Dept of Ophthalmology[2] Kyoto Prefectural Univ of Medicine[3] Japanese Preventive Medicine Society, Tokyo, Japan[4]

1:20 Evaluation Of Conjunctival Inflammatory Status By Confocal Laser Microscopy And Conjunctival Brush Cytology In Patients With Atopic Keratoconjunctivitis (AKC). Murat Dogru, MD,[1,2] ; Osama Ibrahim, MD,[1] ; Yukihiro Matsumoto, MD,[3]; Yoji Takano,MD,[4]; Mari Tanaka, MD,[5]; Yoshiyuki Satake, MD[2]; Kazumi Fukagawa, MD[3]; Hiroshi Fujishima[4]; Kazuo Tsubota[3]. 1) Johnson & Johnson Department of Ocular Surface and Visual Optics, Keio University School of Medicine, Tokyo, Japan. 2) Department of Ophthalmology, Tokyo Dental College, Chiba, Japan. 3) Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan. 4) Department of Ophthalmology, International Welfare University, Mita Hospital, Tokyo, Japan. 5) Ajisai Eye Clinic, Funabashi, Japan.

1:25 An Investigtion Of The Direct Retention And Retention Of Effect Of An Artificial Tear In Dry Eye. Jerry Paugh,[1] Julie S. Hwang,[1] Pochi Huang,[1] Andrew Loc Nguyen,[2]. Southern California College of Optometry,[1] California State University, Fullerton[2].

1:30 Selection Of Compatible Solutes For Inclusion In A Lubricant Eye Drop. Peter A Simmons[1], Joan-En Chang-Lin[1], Joseph G Vehige[1], Quang Chung[2], Devin Welty.[1] Allergan R&D[1], Irvine CA USA; Southern California College of Optometry[2], Fullerton CA USA.

Pathophysiology & Diagnostics: What's New?

Chairpersons - Christophe Baudouin (France), Maurizio Rolando (Italy), Norihiko Yokoi (Japan)

1:35 **Keynote Address:** A Non-Apoptotic Model Of Exocrine Gland Hypofunction. Philip C. Fox. Department of Oral Medicine, Carolinas Medical Center, Charlotte, NC, USA and PC Fox Consulting, LLC, Spello, Italy.

1:50 **Keynote Address:** Building Better Mouse Models To Study Sjögren's Syndrome. Ammon Peck, Department of Oral Biology, College of Dentistry, University of Florida, Gainesville, FL USA.

2:05 **Keynote Address:** Evaluation Of The Ocular Surface In Health And Disease - Based On Confocal In-Vivo Microscopy On The Way From Image Interpretation To Quantification. Rudolf F. Guthoff, Robert Kraak, Oliver Stachs, Joachim Stave, Andrey Zhivov, Universitats-Augenklinik, Rostock, Germany, Univ. Eye Hospital, Rostock Germany

2:20 Tear Hyperosmolarity As The Initiator Of Ocular Surface Damage In Dry Eye. Anthony J. Bron[1], Eamonn A. Gaffney[2] and John M. Tiffany[1] Nuffield Laboratory of Ophthalmology [1] and Mathematical Institute, University of Oxford, U.K.

2:30 Dry Eye In Patients Treated With Continuous Positive Airway Pressure (CPAP). Fabiani C.,[1] Roma R.,[2] Spinelli S.,[2] Fabiani M.,[2] Pivetti Pezzi P.[1] Department of Ophthalmology[1] and Center for the Study of Sleep Apnea, Department of Neurology & Otolaryngology[2], Università di Roma "La Sapienza", Italy.

2:40 Objective Tests In The Differential Diagnosis Of Dry Eye. Alan Tomlinson[1], Santosh Khanal[1], Angus McFadyen[2],Charles Diaper[3]. [1&2]Vision Sciences and Mathematics, Glasgow Caledonian University, Glasgow, [3]The University Hospital Trust, Southern General Hospital, Glasgow, Dept of Vision Sciences, Glasgow, UK

2:50 Discussion

3:05 Poster Session III (with Coffee & Tea)

Treatments: What's on the Horizon?

Chairpersons - Gerd Geerling (Germany), Gary D. Novack (USA), John E. Sutphin (USA)

3:50 **Keynote Address:** The Glucocorticoid Receptor: One Gene, Many Proteins - New Mechanisms For Tissue Specific Anti-Inflammatory Actions Of Glucocorticoids In Health And Disease. John Cidlowski,_ Nick Lu, Christine Jewell, Onard Schoneveld, Danielle Duma, Kathy Gross, Javier Revollo, Robert Oakley. LST, NIEHS, Research Triangle Park, NC, USA

4:05 **Keynote Address:** Reversal Of Sjögren's-Like Syndrome In Non-Obese Diabetic Mice. Denise Faustman[1], Simon Tran[2], Shohta Kodama[3], Beatrijs Lodde[4], Ildiko Szalayova[5], Sharon Key[5], Saeed Khalili[2], Eva Mezey[5]. Massachusetts General Hospital and Harvard Medical School, Boston, MA, McGill University, Montreal, Canada, Brigham and Women's Hospital, Boston, MA, National Institutes of Health, USA and Division of Rheumatology, University of Amsterdam, Netherlands, National Institutes of Health, NIDCR, CSDB, Bethesda, MD.

4:20 **Keynote Address:** Factors Impacting Research On Visual Function And Keratitis In Dry Eye. Abelson MB[1].[1]ORA Clinical Research and Development, North Andover, MA.

4:35 Influence Of Prostaglandin Analogs On The Ocular Surface Of Glaucoma Patients Treated Over The Long Term. Christophe Baudouin, Hong Liang, Pascale Hamard, Antoine Labbé, Françoise Brignole-Baudouin. Depts of Ophthalmology, Quinze-Vingts National Ophthalmology Hospital and Immunotoxicology, University Paris 5, France.

4:45 Effect Of Temporary Collagen Inserts On Ocular Comfort And Osmolality During Contact Lens Wear. U.Stahl,[1,3] M. Willcox,[1,2,3] F. Stapleton[1,2,3]. The VisionCRC,[1] Institute for Eye Research,[2] School of Optometry and Vision Science, University of NSW,[3] Sydney, Australia.

4:55 The Effect Of Hormone Replacement Therapy On Ocular Surface Disease In Women With Premature Ovarian Failure. Janine A. Smith, MD[1], Susan Vitale, PhD, MHS[1], Serena Morrison, MD[1], Linda A. Goodman, COT [1], George F. Reed, PhD,[1] Roula Nashwinter, COA,[1] Vien H. Vanderhoof, RN, CRNP[3], Dessie Koutsandreas, COA[1], Karim A. Calis, [2] Lawrence M. Nelson, MD [3]. [1] Division of Epidemiology and Clinical Research, National Eye Institute, [2] Mark O. Hatfield Clinical Research Center, [3]Developmental Endocrinology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland.

5:05 Discussion

Closing Session

Chairperson: Alan Tomlinson (UK)

5:20 Academic Perspective: Suzanne M. J. Fleiszig (USA)

5:25 Clinical Perspective: J. Daniel Nelson (USA)

5:30 Industry Perspective: Sherryl Frisch (USA)

Closing Remarks

5:35 *Michael A. Lemp (USA)*

Poster Session III

Chairpersons - Murat Dogru (Japan/Turkey), Jason J. Nichols (USA), Teruo Nishida (Japan)

1. Recent Advances In Point Of Care Nanoliter Tear Collection. Benjamin Sullivan[1,2], Steve Zmina[2], Michael Berg[2], Sasha Miu[2], Graeme Bullock[2], Eric Donsky[2]. University of California, San Diego, La Jolla CA,[1] OcuSense Inc., San Diego, CA,[2] USA.

2. The Impact Of Fluorescein Quantity On Tear Film Break Up. Jennifer P Craig[1,2], Andreas Müller[2], Jennifer Mooi[2]. Ophthalmic Research Group, Aston University, Birmingham, UK[1] and Department of Ophthalmology, University of Auckland, New Zealand[2]

3. Comparison Of Hand-Held And Slit-Lamp Mounted Techniques For Assessing Non-Invasive Tear Film Stability. Jennifer P Craig, [1,2,4] Kenneth J Blades,[2] James Farrell,[2] Paul J Murphy,[2.3] Simon J Dean.[4] Ophthalmic Research Group, Aston University, Birmingham, UK[1], Department of Vision Sciences, Glasgow Caledonian University, UK[2], School of Optometry and Vision Science, Cardiff University, UK[3] and Department of Ophthalmology, University of Auckland, NZ[4]

4. A New System For Grading Ocular Surface Staining. Christian Lang[1], Gary N. Foulks[2], Janine A Smith[3], John M.Tiffany[4], Anthony J. Bron[4]. Dept. of Materials[1] and Nuffield Laboratory of Ophthalmology[4], University of Oxford, UK, Dept. of Ophthalmology, University of Louisville [2], National Eye Institute [3], Bethesda MD, USA.

5. Grading Scale For The Time Course Of Corneal Fluorescein Staining. Chris Snyder[1], Mohinder Merchea[2]. [1]Adjunct Professor, University of Alabama at Birmingham School of Optometry, Birmingham, AL, USA; [2]Director, Scientific & Medical Affairs, Bausch & Lomb

6. A Correlation Between Fluorescein Corneal Staining And Ocular Discomfort In Patients Diagnosed With Dry Eye. Juan Guzman[1], Gary Foulks[2], Peter Zhang[1], Satoshi Nakatsu[1], Chau Whatley[1], Ayako Tano[1]. Otsuka Pharmaceutical Development & Commercialization, Inc.[1], University of Louisville, KY[2].

7. Permeability Evaluation In Humans. Vincent C. Fan, MD, Elvin Yildiz, MD, Karuna Bitra, MD, D.Chen, MD, PhD, T.T. Du, MD, P.A. Asbell, MD, Department of Ophthalmology, Mount Sinai School of Medicine, New York, NY, USA.

8. The Effect Of Varying Volumes Of Flourescein On Tear Breakup Time In Dry Eye Subjects. Jerry Paugh,[1] Kashif Qadeer,[1] Hans Steimann,[1] Andrew Loc Nguyen.[2] [1]Southern California College of Optometry, [2]California State University, Fullerton, CA, USA.

9. The Pre-Corneal Residence Time Of Artificial Tears Measured In Dry Eye Subjects. Jerry Paugh,[1], Andrew Loc Nguyen[1,2], David Meadows[3], Mike Christensen[3]. Southern California College of Optometry,[1] California State University, Fullerton[2], Alcon Laboratories, Ft. Worth, TX USA[3].

10. **Discussion:** An Investigtion Of The Direct Retention And Retention Of Effect Of An Artificial Tear In Dry Eye. Jerry Paugh,[1] Julie S. Hwang,[1] Pochi Huang,[1] Andrew Loc Nguyen,[2]. Southern California College of Optometry,[1] California State University, Fullerton[2].

11. A Comparison Of The Numerical Rating Scale (NRS) And The Visual Analog Scale (VAS) In The Assessment Of Ocular Irritation. Jerry Paugh,[1] Robin Sinn,[1] Jennie Fan,[1] Andrew Loc Nguyen,[2]. Southern California College of Optometry,[1] California State University, Fullerton[2].

12. **Discussion:** Implementation Of A New Questionnaire Into The Recently Revised Japanese Dry Eye Diagnostic Criteria. Samantha Ward,[1] M. Dogru, [1] T. Wakamatsu, [1,2] O. Ebrahim, [1] M. Kaido,[2] Y. Matsumoto, [2] N. Yokoi, [3] M. Ueda, [4] A. Tsuyama, [4] K. Tsubota[2] Keio Univ, J&J OSVO,[1] Dept of Ophthalmology[2] Kyoto Prefectural Univ of Medicine[3] Japanese Preventive Medicine Society, Tokyo, Japan[4]

13. Clinically Important Differences In The Symptom Bother Module Of Ideel Questionnaire. Carolyn G. Begley,[1] Robin Chalmers,[1] and Carol Fairchild,[2] Indiana University School of Optometry,[1] Alcon Research Ltd.[2]

14. Simultaneous Tracking Of Visual Performance, Optical Quality And Tear Disruption. Carolyn G. Begley,[1] Haixia Liu,[1] Larry N. Thibos,[1] Robin Chalmers,[1] Colleen Riley,[2] Kurt Moody.[2] Indiana University School of Optometry, Bloomington, IN, Johnson & Johnson Vision Care, Inc., Jacksonville, FL.

15. Dryness And End-Of-Day Comfort With Silicone-Hydrogel Contact Lenses. Noel A Brennan, Chantal M-L Coles, Heather RM Connor, Robert G McIlroy. Brennan Consultants, Melbourne, Australia.

16. Reduced Corneal Sensitivity In Patients With Primary Sjögren's Syndrome. Joon Young Hyon[1,2,5],Yun Jong Lee[1,3,5], Pil-Young Yun[1,4,5]. Seoul National University Bundang Hospital,[1] Department of Ophthalmology,[2] Department of Internal Medicine,[3] Department of Oral and Maxillofacial Surgery Section of Dentistry,[4] and Seoul National University College of Medicine,[5] Seongnam, Korea.

17. Dry Eye Syndrome-Related Quality Of Life In Glaucoma Patients. Gemma CM Rossi[1,2], Carmine Tinelli[3]. [1] UO Oculistica, AO Bolognini, Seriate Bg; [2] University Eye Clinic of Pavia, Pavia; [3] Lab. Epidemiologia e Statistica, IRCCS Policlinico S. Matteo Pavia.

18. The Development Of A Composite Score That Incorporates Both Clinical And Patient Reported Outcomes For The Assessment Of Disease Severity In Dry Eye Patients. Figueiredo FC[1], Steeds CS[2], Figueiredo MS[1], Irwin DE[3], Buchholz P[4]. [1]Royal Victoria Infirmary, Newcastle, UK; [2]CS Consulting, UK; [3]University of North Carolina; [4]Allergan Europe.

19. Tear Volume Assessment Techniques – Repeatability In Normal Patients. Michel Guillon, Cécile Maissa, Aurélie Briffault. OTG Research & Consultancy, London, UK.

20. An Investigation Of The Realtionships Between Tear Ferning, Tear Film Stability And Ocular Comfort. Katharine S. E. Evans, Christine Purslow, Rachel V. North. Contact Lens and Anterior Eye Research Unit, School of Optometry and Vision Sciences, Cardiff University, UK.

21. Effective Of Tests Of Tear Dynamics In Differentiating Between Dry Eye Subtypes. Santosh Khanal,[1] Alan Tomlinson,[1]Angus McFadden,[2] Charles Diaper.[3] Departments of Vision Sciences[1] and Mathematics,[2] Glasgow Caledonian University; South Glasgow University Hospital Trust, Southern General Hospital,[3] Glasgow, Scotland, UK.

22. Diurnal Variation Of Visual Function And Corneal Keratitis In Patients With Dry Eye. [1]Pamela M. Walker, [1]George W. Ousler III, [1]Michael Schindelar, [1]Donna Welch, [1,2,3]Mark B. Abelson. ORA Clinical Research and Development, North Andover, MA[1], Schepens Eye Research Institute, Boston, MA[2]; Harvard Medical School, Boston, MA[3].

23. Correlation Between Diagnostic Tests Guides The Development And Use Of A Dry Eye Diagnostic Algorithm. Jonathan E Moore,[1,2] Joanna E. Graham,[1] Edward Goodall,[1] Darlene A. Dartt,[3] Antonio Leccisotti,[1,4] Stephen C. Downes,[1] Tara CB. Moore[1]. Centre for Molecular Biosciences, University of Ulster, Northern Ireland,[1] Royal Group Hospitals, Belfast, Northern Ireland,[2] Schepens Eye Research Institute, Boston, USA,[3] Générale-de-Santè Toscana, Siena, Italy[1,4].

24. Detection Of Objective Ocular Discomfort In Dry Eye-Related Diseases By fNIRS. Masafumi Ono, Hiroshi Takahashi. Nippon Medical School, Tokyo, Japan.

25. Solution Related Chemical Staining Case Control Study In Silicone Hydrogel Daily Wear. Nicole Carnt[1,2], Vicki Evans[1,2], Thomas Naduvilath[1,2,3], Varghese Thomas[1,2], Mark Willcox[1,2,3], Brien Holden[1,2] Institute for Eye Research[1], Vision Cooperative Research Centre[2], School of Optometry and Vision Science, University of New South Wales[3], Sydney, Australia.

26. Spectroradiometry As An Objective Measure Of Conjuncitval Hyperaemia. Louise C. McCann[1], E. Ian Pearce[1], Colin J. Gafan[1], Kevin Middleton[1], Alexander D. Logvinenko[1], Norman F. Button[1] Department of Vision Sciences, Glasgow Caledonian University, UK[1]

27. An Investigation Of Limbal And Bulbar Hyperaemia In Normal Eyes. Heiko Pult[1], Paul J Murphy[1], Christine Purslow[1], Jeff Nyman[2], Russell L Woods[3, 1]School of Optometry and Vision Sciences, Cardiff, UK; [2]Pennsylvania College of Optometry, Philadelphia, USA; [3]Schepens Eye Research Institute, Harvard Medical School, Boston, USA.

28. The Relationship Between Lid Wiper Epitheliopathy, Lid Parallel Conjunctival Folds And Ocular Surface In Symptomatic And Asymptomatic Contact Lens Wearers**.** Heiko Pult[1], Christine Purslow[1], Monica Berry[2], Jeff Nyman[3], Paul Murphy[1]. [1]School of Optometry and Vision Sciences, Cardiff, UK. [2]Academic Unit of Ophthalmology, Bristol, UK. [3]Pennsylvania College of Optometry, Philadelphia, USA.

29. **Discussion:** Evaluation Of Conjunctival Inflammatory Status By Confocal Laser Microscopy And Conjunctival Brush Cytology In Patients With Atopic Keratoconjunctivitis (AKC). Murat Dogru, MD,[1,2] ; Osama Ibrahim, MD,[1] ; Yukihiro Matsumoto, MD,[3]; Yoji Takano,MD,[4]; Mari Tanaka, MD,[5]; Yoshiyuki Satake, MD[2]; Kazumi Fukagawa, MD[3]; Hiroshi Fujishima[4]; Kazuo Tsubota[3]. 1) Johnson & Johnson Department of Ocular Surface and Visual Optics, Keio University School of Medicine, Tokyo, Japan. 2) Department of Ophthalmology, Tokyo Dental College, Chiba, Japan. 3) Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan. 4) Department of Ophthalmology, International Welfare University, Mita Hospital, Tokyo, Japan. 5) Ajisai Eye Clinic, Funabashi, Japan.

30. In Vivo Confocal Microscopy For Assessing Inflammatory Changes In Ocular Surface Diseases. Christophe Baudouin, Hong Liang, Aude Pauly, Bénédicte Dupas, Antoine Labbé, Françoise Brignole-Baudouin. Depts of Ophthalmology, Quinze-Vingts National Ophthalmology Hospital and Immunotoxicology, University Paris 5, France.

31. Dry Eye Disease In The Young: Relevance Of Corneal Dyrstrophies In The Differential Diagnosis. Gysbert van Setten Sankt Eriks Eye Hospital, Karolinska Insitutet, Stockholm, Sweden.

32. Occult Thyroid Eye Disease In Patients Presenting With Dry Eye Symptoms. Anita Gupta[1], Pooyan B. Sadeghi[2], Esen K. Akpek. [1] Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD[1], University of Cincinnati, College of Medicine, Cincinnati, OH[2].

33. Levels Of Diadenosine Polyphosphates In Sjögren Syndrome. Gonzalo Carracedo, Assumpta Peral[1], Jesús Pintor[2]. Dept. Optica II (Optometría y Visión)[1], Dept. Bioquímica y Biología Molecular IV [2], E.U. Optica (Universidad Complutense), Madrid, Spain.

34. A New Test To Quantify Lipid Layer Behavior In Normal And Keratoconjunctivitis Sicca Patients. Maurizio Rolando, Cristiana Valente, Stefano Barabino. Ocular Surface Research Center, Department of Neurosciences, Ophthalmology, and Genetics, University of Genoa, Genoa, Italy.

35. Psychiatric Diagnosis In Dry Eyes. Johannes Nepp. Department of Ophthalmology, Medical. University Vienna, Austria.

36. Prevalence Of Dry Eye Syndrome Among Japanese VDT Users. Yuichi Uchino[1,2], Miki Uchino[1,2], Murat Dogru[1], Kazumi Fukagawa[1,2], Shigeto Shimmura[1,2],Toru Takebayashi[3], Debra A. Schaumberg[4], Kazuo Tsubota[1,2] Department of Ophthalmology, Keio University School of Medicine,[1] Ryogoku Eye Clinic,[2] Department of Public Health, Keio University School of Medicine, [3] Tokyo, Japan, Division of Preventive Medicine, Brigham and Women's Hospital, and the Schepens Eye Research Institute, Harvard Medical School[4], Boston, MA, USA.

37. How To Win A Staring Competition. E. Ian Pearce,[1] Alan E.C. Bartholomew,[1] Owen McCann,[1] Sara E. Pearce[2] & Glyn Walsh[1] Department of Vision Sciences, Glasgow Caledonian University[1] Kelvindale Primary School,[2] Glasgow, United Kingdom.

38. Development Of A Low Humidity Environment (LHE) Facility For The Clinical Study Of Dry Eye Syndrome. S McCue[1], B Barney[2], P Patel[1], AM Salapatek[1]. Allied Research-Cetero Research,CA[1], Northern Air Environmental Technologies Inc, CA[2].

39. Treatment with Doxycycline Preserves Cell Area after Desiccating Ocular Stress. Cintia S. De Paiva[1], Robert M. Beardsley[1], David Power, Stephen C. Pflugfelder[1]. [1]Department of Ophthalmology, Cullen Eye Institute, Baylor College of Medicine, Houston, TX, [2]Alacrity Biosciences, Inc., Laguna Hills, CA.

40. Preliminary Phase IIb Clinical Trial Results Of Ecabet Sodium For The Treatment Of Dry Eye Syndrome. Ralph Bianca, James A. Gow, Timothy R. McNamara. Department of Clinical Research and Medical Affairs, ISTA Pharmaceuticals®, Inc., Irvine, CA, USA

41. New Formulation Based On Liposomes For Dry Eye Treatment. Tolerance Studies. Rocio Herrero-Vanrell[1], Marta Vicario[1], Beatriz de las Heras[2], Natalia Girón[2], Assumpta Peral[3], Irene T. Molina-Martínez[1]. [1]Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad Complutense, Madrid, Spain. [2]Departamento de Farmacología, Facultad de Farmacia, Universidad Complutense, Madrid, Spain. [3] Departamento de Bioquímica y Biología Molecular IV, E.U. de Optica, Universidad Complutense, Madrid, Spain.

42. Treatment with Liposomes Eyedrops of Dogs with Dry Eye. Jose M. Benitez del Castillo[1], Marta Vicario[2], Alfonso Rodriguez[3], Elisa Gonzalez[3], Ana M. Muñiz[1], Eva Vico[1], Irene T.Molina-Martínez[2]. [1] Departamento de Oftalmología, Hospital Universitario San Carlos, Madrid, Spain.[2] Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad Complutense, Madrid, Spain.[3]Servicio de Oftalmología, Hospital Clínico Veterinario, Universidad Complutense de Madrid.

43. A New Oil-In-Water Emulsion For The Treatment Of Dry Eye. C. Scifo[1], G. De Pasquale[1], M. Pistone[1], S. Barabino[2], A.R. Blanco[1], M.Rolando[2]. [1]Pharma Business Unit S.I.F.I. Spa Lavinaio (Catania), [2]Department of Neurosciences, Ophthalmology, and Genetics University of Genoa[2], Italy.

44. Effects Of An Emulsion And Low/High Viscosity Polymeric Formulations In A Short Term Dry-Eye Model. A.R. Blanco, V. Moschetti, V.Vitale and M.G.Mazzone Pharma Business Unit S.I.F.I. Spa Lavinaio (Catania), Italy.

45. Chemical Denervation Of Lacrimal Pump With Botulinum Toxin Type A For The Management Of Symptomatic Dry Eyes. K. G. Boboridis, D. Mikropoulos, N. S. Georgiadis. 1st Ophthalmology Department, Aristotle University of Thessaloniki, Greece.

46. Comparative Study Of Punctum Plugs Versus Acrylic Smart Plugs And Silicon Canalicular Plugs For The Management Of Dry Eyes. K.G. Boboridis, D. Mikropoulos, N. Ziakas, N.S. Georgiadis 1st Ophthalmology Department, Aristotle University of Thessaloniki, Greece.

47. Long Term Evaluation Of Fci Silicone Punctal Plugs In Dry Eye. Jutta Horwath-Winter, Eva-Maria Haller-Schober, Anna Gruber, Ingrid Boldin, Department of Ophthalmology, Medical University of Graz, Graz, Austria.

48. Punctal Plugs For Patients With Post-Lasik Dry Eye. Ikuko Toda[1], Chikako Sakai[1], Takahiro Yamamoto[1], Yoshiko Hori-Komai[1], Kazuo Tsubota[2]. Minamiaoyama Eye Clinic[1] and Keio University, School of Medicine[2], Tokyo, Japan.

49. Epithelial Damage Of The Conjunctiva_In Sjogren Syndrome Is Not Restored By Standalization Of Tear Volume. Hitoshi Watanabe, MD[1,2] Takeshi Soma, MD[1,], Shizuka Koh[1,] MD, Koji Nishida, MD[1,] , Naoyuki Maeda, MD[1], Osaka University Medical School[1], Osaka, Japan, and Kansai Rosai Hospital[2,] , Hyogo, Japan.

50. Efficacy Of Systane Compared To Hylocomod In The Treatment Of Dry Eye. Elisabeth M. Messmer, Department of Ophthalmology, Ludwig-Maximilians-University, Munich, Germany.

51. A Four Week Therapy With Systane Improves Ocular Surface Parameters In Dry Eye Patients. Piera Versura, Vincenzo Profazio, Emilio C Campos Dept Ophthalmology Alma Mater Studiorum University of Bologna, Italy.

52. A Comparison Of The Effectiveness Of Eyedrops Containing Carbomer And Sodium Hyaluronate In The Treatment Of Moderate Dry Eye. Michael E Johnson,[1,2] Paul J Murphy,[1] Mike Boulton.[1,3] School of Optometry, Cardiff University, UK;[1] Bristol Eye Hospital, Bristol, UK;[2] University of Texas, Galveston, USA.[3]

53. Hyaluronic Acid And *Echinacea Purpurea* Extracts Eye Drops In The Treatment Of Non-Specific Conjunctivitis. Andrea Leonardi, Velika Deligianni, Antonio Manfre', Chiara De Dominicis, Daniele Violato, Iva Fregona. Ophthalmology Unit, Department of Neuroscience, University of Padua, Italy.

54. Hyaluronic Acid Versus Hyaluronic Acid Associated With Echinacea Purpurea Extract (IRIDIUM™) For The Control Of Ocular Surface Disturbances In Patients Treated With Antiglaucoma Polytherapy: A Pilot Study. Piergiorgio Neri, MD, Cesare Mariotti, MD, Manuela Zucchi, Lucia Mercanti, MD, Alfonso Giovannini, MD. The Neurosciences Department, The Eye Clinic, Polytechnic University of Marche, Ancona-Italy.

55. A Comparison Of Two Marketed Artificial Tears In Improvement Of Tear Film Stability As Measured By Tear Film Break-Up Time (TFBUT) And Ocular Protection Index (OPI).

[1]D'Arienzo P, [2]Ousler III GW, [2]Schindelar MR. [1]New York Medical College, Valhalla, NY; [2]ORA Clinical Research and Development, North Andover, MA.

56. Effect Of D–ß–Hydroxybutyrate On Ocular Surface Disorders In A Rat Dry Eye Model. S. Nakamura[1,] H. Nakashima[1], R.Hisamura[1], N.Masuda[1] Y.Saito[1] Y.Yabuno[1], and K. Tsubota[2]. 1 Research center, OPHTECS, Toyooka, Japan; 2 Ophtalmology, Keio University, School of Medicine, Tokyo, Japan.

57. Evaluation Of Optive In Patients Previously Using Systane For The Treatment Of Dry Eye Signs And Symptoms. Rajesh K. Rajpal, M.D.; Lorie A. Logan, O.D. Cornea Consultants, 8180 Greensboro Drive, Suite 140, Mclean, VA.

58. **Discussion:** Selection Of Compatible Solutes For Inclusion In A Lubricant Eye Drop. Peter A Simmons[1], Joan-En Chang-Lin[1], Joseph G Vehige[1], Quang Chung[2], Devin Welty.[1] Allergan R&D[1], Irvine CA USA; Southern California College of Optometry[2], Fullerton CA USA.

59. The Formulation Approach In Ocular Therapy. Valeria Moschetti Letizia Lo Grasso, Elena Solfato, Anna Claudia Scuderi, Pharma Business Unit, S.I.F.I. S.p.A., Catania, Italy.

60. Does Artificial Tear Use Alter The Tear Layer? William H. Ridder, III, James LaMotte, Robin Sinn and Jonathan Q. Hall, Jr. Southern California College of Optometry, Fullerton, CA, USA.

61. Impact Of Administration Angle On The Cost Of Artificial Tear Solutions. Bruce I. Gaynes, Ramesh M. Singa, Gabriel Schaab, Yevgeniva Sorokin. Regenstein Eye Center of Rush University Medical Center, Chicago, IL, USA.

62. Omega-3 Fatty Acids And Dry Eye: Clinical, Interferometric, And Proteomic Considerations. Satiani NG,[1] Green-Church KB,[2] Nichols JJ,[1] King-Smith PE,[1] Nichols KK.[1] The Ohio State University, College of Optometry,[1] Mass Spectrometry and Proteomics Facility[2]

63. The Effect Of An Omega -3 Supplement On Xeropthalmia And Xerostomia In Sjogrens Patients. Athena Papas, Medha Singh and Mabi Singh Tufts University School of Dental Medicine.

64. Boston Type 1 Keratoprosthesis Retention Rates For 3 Ocular Groups (Autoimmune Disease, Chemical Injury, Other): Results From The Boston Type 1 Multicenter Study Group. Joseph B. Ciolino [1,2], Brian L. Zerbe [1], Michael W. Belin [1,3]. Albany Medical College,[1] Albany, NY, Massachusetts Eye and Ear Infirmary, Boston, MA, and Cornea Consultants of Albany [2], Albany, NY, USA.

65. Protective Effect of Permanent Soft Contact Lens Wear with the Boston Keratoprosthesis. Mona Harissi-Dagher, MD, Claes H. Dohlman, MD, PhD. Department of Cornea and External Disease, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA, USA.

66. Anterior Segment Effects Of Intravitreal Bevacizumab (Avastin) Application In Two Cases. Dilek D. Altinors[1] Cem Küçükerdönmez[1], Yonca A. Akova,[1] Baskent University, Department of Ophthalmology, Ankara, Turkey.

67. Prevention of Exposure Keratopathy in Intensive Care Units. Darren G. Gregory. Rocky Mountain Lions Eye Institute. Denver, CO, USA.

68. Selenoprotein P Protect Production Of The Oxidative Stress In The Cornea Of The Dry Eye Model Rat. Akihiro Higuchi[1], Yuri Okubo[1], Kazuo Tsubota[1,2] 6N9 Research Park [1],Department of Ophthalmology[2], Keio University School of Medicine, Tokyo Japan.

5th International Conference on the Tear Film & Ocular Surface: Basic Science and Clinical Relevance

Abstracts

Taormina, Sicily, Italy
September 5-8, 2007

Title Sponsor:

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Factors Impacting Research On Visual Function And Keratitis In Dry Eye. Abelson MB[1].[1]ORA Clinical Research and Development, North Andover, MA.

This presentation will discuss the development of realistic and clinically relevant scales for the assessment of the various instantiations of dry eye. Visual function is quickly becoming a recognized variable in the presence of ocular surface staining, and a new diagnostic test is being used to evaluate inter-blink interval visual acuity decay (IVAD). This ties into the unique anatomical, physiological, and pathological differences between the central and peripheral corneas. MUC patterns and types can vary between regions and patients, which could affect tear film break-up pattern (TFBUP) or other break-up characteristics in dry eye sufferers. Researchers have identified five distinct TFBUPs. After one understands the science underlying visual function alteration in dry eye, it is important to properly select patients in a clinical setting, which requires an understanding of a drug's mechanism of action. Several classes of potential dry eye drugs, including anti-inflammatories and secretagogues, will be described, with specific developmental examples such as diquafosol, ecabet sodium, and sodium hyaluronate. The respective pros and cons of oral versus topical treatment will be touched upon. Successful clinical models for testing dry eye treatments will be compared, with a focus on the controlled adverse environment (CAE). In dry eye, this level of control is desirable for patient selection, because only about 60% of patients have both signs and symptoms of dry eye. Ultimately, in order to develop relevant clinical models, it is vital to understand the nature of the patients being treated, the pharmacology of the investigational product, and the desirable and attainable endpoints that should be incorporated into a study.

Identification Of Biomarkers For Conjunctivochalasis Diagnosis In Tear By 2d-Based Proteomics Approach. Arantxa Acera[1] Tatiana Suárez,[1] Ignacio Rodríguez-Agirretxe,[2] Elena Vecino,[1] Juan A. Durán.[1,2] Basque Country University[1] and ICQO,[2] Bilbao, Spain.

Purpose. To study the differential protein expression in tears from normal and conjunctivochalasis patients, and further to identify new molecular markers for the diagnosis of this pathology. **Methods.** Tears from six normal subjects and six patients with conjunctivochalasis were analyzed by two-dimensional electrophoresis (2DE). Protein concentrations were measured using the EZQ Protein Quantitation Kit. Total protein from tears was separated in the first dimension by isoelectric focusing on immobilized pH gradient strips, 3-10 pH range. The second dimension SDS-PAGE separation was made using a Precast Criterion 8-16% gradient gel in a Dodeca Criterion Cell apparatus. SDS-polyacrilamide gels were stained with SYPRO Ruby. The gels images were captured on a VersaDoc™ Model 4000 Imaging System and the images were analyzed using PDQuest 2D gel image analysis software and Progenesis software. Those spots of interest were manually cut out from the gels and sent to protein identification by MS MALDI-TOF.**Results.** Approximately 200 spot proteins were detected in the whole proteome. Twenty-four spots showed a significant and differential expression between normal and conjunctivochalasis tears samples. Eighteen proteins of these 24 spots were identified including proteins S100 family (A4, A8, A9), fatty acid-binding protein, keratin type I and II, glutathione S-transferase P, cullin-4B, L-lactate dehydrogenase A, between others. **Conclusions.** In the present study a group of proteins were found to be highly expressed in conjunctivochalasis in comparison to normals. The proteins more overexpressed in conjunctivochalasis tear samples were. S100-(A8-A4), cullin-4B, gluthathione S-transferase P, keratine type I cytoskeletal 10, and L-lactate dehydrogenase A, which are involved in inflammation, oxidative and keratinisation processes. In summary, we have identified a number of proteins as potential biomarkers for conjunctivochalasis. *[This research was supported by Basque Government, INTEK program W6088]*

Corneal Epithelial Cell Killing Of Internalized Bacteria. Amanda D. Ackerman, Annette A. Angus, Suzanne Fleiszig, University of California, Berkeley, CA USA

Purpose. The healthy ocular surface has a remarkable capacity to defend itself against infection despite daily exposure to potentially pathogenic bacteria. Understanding these defenses and how they are compromised could lead to new strategies in therapeutics. Here we studied the fate of bacteria internalized by corneal epithelial cells. **Methods.** Human telomerase immortalized corneal epithelial cells (hTCEpi) were inoculated with 10^6 cfu/ml of wild type *Pseudomonas* PA01 or mutant PA01_pscC lacking the type III secretion system (T3SS) needle apparatus. Intracellular survival and replication of bacteria were quantified by viable counts 4 and 8 hours post infection. Phase contrast and fluorescent microscopy were used to visualize bacterial location in cells. Corneal cells were compared to two airway epithelial cell lines (Calu-3 and A549 cells). **Results.** After internalization by hTCEpi cells, wild type PA01 replicated intracellularly while _pscC gradually lost viability. In contrast, both wild type and _pscC replicated in Calu-3 and A549 airway epithelial cells. In hTCEpi cells, wild type PA01 were found to swim freely in cytosolic blebs while _pscC trafficked to paranuclear vacuoles that labeled positive for the lysosomal marker LAMP-3. Wild type bacteria localized to blebs in Calu-3 and A549 cells similarly to corneal cells. Interestingly, _pscC mutants caused blebbing in Calu-3 cells but trafficked to vacuoles in A549 cells. **Conclusions.** Corneal epithelial cells can kill bacteria after internalization, correlating with trafficking to vacuoles that label with a lysosomal marker. Two airway epithelial cell types lacked the capacity to kill the same bacteria despite vacuolar localization within one of the cell types. The ability of corneal epithelial cells to kill internalized bacteria likely contributes to the healthy cornea's remarkable resistance to infection. The fact that *P. aeruginosa* can utilize its T3SS to avoid being killed within corneal epithelial cells may contribute to its success as a corneal pathogen. *[Funding: RO1-EY11221, Alcon, Allergan; T32 AI007620 (ADA), NSF Graduate Fellowship (AAA)].*

Evaluation Of Patients With Dry Eye Syndromes For Associated Medical Conditions. Esen Karamursel Akpek[1], MD; Alena Klimava, BS[1]; Jennifer E. Thorne, MD[2], PhD; Don Martin, MD[3]; Kaevalin Lekhanont, MD[1]; Ann Ostrovsky, MD[1]. [1]The Ocular Surface Diseases and Dry Eye Clinic, and [2]Division of Ocular Immunology, Wilmer Eye Institute, and [3]Rheumatology Division, Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD.

Purpose. To analyze patient characteristics and evaluate the associated medical conditions, particularly Sjögren's syndrome in a cohort of patients with dry eye syndrome. **Methods.** Medical records of patients with a primary diagnosis of tear film insufficiency (ICD code 375.15) or keratoconjunctivitis sicca (ICD code 370.33) were reviewed retrospectively for the presence of an associated medical condition. Two hundred and twenty consecutive patients who had 2 or more visits during a 2-year period (January 2004 to January 2006) were considered. The data gathered were analyzed using a customized database. **Results.** The majority of the patients (75.91%)

were female, with a median age of 59 years (range 10 to 91 years). A total of 57 (26%) patients had an associated rheumatic disease; the most common being rheumatoid arthritis (25 patients, 11.4%) and primary Sjögren's syndrome (24 patients, 10.9%). Of all the patients with primary Sjögren's syndrome, 33.3% carried a diagnosis at the time of presentation, 50% were diagnosed as a result of the initial evaluation, and 16.7% went on to develop the diagnosis during follow-up. An overwhelming majority of the patients with rheumatoid arthritis (96%) carried the diagnosis at the time of presentation. Among patients with no evident rheumatic disease initially, the rate of occurrence of Sjögren's syndrome during follow-up was 4% per person-year. **Conclusions.** Associated medical conditions are common in patients with dry eye syndrome. Sjögren's syndrome appeared to be underdiagnosed and should be the focus of diagnostic evaluation in patients with dry eye syndrome.
Dr. Akpek is supported in part by the William and Mary Greve Scholarship from Research to Prevent Blindness.
Dr. Thorne is supported in part by EY 13707 from the National Eye Institute, The National Institutes of Health, Bethesda, MD.

Contact Lens Associated Fungal Keratitis: What We Know And What We Need To Find Out. Eduardo C.Alfonso, MD., Edward WD Norton Professor of Ophthalmology, Bascom Palmer Eye Institute, University of Miami Miller School of Medicine

Fungal keratitis among soft contact lens wearers (SCL) is rare but may be increasing. Prior to the recent international outbreak of fusarium keratitis, prevalence of SCL-associated fungal keratitis ranged from 2-20%. In a comparative series from one institution SCL-associated fungal keratitis increased from a baseline of 2.2% (3/133, 1969-1977) to 49. 6% (59/119, 2004-2005). Rates for the intervening years were 3.1% (2/65, 1977-1982) and 18.6% (6/32, 2000). Risk factors for contact lens associated fungal keratitis include epithelial micro-trauma, increased corneal staining, noncompliance with recommended cleaning and disinfecting regimens and microbial contamination of lens cases and or solutions. Reduced oxygen levels and presence of corneal infiltrates provide favorable conditions for fungal entry and multiplication. Contact lens polymer matrix may play also be a contributing factor. Studies evaluating the antimicrobial activity of multipurpose disinfecting solutions (MPDS) have focused on the in vitro ability of the solutions to inhibit microbial growth in lens cases and the contact lenses. Others have involved the correlation of declining antimicrobial efficacy with increased absorption of the solutions into the contact lens matrix. Reported results have demonstrated varying resultant antifungal activity dependent on the MPDS and lens type evaluated. Future studies will need to focus on the effect of the contact lens on the tear film. The tear film is responsible for the health of the epithelium and represents the most important immune barrier to infections. Soft contact lens wear disturbs this layer. Hard contact lens is almost never associated with microbial keratitis. The status of the tear film in hard contact lens wear needs to be used as a baseline for the study of the health of the tear film in soft contact lens wear.

Anterior Segment Effects Of Intravitreal Bevacizumab (Avastin) Application In Two Cases. **Dilek D. Altinors[1] Cem Küçükerdönmez[1], Yonca A. Akova,[1] Baskent University, Department of Ophthalmology, Ankara, Turkey**

Purpose. To report the effects of intravitreal Bevacizumab (Avastin) application on the ocular surface in 2 cases. **Methods.** Intravitreal Bevacizumab was applied for age-related macular degeneration (AMD) in one case at baseline and intraoperatively in a penetrating keratoplasty (PKP) case. The AMD case had an occult choroidal neovascular membrane. The patient who underwent a PKP had diffuse neovascularization due to an unknown corneal infection which progressed to diffuse corneal vascularization. In both cases, 0.05 ml (1.25mg) Bevacizumab was administered intravitreally. **Results.** In the AMD case, the next day after the intravitreal Bevacizumab injection, sterile corneal infiltrates occurred in the corneal periphery and they responded to topical steroid treatment. In the penetrating keratoplasty case, 3 weeks after the intraoperative bavacizumab injection, the anterior segment neovascularization subsided prominently. **Conclusions.** Intravitreal Bevacizumab (Avastin) may cause corneal complications like sterile infiltrates as in the case above and it helps regression of the ocular surface neovascularization.
Authors have no commercial relationship with the above material.

Brunescent Cataract Extraction In A Case Of Severe Rheumatoid Arthritis. Dilek D. Altinors[1], Yonca A. Akova[1], Baskent University, Department of Ophthalmology , Ankara, Turkey

Purpose. To report our clinical and surgical approach to a dense cataract surgery in a severe dry eye patient with rheumatoid arthritis. **Methods.** A 68 year-old female patient who had severe dry eye and limbal stem cell deficiency (LSCD) due to rheumatoid arthritis is presented. The patient had an only seeing eye which lost vision gradually due to a dense cataract. Her cornea in that eye was cloudy and the details of the anterior segment could barely be seen. An almost blind planned extracapsular cataract extraction was applied to her using the trypan blue dye to visualize the anterior capsule and the chamber details. All punctae were cauterized intraoperatively. **Results.** On the postoperative first day, some residual cortical debris was observed behind the iris but the intraocular lens was in place, the anterior chamber was formed and it was quiet. Three weeks after the surgery, the patient's vision increased to counting fingers and the cortical material had totally resorbed by topical steroid treatment. **Conclusions.** Trypan blue dye may be a good adjunct to extracapsular cataract surgery in cases where the cornea is cloudy and a penetrating keratoplasty is not planned.
Authors have no commercial relationship with the above material.

Current Applications Of Autologous Serum Drops in Ocular Surface Reconstruction. Dilek D. Altinors[1], Yonca A. Akova[1], Baskent University, Department of Ophthalmology , Ankara, Turkey

Purpose. To report the benefits and risks of topical application of autologous serum drops on ocular surface reconstruction. **Methods.** Autologous serum drops were prepared from the patient and in the case of infants, from their mothers. The pathologies were a case of Stevens-Johnson Syndrome, 2 limbal stem cell deficiency (LSCD) cases, 2 herpetic neurotrophic keratitis cases, a newborn with diffuse keratitis and a child with a hot liquid burn in the cornea. In all cases, except the newborn, punctae were occluded or cauterized before the autologous serum application. **Results.** In all cases, the ocular surface healed faster than any other medication. The inflammation subsided, the dependency on steroids decreased. In 2 cases, mild conjunctivitis which responded to topical fluoroquinolone treatment occurred. **Conclusions**. Autologous serum drops in addition to punctal occlusion provides faster ocular surface healing and reconstruction.
Authors have no commercial relationship with the above material.

Chitinase Levels In The Tears Of Subjects With Ocular Allergic Diseases. Pasquale Aragona[1], Maria Musumeci[2], Adriana Maltese[3], Claudio Bucolo[3], Laura Rania[1], Filippo Drago[3], Salvatore Musumeci[4]. [1]Department of Ophthalmology, University of Messina, Messina, Italy. [2]Department of Hematology, Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome, Italy. [3]Department of Experimental and Clinical Pharmacology, School of Medicine, University of Catania, Catania, Italy. [4]Department of Pharmacology, Gynecology and Obstetrics, Pediatrics, University of Sassari and Institute of Biomolecular Chemistry, National Research Council (CNR), Li Punti (SS), Italy.

Purpose. Chitin is abundant in the structural coatings of fungi, insects, and parasitic nematodes. The host defense against chitin-containing pathogens include production of chitinases. An acidic mammalian chitinase (AMCase) is produced in human epithelial cells of lower airways via a Th2-specific, IL-13-dependent pathway and seems associated to allergic asthma. The role of AMCase in ocular allergic diseases has not been studied previously. **Methods.** Six patients with vernal keratoconjunctivitis (VKC) (mean age±SD 12±4.3), 7 patients with seasonal allergic conjunctivitis (SAC) (age 18±8.8) and 8 healthy controls (age 19±5.8) were enrolled in a study to measure the AMCase activity in tears. Briefly, 10 µl of sample were incubated with 0.1 mL of a solution containing 22 µmol/l of the fluorogenic substrate 4-methylumbelliferyl-β-D-N,N_,N__-triacetyl-chitotriose (Sigma, Milan, Italy) in 0.5 M citrate-phosphate buffer pH 4.5 for 15 min at 37 °C. The reaction was stopped by addition of 2 ml of 0.5 mol/l Na_2CO_3-$NaHCO_3$ buffer, pH 10.7. The fluorescence of 4-methylumbelliferone was read on a spectrofluorimeter Hitachi 2500 (Hitachi, Europe Ltd, Herts, UK), on 365 nm excitation and 450 nm emissions. Chitinase activity was expressed as nanomoles of substrate hydrolyzed per ml per hour (nmol/ml/h) or expressed in mU/ml considering one unit of 4-methylumbelliferyl-β-D N,N',N''triacetylchitotriosio-degrading activity as one unit of fluorescence emission under the assay conditions. **Results.** AMCase activity in tears was increased in patients affected by VKC (33.727 ± 10.843 nmol/ml/h) and SAC (7.310 ± 4.142 nmol/ml/h) compared to healthy controls (1.492 ± 0.320 nmol/ml/h). VKC patients showed a tear AMCase activity statistically significantly higher than SAC patients and normal subjects ($p < 0.0001$). The identification of VKC patients on the basis of AMCase level gave a sensibility and a specificity of 100 %. **Conclusions.** AMCase may be an important mediator in the pathogenesis of Th2 mediated inflammatory eye diseases such as VKC. Additional studies are needed to determine the potential of AMCase as a therapeutic target in these diseases.

Expression Of Metalloproteinase 9 And Transglutaminase 2 In Conjuntival Cells Of Dry Eye Patients. Pasquale Aragona[1], M'hammed Aguennouz[2], Rosaria Spinella[1], Mariagrazia De Pasquale[2], Laura Rania[1], Rossana Di Pietro[1], Sebastiano Giuffrida[3]. [1]Dept. of Ophthalmology and [2]Dept of Neuroscience, University of Messina (Italy) and [3]Bausch and Lomb IOM, Catania (Italy)

Purpose. Recent studies show the inflammation and apoptosis participate to dry eye pathogenesis. Little is know about the different expression of inflammatory mediators in different clinical forms of dry eye. Aim of this paper is to study the expression, in two different forms of dry eye, of metalloproteinase (MMP)9, a marker of inflammation, and tranglutaminase (TG)2, ubiquitously expressed in mammalian tissues and one of the few genes induced during the *in vivo* apoptotic program. **Methods.** The expression of MMP9 and TG2 was examined in conjunctival epithelium specimens obtained by conjunctival imprinting from 10 Sjögren's syndrome (SS) patients (9 F, mean age±SD 61.6 ± 13.6); 10 meibomian gland dysfunction (MGD) patients (7 F, age 55.2±10); and 5 normal subjects (CTRL) (5 F, age 45.2±15.7). All subjects underwent to the following tests: Symptom questionnaire (0-3 score), break up time (BUT), fluorescein corneal stain, lissamin green conjunctival stain, Schirmer's I test, conjunctival imprints from the supero-nasal, superior and supero-temporal sites of the bulbar conjunctiva were obtained to be processed for real time RT-PCR and for immunostaining for MMP9 and TG2. **Results.** The symptom score was 8±3.1 in SS; 8±1.1 in MGD and 0 in CTRL ($p<0.001$ vs SS and MGD). BUT was 2.6±0.9 in the SS group, 4.2±1.7 in the MGD group ($p=0.01$ vs SS) and 12±3.2 in the CTRL group ($p<0.001$ vs SS and MGD). Fluorescein corneal score was 4.3±4.3 in SS, 1.2±0.9 in MGD and 0 in CTRL ($p<0.001$). Lissamin green score was 3.6±2.5 in SS, 2±1.8 in MGD and 0.9±0.2 in CTRL ($p=0.003$ vs SS). Schirmer's I test was 11.2±11.2 in SS ($p<0.001$ vs MGD and controls), 26.7±12.7 in MGD and 28±13.4 in CTRL. The semiquantitative data obtained by real time PCR, normalized to the internal control glyceraldehydes-3-phosphate dehydrogenase (GAPDH) showed that MMP9 expression was 22.5±7.6 in SS ($p<0.001$ vs MGD and CTRL); 12±3.7 in MGD ($p=0.0009$ vs CTRL) and 1±0.02 in CTRL. The expression of TG2 was 33.7±15.7 in SS ($p<0.001$ vs MGD and CTRL), 7.7±6 in MGD ($p<0.05$ vs CTRL) and 2±0.02 in CTRL. **Conclusions.** It appears that the expression of MMP9, marker of inflammation, and TG2, enzyme induced in association with apoptosis, is significantly higher in SS, a disease where inflammation plays a pivotal role, than in patients with a different type of dry eye such as MGD.

O-Glycosylation Of Mucins. Pablo Argüeso. Schepens Eye Research Institute and Department of Ophthalmology, Harvard Medical School, Boston MA.

During the last few years it has become evident that carbohydrates on the cell surface play important roles in determining cell function. Indeed, carbohydrates are major components of the cell surface, dictating the physicochemical properties of the glycocalyx, and modulating a wide variety of cellular events, such as cell-cell and cell-pathogen interactions. The ocular surface glycocalyx is rich in hydrophilic carbohydrates (O-glycans) present on a group of molecules known as membrane-associated mucins. Due to their extremely large size, they extend above other components of the plasma membrane, therefore constituting the outermost interface between the epithelial cell and the external environment. Data from our laboratory have shown that the biosynthesis of mucin O-glycans at the ocular surface is regulated by a group of glycosyltransferases—known as polypeptide GalNAc transferases—that have cell-type and cell-layer specific distributions. In patients with dry eye, the expression of these enzymes, as well as the distribution of mucin O-glycan epitopes within apical cells, are altered, suggesting that O-linked glycans contribute to the maintenance of a wet surfaced phenotype. Using functional assays, we have also shown that mucin O-glycans form a protective barrier on the epithelial glycocalyx through a galactose-dependent association with galectin-3, a 35 kDa mammalian lectin expressed by corneal and conjunctival epithelial cells. Abrogation of galectin binding by competitive inhibition results in rose bengal dye penetrance in corneal epithelial cells. Finally, the role of mucin O-glycans in preventing apical cell surface adhesion and bacterial infection at the ocular surface will be discussed.

Supported by NIH/NEI R01EY014847

Ocular Trust 2: Longitudinal Nationwide Surveillance Of Antimicrobial Susceptibilities In Ocular Isolates. Penny A. Asbell,[1] Daniel F. Sahm[2] for the Ocular TRUST Study Group. [1]Mount Sinai School of Medicine, New York, NY, USA and [2]Eurofins Medinet Inc., Anti-Infective Services, Herndon, VA, USA

Purpose: To report the results of the second annual nationwide surveillance of antimicrobial susceptibility in ocular isolates. **Methods**: Isolates from geographically distributed centers were tested by an independent centralized laboratory (Eurofins) for in vitro susceptibility to 9 antimicrobials. **Results**: *H. influenzae* isolates were 100% susceptible to fluoroquinolones (FQs) and to azithromycin; susceptibility to trimethoprim (TMP) was 76%. In *S. pneumoniae*, susceptibilities were: FQs 100% > TMP 73.8% > penicillin 65.5% > azithromycin 59.5%. Susceptibilities of *S. aureus*, coagulase-negative staphylococci, and *P. aeruginosa* varied by antimicrobial class, including high FQ resistance in methicillin-resistant *S. aureus* (MRSA). **Conclusions**: Ocular TRUST is the only national program in the U.S. specifically testing ocular isolates from eye centers and community hospitals. The Ocular TRUST 2 study indicates consistently high and equivalent susceptibility of *S. pneumoniae* and *H. influenzae* to levofloxacin, gatifloxacin, and moxifloxacin. Equivalent but limited susceptibility to newer fluoroquinolones was also seen in MRSA ocular isolates. Ophthalmic FQs generally remain drugs of choice for empiric therapy in ocular infections due to broad-spectrum activity.
[This study was supported by a grant from Vistakon Pharmaceuticals, L.L.C., Jacksonville, Florida. Dr. Asbell serves as a Speaker Bureau consultant for Vistakon Pharmaceuticals, L.L.C. Dr. Sahm is Global Head/Vice President, Anti-Infective Services for Eurofins Medinet, Inc.]

Impact Of Refractive Surgery On The Tear Film And The Ocular Surface. Dimitri Azar, MD. University of Illinois at Chicago.

Several studies have demonstrated that dry eye disease is exacerbated by LASIK and other refractive surgical procedures. In this presentation we will review the basic and clinical findings of dry eye after LASIK and PRK. We will examine the effect of LASIK on tear production, corneal sensation and innervation as well as postoperative dry eye symptoms. We will also describe the pattern of trigeminal regeneration after PRK and LASIK. Review of data evaluating the effect of surgery on the blink rate, ocular surface inflammation, and tear film physiology will be followed by diagnostic and management options. Future directions for research in this field will also be discussed.

Effect Of Protein Kinase Ca And P42/P44 Mapk On Egf-Stimulated Rat Cultured Conjunctival Goblet Cell Proliferation. Jeffrey A. Bair, Marie A. Shatos, Robin R. Hodges and Darlene A. Dartt. Schepens Eye Research Institue, Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School. Boston, MA.

Purpose. To determine the role protein kinase C (PKC)a and p42/p44 mitogen-activated protein kinase (MAPK) on EGF-stimulated cultured conjunctival goblet cell proliferation. **Methods.** For all experiments, cultured P1 rat goblet cells were grown to 75% confluence and serum-starved for 24h in RPMI-1640 medium containing 0.35% BSA. The colorimetric WST-8 cell counting assay was used to determine cell proliferation. To overexpress PKCa, an adenovirus containing a gene for constitutively active PKCa (MyrPKCa, 10^7 PFU) was incubated with goblet cells. Cells were grown on coverslips, and treated with EGF (10^{-7} M) for 0 – 24 hrs. Cells were fixed in 4% paraformaldehyde for immunocytochemical determination of PKCa and MAPK localization. Coverslips were visualized with a Nikon Eclipse microscope. **Results.** PKCa was uniformly distributed throughout the cytoplasm of untreated goblet cells. Stimulation with EGF (5 min) caused a distinct, intensive perinuclear concentration of PKCa. Overexpression of PKCa also resulted in less expression around the periphery of the cell and more in the cytoplasm surrounding the nucleus. EGF and overexpression of PKCa significantly increased goblet cell proliferation to 1.4 ± 0.1 and 1.5 ± 0.2 fold increase above basal, respectively, when assayed by WST-8. EGF caused the translocation of MAPK to the nucleus in 96% of the goblet cells after 1 min. Inhibition of MAPK by the inhibitor U0126 (10^{-6} M) inhibited both translocation of MAPK and goblet cell proliferation induced by EGF. MyrPKCα caused MAPK translocation to the nucleus in 13% of the goblet cells after 24h treatment as well as an increase in goblet cell proliferation, which was also significantly inhibited by UO126. **Conclusions.** We conclude that activation of PKCa stimulates goblet cell proliferation by increasing MAPK activity. Furthermore, EGF induces goblet cell proliferation by activating PKCa that in turn stimulates MAPK.
Supported by NIH EY9057.

Tear Function And Lipid Layer Alterations In Chronic Graft-Versus-Host Disease. Yumiko Ban,[1] Yoko Ogawa,[1] Eiki Goto,[1,2] Miki Uchino,[1] Naoki Terauchi,[1] Maiko Seki,[1] Mika Nakaya,[1] Megumi Saeki,[1] Murat Dogru,[1] Kazuo Tsubota.[1] Department of Ophthalmology, School of Medicine, Keio University, Tokyo, Japan,[1] Department of Ophthalmology, School of Dental Medicine, Tsurumi University, Kanagawa, Japan.[2]

Purpose. To assess the tear function and alterations of the tear film lipid layer in dry eye patients with chronic graft versus host disease (cGVHD) in an observational case-control study. **Methods.** Twenty eyes of 11 patients diagnosed as dry eye associated with cGVHD, as well as 20 eyes of 11 normal controls were enrolled. Tear film interference images obtained by a DR-1 tear lipid layer interferometry was used to investigate the tear film lipid layer, and Yokoi's grading were analyzed. Schirmer test with or without nasal stimulation, fluorescein and rose-bengal vital staining, tear film break up time (TBUT), grading of ease of meibum expression, and cicatricial change of conjunctiva were also examined. **Results.** The mean DR-1 grade (cGVHD 3.9 ± 0.9 vs. controls 1.2 ± 0.5), grading of ease of meibum expression (cGVHD 2.5 ± 0.6 points vs. controls 0), Schirmer test (cGVHD 2.2 ± 1.7 mm vs. controls 15.0 ± 9.4 mm), TBUT (cGVHD 3.5 ± 1.3 seconds vs. control 9.3 ± 2.8 seconds), fluorescein staining scores (cGVHD 4.4 ± 1.8 points vs. control 0.1 ± 0.3 points), and rose-bengal staining scores (cGVHD 3.3 ± 2.6 points vs. control 0.2 ± 0.5 points), respectively ($p < 0.05$). Cicatricial changes were observed exclusively in cGVHD patients. Patients with cicatricial conjunctivitis had significantly higher DR-1 grading (4.4 ± 0.9) than patients without cicatricial conjunctivitis in cGVHD (3.4 ± 0.7) ($p < 0.05$). **Conclusions.** Tear film lipid layer in dry eye patients with cGVHD was highly affected. The DR-1 tear lipid layer interferometry may be a useful non-invasive tool for the monitoring and assessment of the conditions of dry eye in patients with cGVHD before and after HSCT.
(This study was supported by grants #17791254 and #18591932 from the Japanese Ministry of Education, Culture, Sports, Science, and Technology)

Steroid Hormones In The Tear Film. Linda Banbury[1], Carol Lakkis[2], Carol Morris[1], [1]Centre for Phytochemistry and Pharmacology Southern Cross University, Lismore, NSW, Australia, [2]Clinical Vision Research Australia, University of Melbourne, Melbourne VIC Australia.

Purpose. To analyse tear fluid for the presence of two steroid hormones and to investigate day to day variation and correlation with levels in saliva and serum. **Methods.** Tear, saliva and serum samples were collected within a 1 hour period from 54 subjects and analysed for the presence of cortisol and DHEA. For comparison with saliva and serum levels, a cross-sectional study was done on all subjects (n=54), and day-to-day variation was assessed on 10 separate days (n=6). Free hormones in saliva and tears were assayed by enzyme immunoassays (EIAs) developed for saliva. Total hormone EIAs were used for serum. **Results.** Cortisol and DHEA were present in tear fluid, with normal ranges of 5.0 – 30.7 ng/mL (cortisol) and 0.07-2.10 ng/mL (DHEA). These concentrations were comparable to saliva levels in this study and in the literature. For single time points in a population of 54, correlations were observed between the levels of hormones in tears and in saliva and serum (tear/saliva cortisol $r = 0.65$, $p<0.0001$; tear/serum cortisol $r = 0.43$, $p = 0.001$; tear/saliva DHEA $r = 0.69$, $p<0.0001$; tear/serum DHEA $r = 0.51$, $p <0.0001$).Gender differences were observed in degree of correlation for cortisol and DHEA (less strong) e.g. tear/serum cortisol, females $r = 0.776$, $p<0.0001$; males $r = 0.232$, $p = 0.245$. For multiple time points in individuals, 3/6 had significant correlations for tear/saliva cortisol ($r = 0.73$-0.77, $p = 0.009 – 0.025$) and 5/6 for tear/serum cortisol ($r = 0.63$-0.97, $p = 0.0001$-0.05). Both hormones decreased significantly with age in all fluids, except tear cortisol, which approached significance ($r = -0.26$, $p = 0.06$). **Conclusions.** This study has established the presence and concentration of two steroid hormones, cortisol and DHEA, in the human tear film. These and other steroid hormones play a significant role in tear physiology and maintenance. Our results suggest that monitoring tear hormone levels may potentially have ocular applications.
This work was supported in part by a grant from CIBAVision GmbH, and the Australian Government (PhD Scholarship).

Ocular Surface Inflammation And Corneal Changes In Patients With Cicatricial Pemphigoid. Stefano Barabino,[1] Cristina Mingari,[2] Marina Papadia, [1,3] Marina Bertolotto,[1] Paola Vacca,[2] Federico Solignani,[1] Cristiana Valente,[1] Maurizio Rolando.[1,3] [1]Ocular Surface Research Center, Department of Neurosciences, Ophthalmology, and Genetics, University of Genoa, [2]DIMES, University of Genoa, [3] IS.PRE Oftalmica, Genoa, Italy.

Purpose. To test the hypothesis that patients with ocular cicatricial pemphigoid (OCP) have a significant degree of conjunctival inflammation and anatomical and immunological changes of the cornea. **Methods.** Early stages OCP patients were identified, and Schirmer test, fluorescein and lissamine green staining, tear break-up time, and impression cytology of the conjunctiva were performed in 8 patients and in 8 age-matched controls. HLA-DR expression on conjunctival cells was measured by flow cytometry. The central cornea was examined by *in vivo* confocal microscopy using a 40x lens and an axial resolution of 5 µm in both groups. **Results.** Statistically significant changes in corneal fluorescein staining ($p<0.005$), lissamine green conjunctival staining ($p<0.005$) and HLA-DR expression ($p<0.005$) occurred in the study group compared to controls, while tear secretion did not show any differences. In patients with OCP confocal microscopy images obtained showed a significant lower number of epithelial cells (1238 ± 375 cc/mm^2) compared to controls (1691 ± 190 cc/mm^2, $p<0.0001$), superficial epithelial cell area considerably higher than normal, reduced nucleus/cytoplasm ratio, halos around the nuclei, and sharp borders. Numerous highly reflective dendritic-like cells were present in the epithelial cell basal layer, and their density correlated with HLA-DR expression. The stroma showed loss of keratocytes, the presence of lacunae, and tortuous subbasal nerves. No differences were recorded in endothelial cells density. **Conclusions.** In OCP the ocular surface is characterized by inflammation of the conjunctiva and immunological and structural changes of the cornea which confirm the importance of local and systemic anti-inflammatory therapy in the early stages of the disease.

The Perception Of Pain. Linda M. Bartoshuk. Center for Smell and Taste, University of Florida, Gainesville, FL. USA

Problem. Comparisons of pain sensations across groups are commonly made with labeled scales (e.g., visual analogue scale, VAS, labeled "no pain" at one end and "strongest pain ever experienced" at the other). The validity of these comparisons depends on the assumption that the labels denote the same pain intensity, on average, to each of the groups to be compared. **Solution.** Magnitude matching is based on our ability to match sensory intensities across modalities. If two modalities are unrelated, we can use one of them as a standard. For example, we asked subjects to rate the most intense pain ever experienced and the brightest light ever seen on a common scale. Women who selected childbirth as their most intense pain rated it 27% more intense than the brightest light. Men rated their most intense pain as about equal to the brightest light. Assuming that men and women experienced, on average, the same brightness from the brightest light, we conclude that the childbirth pain was 27% more intense than the most intense pain for men. Note that these results demonstrate the pain VAS error. Had we asked subjects to rate their most intense pain on the pain VAS, all would have rated their most intense pain at the top. Yet, by using an unrelated standard, we could see that the top of the scale for the women for whom childbirth was the most intense pain was actually 27% higher than the top of the scale for the men. **Recommendations.** Some sensory scaling methods were developed by measurement theorists (e.g., magnitude estimation); others were developed as an answer to practical needs (e.g., VAS). From multiple approaches we have tried to devise user-friendly scales that assess various sensations on a common scale. Most recently, we have focused on a simple modification of the conventional VAS: the global VAS. This gVAS can be used in the form of a line with "no sensation" at one end and "most intense sensation of any kind" at the other end. Similarly, subjects can be asked to imagine a scale from 0 to 100 where 0="no sensation" and 100="most intense sensation of any kind." We recommend the inclusion of multiple potential standards (remembered sensations as well as tested stimuli).

***In Vivo* Confocal Microscopy For Assessing Inflammatory Changes In Ocular Surface Diseases.** Christophe Baudouin, Hong Liang, Aude Pauly, Bénédicte Dupas, Antoine Labbé, Françoise Brignole-Baudouin. Depts of Ophthalmology, Quinze-Vingts National Ophthalmology Hospital and Immunotoxicology, University Paris 5, France

Purpose. New investigation techniques have been developed at the ocular surface level and may now provide histological-like images in a noninvasive way. We evaluated the potentials of a new-generation *in vivo* confocal microscopy technique in the ocular surface of a series of patients suffering from various ocular surface diseases and in animal models related to inflammation or angiogenesis. **Methods.** The Rostock Cornea Module of the Heidelberg Retina Tomograph was used to examine ocular epithelia in 20 patients with corneal inflammatory diseases and/or neovascularization. Impression cytology specimens

were taken and processed for immunostaining in order to allow comparisons with *in vivo* patterns. Animal models of corneal inflammation after scraping and instillation of lipopolysaccharide, and of neovascularization after instillation of benzalkonium were also developed for confocal microscopy assessment. **Results.** Using this technique, we could identify in various inflammatory eye diseases cell processes at a level allowing detection of inflammatory cell rolling, live diapedesis, chromatin fragmentation, or quantification of dendritic cell infiltration, all of which being well correlated with cytological patterns. The animal models we developed also confirmed the usefulness of noninvasive confocal microscopy for inflammation or angiogenesis models. **Conclusion.** The most recently developed *in vivo* confocal microscopy technology may now provide in a noninvasive way excellent histologic-like patterns of the ocular surface epithelia. This technology could thus become a new routine method to explore ocular surface disorders and also has many promising applications in immunology, pharmacology, and angiogenesis research.
No commercial interest

Influence of Prostaglandin Analogs on the Ocular Surface of Glaucoma Patients Treated over the Long Term. Christophe Baudouin, Hong Liang, Pascale Hamard, Antoine Labbé, Françoise Brignole-Baudouin. Depts of Ophthalmology, Quinze-Vingts National Ophthalmology Hospital and Immunotoxicology, University Paris 5, France.

Purpose. To investigate the expression of CCR5 and CCR4, two chemokine receptors, as markers of the T helper (Th)1 and Th2 pathways, respectively, and class II antigen HLA-DR as a hallmark of inflammation, on conjunctival cells obtained from patients on long-term glaucoma treatment. **Methods.** In the present case-controlled study, a total of 18 normal subjects and 70 glaucoma patients treated with topical antiglaucoma drugs for more than 1 year: 14 receiving a beta blocker as monotherapy; 38 with a prostaglandin analog alone (19 with latanoprost, six with travoprost, 13 with bimatoprost), and 18 receiving multiple treatments. Impression cytology specimens (ICS) were taken from one eye of the patients and processed for flow cytometry. Conjunctival cells were extracted and incubated with monoclonal antibodies against CCR4, CCR5, HLA-DR or their specific controls to measure, in a masked manner, the percentages of conjunctival cells positive for the three markers. **Results.** Compared to all other groups, HLA-DR expression was significantly raised in the multitreatment group, whereas all monotherapies showed slight and nonsignificant increases. Both CCR4 and CCR5 were significantly increased in all five glaucoma groups compared with normal subjects, with no between-group differences. **Conclusion.** This study demonstrates the overexpression of two chemokine receptors in the conjunctival epithelium of glaucoma patients treated over the long term. Our results show the simultaneous overexpression of CCR4 and CCR5, suggesting that the chronic use of topical treatments may stimulate both the Th1 and Th2 systems at the same time. They suggest inflammatory mechanisms combining allergy with toxicity and illustrate the complexity of inflammatory reactions occurring in the ocular surface of glaucoma patients.
No commercial interest.

Clinically Important Differences In The Symptom Bother Module Of IDEEL Questionnaire. Carolyn G. Begley,[1] Robin Chalmers,[1] and Carol Fairchild,[2] Indiana University School of Optometry,[1] Alcon Research Ltd.[2]
Purpose. The Impact of Dry Eye on Everyday Life questionnaire (IDEEL) is a valid, reliable questionnaire with 3 modules; Symptom Bother (SB), Quality of Life, and Treatment Satisfaction. The SB module discriminates well between dry eye (DE) subjects. This study was conducted to test the utility of the IDEEL-SB to distinguish self-assessed severity in KCS subjects and to determine the clinically important difference (CID) in SB that relates to a global improvement (GI) in condition with treatment. **Methods.** KCS subjects completed the IDEEL SB at baseline, 1 and 4 weeks after starting a QID tear replacement regimen. At weeks 1 and 4 they also completed GI questions on status of general health and dry eye condition on a 5-point Likert scale. The SB score was based on an unweighted mean score x 25. CID was determined by an anchor-based GI question, receiver-operator curve (ROC) analysis and an effect size (ES) method based on distribution. **Results.** The 74 subjects rated their DE severity as mild (40%), moderate (50%) or severe (9%). Habitually, 56.3% of them rarely/never used tear replacement drops and 43.7% used drops more often. Baseline SB score was 40.0 (SD=7.5) for mild, 50.6 (SD=11.0) for moderate, and 64.3 (SD=8.0) for severe subjects (p=0.001). After 4 weeks use of QID drops, the change in SB for subjects who reported GI as "improved" averaged 13.3 + 10.9 while those who reported "same" had a shift of 4.7 + 9.4, and those who "worsened" change -1.4 + 11.1. ROC analysis results and the pattern of change in SB with treatment for all severity and improvement groups and indicate that a 12-point change in SB is a CID with an ES = 1.14. **Conclusions.** A 12-point shift in the Symptom Bother module of the IDEEL appears to be a clinically important difference based on effect size and the distribution across severity groups compared to their global assessment of change in condition.
This project was supported by Alcon Research, Ltd.

Simultaneous Tracking Of Visual Performance, Optical Quality And Tear Disruption. Carolyn G. Begley,[1] Haixia Liu,[1] Larry N. Thibos,[1] Robin Chalmers,[1] Colleen Riley,[2] Kurt Moody.[2] Indiana University School of Optometry, Bloomington, IN, Johnson & Johnson Vision Care, Inc., Jacksonville, FL.

Purpose. In this study, we demonstrate simultaneous tracking of objective visual performance and subjective optical quality over a soft contact lens, augmented with retro-illumination (RI) to simultaneously monitor tear film disruption and *in vivo* lens wetting of the contact lens surface. **Methods.** A confocal imaging system incorporates a range-defining aperture (RDA) that continuously measures light reflected through the pupil from an infrared laser beam focused on the retina. Light intensity (LI) passing through the RDA is a measure of the fraction of reflected light for which wavefront slope < 6.5 milliradians, which measures dynamic optical quality through changes in light scatter. A monitor, viewed through a beam splitter, displayed letter targets 20/40 in size for which contrast sensitivity (CS) was measured continuously and simultaneously with optical measurements. An infrared light source was used to retro-illuminate the pupil to simultaneously visualize the tear film over the lens surface in the area of pupil. Five hydrogel contact lens wearers were asked to keep one eye open for approximately 35 sec, while CS, LI and RI were collected over 3 trials. **Results.** LI reduction and CS loss were highly correlated (average r = 0.80±0.53). Ten sec after eye opening trials, CS declined by 40.65%±26.44% and LI was reduced 9.65%±8.26%, and after 35 sec CS declined 68.60%±17.81%. and LI by 28.59%±22.59%. The decline was statistically significant (p<0.001, ANOVA) for both parameters. The distribution of tear breakup over the lens surface as viewed by RI was spatially correlated with the level of LI and CS loss. Centrally located tear breakup appeared to produce a greater loss in LI and CS than peripherally located breakup. **Conclusions**. This method allowed simultaneous and dynamic tracking of the deterioration of objective optical quality of the eye and subjective visual performance while

viewing surface tear disruption and provides a procedure for determining *in vivo* lens wetting and its effect on vision.
This project was supported by a grant from Johnson & Johnson Vision Care, Inc.

The Relation Between Tear Break-Up And Blinking. Carolyn G. Begley, Nikole L. Himebaugh, Indiana University School of Optometry, Bloomington, IN, USA

Purpose. Blink rate has been studied by many investigators and is known to increase in dry eye, presumably in response to ocular surface drying. However, blink rate is also modulated by internal factors, thus the contribution of tear instability to blinking is unknown. The purpose of this study was to determine the relation between tear break-up and blinking in dry eye (DE) and control (C) subjects engaged in visual tasks. **Methods**. Sixteen DE and 16 C subjects performed 4 tasks (3 minute duration) requiring varying amounts of attention while the area of fluorescein tear break-up (AB) and blink frequency and amplitudes were videotaped and later quantified using ImageJ. **Results**. DE subjects showed a significantly higher and more irregular blink rate than C ($p<0.05$, Mann Whitney), but the blink rate of both groups significantly decreased with tasks requiring greater attention ($p<0.003$, Repeated Measures ANOVA). Incomplete blinking was common, with an average amplitude of 69% for both groups. Only 56% of blinks were preceded by tear break-up in DE and 49% in C subjects. The AB before a blink was significantly greater ($p<0.05$, Mann Whitney) for DE (AVG=6.3%, range 0.10-80%) than for C (AVG=3.4%, range 0.04%-56%), with most tear break-up occurring over the inferior cornea. Tear break-up over the central and superior cornea, which was more common in DE, was more likely to stimulate a fuller blink than tear break-up occurring in the inferior cornea. **Conclusions**. Blinking is a complex phenomenon that may occur in response to ocular surface conditions, but it is often incomplete and highly irregular. Large areas of inferior tear break-up were common and persistent in both DE and C subjects, who blinked enough to cover the pupil but not to clear the break-up, whereas central and superior break-up often stimulated a rapid, full blink. These data suggest that blinking to maintain a smooth tear film over the pupil has priority, presumably to ensure optimum vision.
This project was supported by Dr. Himebaugh's Bausch & Lomb/American Optometric Foundation William C. Ezell Fellowship.

The Sensation Problem: The Biology And Psychology Of Pain And Irritation. Carolyn G. Begley, Indiana University School of Optometry, Bloomington, IN, USA

According to the 2007 DEWS definition and report, an inadequate and/or unstable tear film is an early or initiating event in the mechanism of dry eye and is linked to symptoms of ocular discomfort and visual disturbance. Tear film instability is thought to exacerbate tear hyperosmolarity through evaporation or tear break-up, which presumably leads to cellular damage in more severe cases of dry eye. This model underscores the idea that the tear film is a dynamic, reactive barrier that responds to stress, either from extrinsic or intrinsic factors, and that this stress is transferred to the ocular surface and its associated glands, presumably leading to dry eye symptoms. Visual disturbances, which can range from annoying to disabling, provide evidence that tear film instability is ongoing in the dry eye patient. Symptoms of ocular discomfort may be inflammatory and/or neurological in origin as the surface epithelium and underlying nociceptors respond to fluctuating hyperosmolarity and increased shear stress with inflammation. This dynamic model of tear instability and chronic stress to the ocular surface provides an explanation for increased end of the day symptoms in dry eye, and for the often reported weak correlation between dry eye symptoms and clinical signs. Future research should be directed toward this cycle of chronic stress in dry eye to provide further insight into the causes of dry eye symptoms and to develop objective clinical tests that predict symptoms.

The Neural Basis Of Sensation In Intact And Injured Corneas. Carlos Belmonte. Insituto de Neurociencias de Alicante. Universidad Miguel Hernandez-CSIC, San Juan de Alicante, Spain.

The characteristics and neural basis of corneal and conjunctival sensations is developing in recent years due to the high incidence of discomfort and altered sensitivity of the cornea following ocular dryness, refractive surgery or use of contact lenses. Corneal nerves are functionally heterogeneous: about 20% respond exclusively to noxious mechanical forces (mechano-nociceptors); 70% are additionally excited by extreme temperatures, exogenous irritant chemicals and endogenous inflammatory mediators (polymodal nociceptors), and 10% are cold-sensitive and increase their discharge with moderate cooling of the cornea (cold receptors). Each of these types of sensory fibres contributes distinctly to corneal sensations. Mechano-nociceptors mediate, sharp acute pain produced by touching of the cornea. Polymodal nociceptors elicit the sustained irritation and pain that accompany corneal wounding; cold receptors evoke cooling sensations and may also mediate sensations of ocular dryness. Depending on the relative activation by the stimulus of each subpopulation of corneal sensory fibres, different subqualities of irritation and pain sensations are evoked. Corneal sensations can be explored experimentally in humans with a gas esthesiometer that applies controlled mechanical, chemical and thermal stimuli to the corneal surface. When the cornea is injured or irritated, corneal nerves are excited and eventually severed in a variable degree and local inflammation is produced.They also become sensitized by local inflammatory mediators, such as prostaglandins or bradykinin and thus exhibit spontaneous activity, lowered threshold and enhanced responses to new stimuli. This leads to discomfort, spontaneous pain and hyperalgesia. Nerves destroyed by injury soon start to regenerate and form microneuromas that exhibit abnormal responsiveness and spontaneous discharges, due to an altered expression of ion channel proteins in the neuron cell body and its regenerating nerve terminals. Presumably, this altered excitability is the origin of the lowered sensitivity, spontaneous pain, dry eye sensations and other disaesthesias reported in patients following refractive surgery.
Supported by the Fundación Marcelino Botin and Grant BFU2005-08741 of the MEC, Spain

Treatment With Liposomes Eyedrops Of Dogs With Dry Eye. Jose M. Benitez del Castillo[1], Marta Vicario[2], Alfonso Rodriguez[3], Elisa Gonzalez[3], Ana M. Muñiz[1], Eva Vico[1], Irene T.Molina-Martínez[2]. [1] Departamento de Oftalmología, Hospital Universitario San Carlos, Madrid, Spain.[2] Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad Complutense, Madrid, Spain.[3]Servicio de Oftalmología, Hospital Clínico Veterinario, Universidad Complutense de Madrid.

Purpose. To evaluate in an spontaneous dry eye model the effect of a new eyedrop containing thehalose, hyaluronate and liposomes with vitamine E and phosphatidilcoline (NED). **Methods.** A preservative free formulation containing aqueous and lipid constituents was prepared. The lipid components were neutral liposomes made from phosphatidylcholine, cholesterol and vitamin E in a ratio (8:1:0.08).

Liposomes were suspended in an aqueous solution of trehalose and hyaluronic acid. Five dogs with dry eye (Schimer´s test <10 mm) treated with cyclosporine, dexamethasone and hyaluronate were switched to cyclosporine, dexamethasone and the NED. Schirmer´s test (ST), tearfilm breakup time (BUT), lissamine green staining, hyperemia and the presence of secretions were evaluated. Follow-up was between 30 and 120 days. The worst eye was analysed (lower ST). **Results.** Final ST and BUT were higher than baseline (6.4 SD 7.0 vs 10.2 SD 4.0 and 20 SD 10 vs 26 SD 7 sec). Lissamine green staining, hyperemia and secretions decreased over time. **Conclusions**. Althought preliminary these data show that the NED provides a better relief than sodium hyalurate alone.
Acknowledgements. Research Group UCM (CAM 920415, CCG06-UCM/BIO-1304).

Mucins In Symptomatic And Asymptomatic Contact Lens Wearers. Monica Berry[1], Heiko Pult[2], Christine Purslow[2], Jeff Nyman[3], Paul J Murphy[2].[1]Academic Unit of Ophthalmology, Bristol, UK; [2]School of Optometry and Vision Sciences, Cardiff, UK; [3]Pennsylvania College of Optometry, Philadelphia, USA.

Purpose Lubrication of the ocular surface – one of the functions ascribed to mucins – is pivotal in contact lens comfort. We investigate the relationship between surface mucins, dry eye symptoms, lid wiper epitheliopathy (LWE) and lid parallel conjunctival folds (LIPCOF). **Methods** Sixty-one experienced contact lens wearers (23M, 38F; age range 18-55 years) were recruited for the study. Ocular surface mucin samples were collected by gently pressing Schirmer strips onto the temporal bulbar conjunctiva. The worn contact lenses and strips were kept frozen until tested, when they were individually extracted in 4MGuHCl with protease inhibitors and RIPA buffer, respectively. Reactivity with antibodies against mucin peptide cores was probed in dot-blots and in western blots after electrophoresis on NuPage bis-tris gels, visualised with fluorescent substrates. **Results** Subjects were divided into two groups, asymptomatic or symptomatic, according to their responses to the Contact Lens Dry Eye Questionnaire. Similar amounts of material, assessed by absorbances at 210 and 280nm, adhered to contact lenses and impressions irrespective of dry eye symptoms (Kruskal-Wallis and Dunn post hoc tests, ns.). However, these absorbencies are significantly negatively correlated to PLBUT ($p<0.006$). All mucins described at the ocular surface could be detected, in different ratios in individual extractions. Dry eye symptoms could not be related to individual mucin species, i.e. MUC1, MUC2, MUC5AC, MUC5B or MUC7, presence or reaction intensity. MUC5B, a highly self-aggregating mucin, was positively correlated to LIPCOF ($p=0.006$) and LWE ($p=0.022$). MUC2 was more often undetectable in Schirmers of asymptomatics. **Conclusions** In soft contact lens wearers, dry eye symptoms could not be simply related to mucin coverage of the ocular surface. The correlation of MUC5B to ocular surface pathology and tendency to higher MUC2 in symptomatics suggest that the mucin-species composition of the preocular fluid reflects, and may influence, specific ocular symptoms and signs.

Tear "Omics": Application To Clinical Problems. Roger W. Beuerman,[1,2,3] Lei Zhou, [1,2] Singapore Eye Research Institute,[1] Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore,[2] School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore.[3]

Purpose. The tears are an extra-cellular fluid containing molecular and cellular information derived from the several sources that form the tears and signaling the cells of the ocular surface. The purpose of this study was to continue the proteomics approach to dry eye and to report our metabolomics approach to ocular surface inflammation and disease. Proteomics uncovers peptides and proteins while metabolomics reveals small molecules that are a reflection of the physiology of the cell. **Methods.** Tears were collected by either fire-polished micro-capillaries or Schirmer's strips. NanoLC-MS/MS was used to analyze the tear metabolites from both diseased and control samples. Principal component analysis (PCA) was used to detect patterns in tear metabolites. For proteomic analysis, iTRAQ combined with 2D-nanoLC-MS/MS was performed to determine quantitative changes of tear proteins. **Results.** Metabolomic analysis was used with tears from patients with climatic droplet dystrophy. PCA analysis of metabolomic profiles showed distinct patterns between CDK and control tears. Classification of CDK and controls was possible using this approach. The levels of tear metabolites with molecular weight of 109.0, 192.0, 335.1, 339.1, 343.2, 361.1, 362.2, 383.1, 384.1, 385.1, 395.1, 399.1, 412.2, 452.2, 456.2, and 481.1 showed significant differences between CDK and controls. Proteomics, using iTRAQ quantitation (relative to normal controls) showed that S100 A8 (2-fold), S100 A9 (2-fold), alpha-enolase (2.5-fold) and alpha-1-acid-glycoprotein 1 (10-fold) were up-regulated in tears of patients with dry eye, whereas, lactotransferrin (1.5-fold), lysozyme (1.8-fold), prolactin-inducible protein (2-fold) and antileukoproteinase 1 (1.5-fold) were down-regulated in tears of patients with dry eye. In total 10 proteins were differentially expressed between the dry eye group and normal control group with 6 proteins up-regulated and 4 proteins down-regulated in dry eye patients. **Conclusions.** Qualitative and quantitative tear "omics" are powerful techniques that expand the coverage of the human tear proteome and metabolome to discover biomarkers associated with eye diseases and providing insights into the pathogenesis of disease.
Supported by NMRC grants /0808/2003, /CPG/002/2003 and IBG.

Preliminary Phase IIb Clinical Trial Results Of Ecabet Sodium For The Treatment Of Dry Eye Syndrome. Ralph Bianca, James A. Gow, Timothy R. McNamara. Department of Clinical Research and Medical Affairs, ISTA Pharmaceuticals®, Inc., Irvine, CA, USA

Purpose. Mucin is a glycoprotein component of the tear film that lubricates while retarding moisture loss from tear evaporation. Ecabet sodium represents a new class of agent that increase the quantity of mucin produced by conjunctival goblet cells and corneal epithelia. Ecabet sodium is already marketed in Japan as an oral agent for the treatment of gastric ulcers and gastritis. In an earlier phase IIa ophthalmic clinical trial, the ecabet sodium treatment group demonstrated strong and positive trends for the relief of dry eye syndrome. The purpose of this phase IIb clinical trial was to confirm earlier observations of efficacy endpoints for potential use in phase III studies. **Methods.** A total of 112 eligible subjects were assigned randomly to receive either ophthalmic ecabet sodium or placebo QID for 90 days. There were 4 primary efficacy endpoints: 2 objective signs (blink rate and corneal staining) and 2 subjective symptoms [the subject's most bothersome symptom via a diary and the subject's response to the Allergan Ocular Surface Disease Index© (OSDI©)]. Subjects were evaluated pre- and post-exposure to a dry eye chamber on Day 1 and on Day 91. **Results.** The ecabet treatment group demonstrated strong trends toward efficacy for blink rate and OSDI, and a positive trend toward efficacy in the subjects' most bothersome symptom. While this study was not powered to show statistical significance, the ecabet treatment group demonstrated statistically greater reductions in OSDI. Furthermore, 14% of the ecabet group reported increased lacrimation, compared to 1.8% of the placebo group. No serious ocular adverse events were reported. **Conclusions.** Ecabet

sodium demonstrated trends toward efficacy for a sign and two symptoms of dry eye syndrome. Further analyses of the phase IIb clinical trial results are ongoing. This research was supported by ISTA Pharmaceuticals, Inc.

Effects Of An Emulsion And Low/High Viscosity Polymeric Formulations In A Short Term Dry-Eye Model. A.R. Blanco, V. Moschetti, V.Vitale and M.G.Mazzone Pharma Business Unit S.I.F.I. Spa Lavinaio (Catania), ITALY

Purpose. The aim of this study was to test the efficacy of same tear substitutes with different viscosity and composition in the prevention of the corneal damage induced by a simple short term dry eye model (Fujihara et al, JOPT 1995). **Methods**. 32 anesthetized New Zealand rabbits were divided in 4 groups of 8 animals each and treated (1 drop) with: 1) BSS; 2) 0.2% sodium hyaluronate (NaHa) eye drops (low viscosity formulation); 3) 0.15% NaHa/1% xanthan gum hydrogel (high viscosity formulation); 4) oil-in-water emulsion containing natural apolar triglycerides and phospholipids. Soon after treatment, rabbit's eyes were held open with a speculum for 3 hours to prevent blinking. Morphological alterations and cell damage of the conjunctiva and cornea were evaluated by impression cytology and methylene blue staining, respectively. Ferning test was also performed to assess the crystallization pattern of tears. A scoring system was used for all tests. **Results**. The stress evaporative model used herein produced only a slight morphological conjunctival alteration in all groups of animals. On the other hand, the corneal damage induced by was evident in BSS group; all other tested treatments were effective in preventing this injury ($p<0.001$ for emulsion, $p<0.05$ for the other groups, ANOVA). Moreover, samples of tears obtained from the BSS treated group lost the normal fern-like crystallization pattern and showed a type III/IV ferns typical of tear film alterations (Rolando et al., 1985). On the contrary, samples obtained from the other groups revealed a normal pattern (type I/II ferns), reaching a statistically significance for the emulsion ($p<0.05$, ANOVA) and eye gel ($p<0.01$). **Conclusions.** The data obtained in this experimental model of evaporative dry eye suggest that the lipid emulsion and the NaHa-xanthan gum based hydrogel are able to maintain a normal tear fern-like crystallization pattern and to prevent the epithelial corneal damage consequent to dryness. The low viscosity formulation containing NaHA could be useful in mild condition of ocular surface dryness.

Chemical Denervation Of Lacrimal Pump With Botulinum Toxin Type A For The Management Of Symptomatic Dry Eyes. K. G. Boboridis, D. Mikropoulos, N. S. Georgiadis. 1st Ophthalmology Department, Aristotle University of Thessaloniki.

Purpose. We present our experience with the use of botulinum toxin type A (Botox, Dysport) for the chemical denervation of lacrimal pump and management of dry eyes with the reduction of tear drainage. **Methods.** We have included 18 consecutive patients over a five month period with symptomatic dry eyes, mean Schirmer's test 6 mm, tear brake up time<5sec not amenable to topical lubricating medications. Three injections of 0,1 ml (4 IU Botox and 20 IU Dysport) were applied 0,5 mm superior to the upper and inferior to the lower lacrimal punctum and 1 cm inferolaterally to the lateral canthus. The patients were followed up the first, second week and every month. **Results.** The maximum toxin action was observed within the first 6 days with mean duration 10 weeks. Delayed lacrimal pump function was observed in every patient with improved mean Schirmer's test of 13 mm and subjective improvement of symptoms in 15/18 (83,3%) cases. There were no side effects or complications in the minimum follow up period of 12 months. No statistical significant difference between the two preparations of botulinum toxin was recorded. **Conclusions.** Chemical denervation of lacrimal pump with botulinum toxin results in significant tear retention and improvement of dry eye symptoms. It is a safe, fast and effective method with a mean duration of 10 weeks with a relatively high cost of botulinum toxin preparation.

Comparative Study Of Punctum Plugs Versus Acrylic Smart Plugs And Silicon Canalicular Plugs For The Management Of Dry Eyes. K.G. Boboridis, D. Mikropoulos, N. Ziakas, N.S. Georgiadis 1st Ophthalmology Department, Aristotle University of Thessaloniki, Greece.

Purpose. Lacrimal occlusion has become an established method of dry eye management. We present the results from a comparative study of punctum plugs versus acrylic lacrimal plugs. **Methods.** Retrospective study of 32 patients with aqueous tear deficiency managed with lower lacrimal occlusion over one year period (2005). Eleven patients (group A) had silicon punctum plugs whereas 15 patients (group B) had acrylic lacrimal plugs and 6 patients (group C) had canalicular plugs. In the minimum follow up period of 12 months we recorded the symptoms and signs of dry eyes, the effectiveness of occlusion and possible complications. **Results.** In group A were used plugs of 0,6 and 0,8 mm diameter after punctum dilatation. Mean increase of Schirmer's test was 9 mm and 8/11 (73%) patients reported subjective improvement after the second week. In 7/11 (63%) cases spontaneous loss of at least one plug was recorded within the first three months. Pyogenic granuloma of the punctum developed in 2 cases whereas further 3 had conjunctival irritation. In group _ no punctum dilatation was required, mean increase of Schirmer's test was 5 mm and 13/15 (86%) patients reported subjective improvement of symptoms. In group C the plug was inserted following punctum dilatation, mean increase of Schirmer's test was 7 mm and one case developed canalicular inflammation which was treated conservatively No further complications were recorded. **Conclusions.** Punctum plugs require size selection and offer complete occlusion of the lower system with low subjective improvement due to conjunctival irritation and spontaneous loss. On the contrary, the acrylic lacrimal plugs do not require punctum dilatation and achieve a high subjective improvement rate with minimal discomfort. Finally, the canalicular plugs offer the maximum occlusion with high patient satisfaction but are related to minor transient complications.

Management Of Symptomatic Evaporative Dry Eye Secondary To Meibomian Gland Dysfunction. K.G. Boboridis, D. Mikropoulos, A.G.P. Konstas, N.S. Georgiadis. 1st Ophthalmology Department, Aristotle University of Thessaloniki, Greece.

Purpose. Meibomian gland dysfunction with disturbance of the tear lipid layer may cause symptoms of evaporative dry eye. We present the results from the conservative management of symptomatic dry eye cases related to blepharitis. **Methods.** A retrospective study of 224 dry eye cases who presented in our outpatient oculoplastic service over a 2 year period (2003-2005) identified 117 patients with symptomatic dry eyes related to blepharitis. The management protocol included oral doxycycline and topical antibiotic ointment for a month, mild steroid eye drops for 2 weeks with intensive lid hygiene and lubricating treatment.
In the minimum observation of 6 months we've recorded the alterations of symptoms and signs of dry eyes, the effectiveness of treatment and possible complications. **Results.** Significant improvement of symptoms was reported by 103/117 (88%) cases at the end of the first month. The increase in mean Schirmer's test was 3 mm (from 11 to 14 mm) and for

tear brake up time was 4 sec (from 3 to 7 sec) at the same time with cure of the exposure keratopathy in all cases. Complete resolution of blepharitis was recorded in 89/117 (76%) cases whereas improvement only in the remaining. In 11 (9,4%) cases we've recorded mild stomach ache from oral doxycycline and in 3 (2,5%) cases topical allergic reaction to antibiotic ointment was noted. No other complications of intraocular pressure elevation were recorded. Finally, in the majority of cases there was a significant compliance problem with lid hygiene and topical lubricating medication. **Conclusions.** Blepharitis and Meibomian gland dysfunction is often misdiagnosed as a cause of evaporative dry eyes. Epiphora is the main misleading symptom of patients and the complex management requires persistence and duration. There is a significant compliance problem with lid hygiene and lubricating medication resulting in incomplete resolution of Meibomian gland dysfunction and frequent recurrence of symptoms.

Characterization Of Human Meibum In Relation To Age And Dry Eye Using Infrared Spectroscopy. Douglas Borchman, Gary N Foulks, Donghai V. Ho, Jonathan Mathews, Eric M. Schwietz. Department of Ophthalmology and Visual Science, University of Louisville, 301 E. Muhammad Ali Blvd., Louisville, KY 40202, USA

Purpose. Infrared spectroscopy was applied to quantify the molecular structure and conformation of lipid moieties in human meibum. **Methods.** Meibum was collected from 14 normal human subjects, 3 to 62 years of age, and from 20 subjects with evaporative dry eye symptoms, 8 to 87 years of age. Meibum lipid composition, conformation and structure were measured using Fourier transform infrared spectroscopy. Lipid phase transitions were described by a two state sigmodal equation consisting of four parameters: minimum order, magnitude of change, transition temperature and cooperativity. **Results.** The relative area of the infrared C=O, C=C and C-O bands increased by about 40% with age indicating lipid compositional changes. Concomitant, conformational, structural and thermodynamic changes with age were also evident. The frequency of the C=O band and hydrocarbon chain order at 34.4oC decreased and the C=O bandwidth increased and with age. Infrared spectra of meibum from subjects with evaporative dry eye symptoms were compared to meibum from age matched normal subjects. The intensity of the C=C and C=O bands were lower in 95% of the meibum samples collected from subjects with dry eye symptoms. Hydrocarbon chain order at 34.4oC was higher for 80% of the subjects with dry eye symptoms. **Conclusions.** Meibum lipid compositional changes with age and evaporative dry eye symptoms reflect changes in hydrocarbon chain conformation. This work highlights the power of infrared spectroscopy to characterize molecular structure/conformation, and packing of human meibum lipids and provides a basis for future study of tear film lipid composition-structure-function relationships and lipid-protein interactions in relation to age, sex and dry eye symptoms. The lipid carbonyl and C=C bands may be useful as markers for dry eye symptoms.
Commercial Relationships: None. Supported by: Supported by Public Health Service research grant EY017094, the Kentucky Lions Eye Foundation and an unrestricted grant from Research to Prevent Blindness Inc.

Mechanotransduction Of Hydration Of Pulmonary Surfaces Mediated By Extracellular ATP. Richard C. Boucher, M.D., Cystic Fibrosis/Pulmonary Research and Treatment Center The University of North Carolina at Chapel Hill, Chapel Hill, NC

Proper hydration is required for efficient mechanical clearance of mucus from pulmonary surfaces, a key component of innate defense of the lung. Hydration of airway surfaces is mediated by active ion transport processes located within pulmonary epithelia. Pulmonary epithelia are quite water permeable, so the volume of pulmonary surface liquid (i.e., 'hydration') is determined by the mass of NaCl transported onto pulmonary surfaces. The mass of salt on pulmonary surfaces reflects the balance between active Na^+ absorption vs. active Cl^- secretion. The major regulator of Na^+ absorption and Cl^- secretion on pulmonary surfaces is extracellular ATP. ATP interacts with luminal $P2Y_2$ receptors to inhibit Na^+ absorption and activate Cl^- secretion by distinct mechanisms. ATP is secreted onto pulmonary surfaces by both constitutive and regulated processes. In the absence of ATP (or its metabolite adenosine) on pulmonary surfaces, pulmonary epithelia absorb salt and water. Constitutive rates of ATP release are sufficient to hydrate pulmonary surfaces for basal activities. Regulation of ATP release, and hence hydration, is mediated by mechanical stresses imparted to pulmonary surfaces. These mechanical stresses include the airflow-dependent surface shear stress, transmural compression, and stretch associated with tidal breathing. This mechanotransduction mechanism allows pulmonary surfaces to maintain adequate hydration in response to a variety of stresses to the lung. In diseases characterized by inadequate surface hydration, e.g., cystic fibrosis, pharmacologic modulation of the ATP-$P2Y_2$-R system appears therapeutically beneficial.

Detection Of Surfactant Proteins A, B, C And D In The Human Lacrimal System And In Tear Fluid. Lars Bräuer,[1] Christian Kindler,[1] Kristin Jäger,[1] Saadettin Sel,[2] Bernhard Nölle,[3] Uwe Pleyer,[4] Matthias Ochs,[5] Friedrich P. Paulsen[1], [1]Department of Anatomy and Cell Biology and [2]Department of Ophthalmology, Martin Luther University of Halle-Wittenberg, Germany; [3]Department of Ophthalmology, Christian Albrecht University Kiel, Germany, [4]Department of Ophthalmology, Charite, Berlin, [5]Department of Anatomy, University of Bern, Switzerland

Purpose. To evaluate the expression and presence of the surfactant proteins (SP) A, B, C and D in the lacrimal apparatus, at the ocular surface and in tears in healthy and pathologic states. **Methods.** Expression of mRNA for SP-A, -B, -C and -D was analyzed by RT-PCR in healthy lacrimal gland, conjunctiva, cornea and nasolacrimal ducts as well as in immortalized conjunctival and corneal epithelial cell lines. Deposition of all surfactant proteins was determined by Western blot, dot blot and immunohistochemistry in healthy tissues, in tears, aqueous humor as well as in sections of different corneal pathologies. **Results.** The presence of SP-A, -B, -C and -D on mRNA and protein level was evidenced in healthy lacrimal gland, conjunctiva, cornea and nasolacrimal duct samples. Moreover, all proteins were present in tears but were absent in aqueous humor. Immunohistochemistry revealed production of the four peptides by acinar epithelial cells of the lacrimal gland as well as epithelial cells of the conjunctiva and nasolacrimal ducts. Healthy cornea revealed weak reactivity on epithelial surface cells only. In contrast, SP-A and SP-D (but not SP-B and -C) revealed strong reactivity in cases of herpetic keratitis and corneal ulceration. Reactivity in corneal epithelium and endothelium was also seen in cases of keratoconus. Cell culture experiments revealed that SP-A and SP-D are produced by both epithelial cell lines without and after stimulation with cytokines and bacterial components. **Conclusions.** Our results show that, in addition to SP-D, SP-A, SP-B and SP-C are peptides of the tear film. Based on the known direct and indirect antimicrobial effects of collectins, the surfactant-associated proteins A and D seem to be involved in several ocular surface diseases.

Computing Tear Film Dynamics: Blinkcycles, Evaporation, Reflex Tearing And Dewetting. R.J. Braun,[1] K.L. Maki,[1] T.A. Driscoll,[1] L.P. Cook,[1] and P.E. King-Smith.[2] Department of Mathematical Sciences, University of Delaware, Newark, DE 19716-2553 USA.[1] College of Optometry, The Ohio State University, Columbus, OH 43210-1280 USA.[2]

Purpose. To solve mathematical models for tear film evolution over multiple blink cycles and a variety of conditions. **Methods**. A mathematical model is derived and solved using new numerical methods for this problem (a moving overset grid method). Surface tension, viscosity, evaporation, slip, gravity and van der Waals effects are included. In the limit of a very strong insoluble (the uniform stretching limit), the model results in a single partial differential equation for the tear film thickness. The film thickness depends on one space dimension and time; fluxes through the ends model tear film supply (from exposing a pre-existing film and the lacrimal gland) and drainage (through the puncta). Appropriate van der Waals forces are used to mimic dewetting; tear film break up is mimicked by a small submicron tear film thickness below which the tear film cannot go. **Results**. Successfully solving for tear film evolution over multiple blink cycles is an advance in modeling the tear film. To this capability we add physiological effects that are seen in eyes. Evaporation may be important in thin regions of the tear film. Reflex tearing is approximated by an increased influx from the film end corresponding to the upper lid; the results are compared with *in vivo* observation (P.E. King-Smith et al, IOVS (2000) 41(11):3348-59). Gravity aids the addition of tears through reflex tearing. Furthermore, the inclusion of van der Waals forces is required to qualitatively capture the evolution of dry spots as observed in dewetting. **Conclusions**. Suitable tear supply qualitatively models increased tear supply for reflex tearing. Evaporation may have an important effect in thin regions of the film.
[The authors thank Dr. M. Doane for helpful conversations.
This work is supported by a grant from the NSF Division of Mathematical and Physical Sciences' Program in Mathematical Biology.]

Modeling Tear Film Evolution During Multiple Blink Cycles And Partial Blinks. R.J. Braun,[1] A. Heryudono,[1] T.A. Driscoll,[1] K.L. Maki,[1] L.P. Cook,[1] and P.E. King-Smith.[2] Department of Mathematical Sciences, University of Delaware, Newark, DE 19716-2553 USA.[1] College of Optometry, The Ohio State University, Columbus, OH 43210-1280 USA.[2]

Purpose. To solve mathematical models for tear film evolution over multiple blink cycles and for partial blinks and to help identify necessary physical and chemical effects for tear dynamics in half blinks. **Methods**. A mathematical model is derived and solved using new modified spectral collocation methods for this problem. Surface tension, viscosity, and slip at the corneal surface are included. Two limits of the effect of an insoluble surfactant are considered and both result in a single partial differential equation for the tear film thickness. In one limit, the surfactant is absent (stress-free limit); in the other, the effect of the insoluble surfactant is very strong (uniform stretching limit). The film thickness depends on one space dimension and time; fluxes through the ends model tear film supply (from exposing a pre-existing film and the lacrimal gland) and drainage (through the puncta). **Results**. Successfully solving for tear film evolution over multiple blink cycles is an advance in modeling the tear film, which allows us to study both full and partial blinks over multiple blink cycles. Good comparison is found between measured *in vivo* tear film thicknesses after a half blink and the computational results from our model. **Conclusions**. Appropriate choices of the fluxes from the ends, together with viscous and surface tension effects, result in good agreement with *in vivo* measurements. The uniform stretching limit appears to best fit the experimental results. Fluxes from the lacrimal gland and puncta appear to be necessary to match the tear film thickness from a half blink in this model; fluxes used by previous researchers solely from exposing a pre-existing film appear to be insufficient to match experimental tear film profiles for a half blink. [The authors thank Dr. M. Doane for helpful conversations.
This work is supported by the NSF Division of Mathematical and Physical Sciences' Program in Mathematical Biology.]

Dryness And End-Of-Day Comfort With Silicone-Hydrogel Contact Lenses. Noel A Brennan, Chantal M-L Coles, Heather RM Connor, Robert G McIlroy. Brennan Consultants, Melbourne, Australia.

Purpose. There is a perception that silicone-hydrogel lenses lead to less dryness and better end-of-day comfort than traditional hydrogels. Here we examine this perception by comparing various silicone-hydrogel lenses and traditional hydrogel lenses for their effects on symptoms of dryness and end-of-day comfort.
Method. This investigation was a retrospective analysis of 586 separate wearing trials of 1- month duration. A total of 290 subjects were involved. Lens types comprised balafilcon A (S1: N=94), comfilcon A (S2: N=53), etafilcon A (H1: N=137), hioxyfilcon A (H2: N=35), lotrafilcon B (S3: N=121), omafilcon A (H3:N=37), polymacon (H4: N=31), and senofilcon A (S4: N=78). Subjects scored initial comfort, and 1-month comfort, dryness, and end-of-day comfort on 100-point visual analog scales. **Results**. There was a high correlation between initial comfort and the 1-month dryness ($r = 0.48$) and end-of-day comfort ($r = 0.45$) scores. All lenses showed 1-month end-of-day comfort was significantly ($p < 0.05$) decreased compared to average comfort except for S4. Median (10-90th percentiles) 1-mnth dryness scores for the various lenses were as follows: H1 93.5 (68.6-98.7), H2 93.2 (81.1-98.7), H3 89.5 (68.5-99.5), H4 85.5 (55.0-98.7), S1 88.2 (52.6-97.4), S2 89.0 (74.0-98.0), S3 90.4 (69.1-98.8), S4 94.0 (75.0-99.0). Median (10-90th percentiles) 1-mnth end-of-day comfort scores for the various lenses were as follows: H1 91.0 (64.3-98.7), H2 89.3 (69.6-94.7), H3 89.0 (64.2-96.8), H4 76.3 (23.4-96.8), S1 83.1 (48.8-97.4), S2 90.0 (60.6-98.0), S3 87.5 (49.7-99.0), S4 94.0 (68.5-99.0). **Conclusions**. There was considerable overlap between the comfort scores generated for the silicone-hydrogel lenses and the traditional hydrogel lenses. It is apparent that factors other than oxygen transmissibility have a predominant effect on dryness and end-of-day comfort symptoms during contact lens wear.

Tear Hyperosmolarity As The Initiator Of Ocular Surface Damage In Dry Eye. Anthony J. Bron[1], Eamonn A. Gaffney[2] and John M. Tiffany[1] Nuffield Laboratory of Ophthalmology [1] and Mathematical Institute, University of Oxford, U.K.

Purpose. To explore factors influencing expression and effect of hyperosmolarity at the ocular surface in dry eye. **Methods.** Literature review and mathematical modelling. **Results.** Compartmental factors could influence the distribution of tear osmolarity at the ocular surface, including: a differential osmolarity between tear film and meniscus (with a higher value in the film), regional differences in air flow and surface temperature and variations in ambient temperature and humidity. Other factors include imperfect tear mixing, differential corneal and conjunctival exposure and individual differences in reflex tear flow, evaporation rate and blink interval. We hypothesise that interactions of these factors determine the dry eye phenotype.

This gives rise to different expectations in aqueous deficient dry eye (ADDE) and evaporative dry eye (EDE). In ADDE, lacrimal gland

failure leads to a condition of low volume hyperosmolarity but retention of reflex tearing in EDE predicts a high volume hyperosmolar state. This could influence the clinical outcome.

This background is also relevant to LASIK 'dry eye'. Waking tear flow is driven reflexly by afferent impulses from ocular and nasal mucosae. The ocular component arises chiefly from the exposed ocular surface, since daytime tear flow is only established on eye opening. The loss of reflex drive caused by refractive surgical denervation, is likely to depend on the size, location and completeness of the central corneal incision, relative innervation densities across the exposed ocular surface and the level of spontaneous firing of injured and recovering corneal nerves. This will influence lacrimal secretion, blinking, symptoms and the balance of drying and trophic factors leading to a dry eye or a neurotrophic phenotype. **Conclusions**. Hyperosmolarity at the ocular surface is affected by multiple factors, each of which may make different contributions to risk in a given individual. This may determine the frequency, phenotype and severity of dry eye in a population.
Disclosure. A.Bron, I, P; E Gaffney, N; J Tiffany, N.

Lipids Of Human Meibum Revisited – Historical Survey And Recent Developments. Igor A. Butovich and James P. McCulley. UTSouthwestern Medical Center, Dallas, TX, USA

Purpose. The purpose of this study was to conduct a critical, in-depth analysis of previously published information on the lipids of human meibomian gland secretions (MGS) and to provide insight on newly emerging data pertinent to the field. It is believed that the lipids of MGS play a critical role in the formation and functioning of the tear film lipid layer (TFLL). In multiple studies, MGS lipid deficiencies and abnormalities were linked to various forms of dry eye. Therefore, lipidomic analysis of TFLL can provide vital information on the development and progress of dry eye, as well as mechanisms underlying the disease(s). The report is concerned primarily with meibum from healthy subjects. **Methods.** Ten samples of normal MGS (5 males, 5 females, median age 34 years, average sample size 0.5 mg, dry weight,) were quantitatively and qualitatively characterized using normal phase high-performance liquid chromatography (NP HPLC) and atmospheric pressure ionization ion trap mass spectrometry (API MS^n). **Results.** The major lipid species in MGS were found to be oleic acid-based wax esters, cholesteryl esters, and triacylglycerols. A minor amount of free cholesterol and oleamide (less than 0.5 and 0.1%, respectively; w/w, dry weight) were detected in MGS. No appreciable amounts of diacylglycerols or ceramides were found in MGS. NP HPLC and API MS^n methods used in the study were capable of resolving and analyzing model mixtures of authentic phospholipids (e.g. phosphatidylglycerol, phosphatidylethanolamine, phosphatidic acid, phosphatidylinositol, phosphatidylserine, phosphatidylcholine, and sphingomyelin) at nanogram levels, but showed little or no presence of these species in the MGS samples. Phosphocholine-based lipids were found in MGS in quantities less than 0.01% (w/w, dry weight), if any. **Conclusions.** These observations suggest that MGS are a major source of the nonpolar lipids of the wax esters and cholesteryl esters families for the tear film lipid layer (TFLL), but not of the previously reported phospholipid, ceramide, and oleamide components of the TFLL. The impact of these findings on the current models of TFLL and dry eye will be discussed.
Acknowledgement. The project was supported by an unrestricted grant from the Research to Prevent Blindness, Inc. (New York, NY, USA).

Tear Flow And Muc16 Expression In Sjögren's Syndrome, KCS And Normals. Barbary Caffery[1], Elizabeth Joyce[1], Miriam L Heynen[1], Robert Ritter III[2], Lyndon Jones[1], Trefford Simpson[1], Allan Slomovic[3], Daniel A. Gamache[2], Michelle Senchyna[2]. [1]Center for Contact Lens Research, School of Optometry, University of Waterloo, Ontario, Canada, [2]Alcon Research Ltd, Fort Worth, Texas, USA. [3]Toronto Western Hospital, Toronto, Ontario, Canada.

Purpose. To investigate the relationship between tear flow and the expression of MUC16 protein and mRNA from non-dry eyed subjects (NORM) compared to two distinct groups of dry eyed subjects: those with keratoconjunctivitis sicca (KCS) and those with Primary Sjögren's Syndrome (SS). **Methods.** 76 subjects were recruited for this study: 25 SS (confirmed via American-European Consensus Criteria 2002); 25 KCS (confirmed by symptoms and Schirmer scores ≤ 10mm) and 26 NORM. Tear flow was measured by the Schirmer test without anesthesia for 5 min. Tears were collected by saline eye wash. Protein and RNA were isolated from conjunctival epithelial cells collected via impression cytology. Soluble and membrane bound MUC16 were quantified via Western blotting, using a standard curve of MUC16 standard (CA125, Biodesign) for quantitation. MUC16 mRNA was quantified by relative real time PCR. Data was analyzed by ANOVA, Dunnett's two-tailed comparison of means and linear regression (α = 0.05). **Results.** The SS group expressed significantly increased soluble MUC16 protein and MUC16 mRNA compared to both KCS and NORM groups ($p < 0.05$). No difference between KCS and NORM groups was found ($p>0.05$). No difference between membrane bound MUC16 was found between any group ($p>0.05$). No significant correlation was found between mean Schirmer values compared with any measure of MUC16 expression. **Conclusions.** Our data demonstrate that only the SS subjects displayed a significant increase in both soluble MUC16 and MUC16 mRNA expression. No correlation was found between tear flow and MUC16 expression. Further research is required to investigate the potential use of biomarkers such as MUC16 in the characterization of dry eye disease.
Funded by Alcon Research Ltd.

Corneal And Intraocular Inflammatory Responses To Endophthalmitis In Toll-Like Receptor-Deficient Mice. M.C. Callegan[1-3], B.D. Novosad[2], R.T. Ramadan[3]. Departments of Ophthalmology[1] and Microbiology/Immunology[2], and Oklahoma Center for Neuroscience[3], University of Oklahoma Health Sciences Center and Dean A. McGee Eye Institute, Oklahoma City OK, USA.

Purpose. Recognition of bacteria during the acute stage of infection is critical in mounting an effective immune response. One of the hallmarks of *Bacillus* endophthalmitis is the corneal ring abscess of inflammatory cells infiltrating into the cornea in response to infection. Because *Bacillus* posesses known ligands for recognition and inflammation via toll-like receptor (TLR)-mediated mechanisms, we analyzed whether TLR deficiencies altered the corneal and overall inflammatory response to *Bacillus* infection of the posterior segment. **Methods.** 100 colony forming units (cfu) of *B. cereus* were injected into the midvitreous of wild type C57BL6/J, homozygous $TLR2^{-/-}$, or homozygous $TLR4^{-/-}$ knockout mice. Infections were analyzed by bacterial quantitation and histology ($N \geq 4$ eyes per assay per time point, mean ± SEM). **Results.** Corneal ring abscess formation and anterior segment inflammation in $TLR2^{-/-}$ and $TLR4^{-/-}$ knockout mice were delayed compared to that of wild type mice, a result reflective of the overall evolution of inflammation in each strain of mouse. The initial response in $TLR2^{-/-}$ and $TLR4^{-/-}$ knockout eyes was one of albumin infiltration only. Although bacteria grew similarly overall in eyes of wild type, $TLR2^{-/-}$, and $TLR4^{-/-}$ knockout mice, no bacteria were observed in the anterior segment or corneas of $TLR2^{-/-}$ and $TLR4^{-/-}$ knockout mice. **Conclusions.** The results suggested that acute cellular responses to *Bacillus* endophthalmitis were altered by loss of TLR2 or

TLR4 during infection, indicating an important role for these receptors in inflammation.
[The authors thank Eric Pearlman (Case Western, Cleveland OH) for the TLR2$^{-/-}$ and TLR4$^{-/-}$ knockout mice and Paula Pierce (Excalibur Pathology, OKC) for histology. This research was supported in part by grants from National Institutes of Health (R01EY12985) and Research to Prevent Blindness (Lew R. Wasserman Award).]

Calcium And Cyclic Amp Alterations In Purinergic Regulation Of Rabbit Lacrimal Gland Acinar Cell Secretion. Stina K. Carlsson[1], Maria C. Edman[1], Sarah Hamm-Alvarez[2], J. Peter Gierow[1]. University of Kalmar, Kalmar, Sweden[1] and University of Southern California, Los Angeles, CA, USA[2].

Purpose. We have previously shown that rabbit lacrimal gland acinar cells increase their secretion when stimulated with adenosine, or agonists of the A1 and the A2 receptors (Gierow et al., Invest. Ophthalmol. Vis. Sci. 47, E1943, 2006). To further characterize the signaling transduction by which this increase occurs, the levels of Ca2+ and cAMP was measured. **Methods.** Rabbit lacrimal gland acinar cells were isolated from rabbits and cultured according to our standard procedure (Andersson et al., Exp. Eye Res. 83, 543-553, 2006). For Ca2+ measurement, cells were cultured on 35 mm glass-bottomed, live cell dishes and loaded with the fluorophore Ca-green. Ca2+ fluxes were monitored with a cell culture equipped LSM 510 Meta microscope. For cAMP measurement, cells were cultured for 2 days on Matrigel-covered plates, preincubated and incubated in absence or presence of secretagogues. Cells were then lysed and cAMP levels were measured (Parameter cAMP Assay, R & D Systems). **Results.** The calcium measurements after stimulation with adenosine revealed a broader but less prominent peak than that obtained when cells were stimulated with carbachol. When cells were stimulated with both adenosine and carbachol, the peak appeared more prolonged than for both adenosine and carbachol alone, and higher than for adenosine alone. The A1 receptor agonist CPA (cyclopropyladenosine) and the A2 receptor agonist CPCA (cyclopropylcarboxamidoadenosine) gave shorter responses than adenosine but with higher peaks. The effects of both receptor specific agonists were abolished by respective antagonists. Measurements of cAMP have revealed small changes in concentration after stimulation with adenosine and adenosine receptor agonists. **Conclusions.** Ca2+ appears to have a key role in cellular signaling transduction after adenosine receptor activation, while the role of cAMP appears to be less important.
Support: University of Kalmar Faculty Research Grant, Karin Sandqvists Foundation, and Crown Princess Margareta's Eye Research Fund. No commercial relationships.

Influencing Factors Of Tear Film Deposition On Silicone Hydrogel Lenses: Clinical Relevance. FP Carney, WL Nash, C Amos, CH Wang, KB Sentell. CIBA Vision Corporation, Duluth, Georgia, USA

Purpose: To investigate the major drivers of protein and lipid deposition on 4 silicone hydrogel lens types and their potential clinical implications. **Methods:** 80 patients randomized into 4 groups wore either lotrafilcon B, galyfilcon A, senofilcon A or balafilcon A, for 2 weeks daily wear using ClearCare®. 20 lenses from each group were extracted for lipid using a chloroform : methanol (1:1) extraction and cholesterol quantified by a commercially available assay (Cayman). 10 lenses from each group were extracted for total protein using acetonitrile : trifluoroacetic acid: water (499:1:500) mixture, followed by a BCA assay. Data was analyzed for correlation of deposition with clinical variables and lens material type. The normal range of deposition was determined by the average of all lens types combined, plus or minus the standard deviation. Above this range was considered a 'high' depositor and below a 'low' depositor. All data was expressed in µg/lens. **Results:** Total protein of all groups combined exhibited a normal range of 6.52 – 90.74 µg/lens. Balafilcon A showed 'high' protein deposition compared to all other lenses (110.1±26.1; $p<0.05$). There was no statistical difference between the remaining 3 lens types although a large patient to patient variability was seen. No correlations to any clinically observed symptom was found; however, this may be due to the short term nature of this study. Cholesterol testing showed, lotrafilcon B exhibited lower adsorption (2.04±1.4; $p<0.05$) and no statistical difference between balafilcon A (3.1±1.2), galyfilcon A (3.83±1.6) and senofilcon A (3.1±1.8). Combining all cholesterol data resulted in an average of 3.03±1.62 with above 4.65 or below 1.41 µg/lens being either a 'high' or 'low' depositor respectively. A tight correlation of 'high' depositors to decreased visual acuity ($p<0.05$) was observed for cholesterol. No other correlations to lipid were seen. **Conclusions:** Lipid deposition above 4.66 µg/lens resulted in a clinical impact on visual acuity, however, no short term clinical impact from high levels of protein was found. High variation in protein adsorption within a specific lens type and between lens types indicates both the patient and material are both dominant factors in deposition. Lens material and tear/surface interfaces appear to be a greater driving force for lipid deposition.

Solution Related Chemical Staining Case Control Study In Silicone Hydrogel Daily Wear. Nicole Carnt[1,2], Vicki Evans[1,2], Thomas Naduvilath[1,2], Varghese Thomas[1,2], Mark Willcox[1,2,3], Brien Holden[1,2,3], Institute for Eye Research[1], Vision Cooperative Research Centre[2], School of Optometry and Vision Science, University of New South Wales[3], Sydney, Australia

Purpose. Contact lens solution related chemical staining (CS) is known to peak between 2-4 hours of lens insertion, and depends on the lens/solution combination. We aim to determine additional patient and lens related factors associated with CS in silicone hydrogel (SiH) daily wear (DW). **Methods.** 371 subjects that wore one of a range of commercially available SiH lenses bilaterally with overnight disinfection with one of five commercially available solutions with fortnightly or monthly disposal for up to three months were analysed retrospectively. 83 (22%) subjects experiencing CS were compared to 288 controls. **Results.** A higher proportion of cases had higher levels of palpebral redness ($p=0.007$) and greater discrete front surface deposits ($p=0.032$) at dispensing compared to controls. During lens wear, cases exhibited poorer lens wetting ($p=0.001$), higher front surface haze ($p=0.001$), higher back surface debris/deposits (<0.001) and presented for evaluation closer to the time of insertion compared to controls. This surface effect during lens wear was found for the cases at the time of event compared to controls ($p<0.05$) but not compared to other visits for the cases. Lens wear time at evaluation was lower for cases at the event compared to the controls ($p<0.001$) and other visits ($p=0.001$), however, there was no difference in the proportion of subjects that had at least one morning visit in the case and control groups ($p=0.560$). When CS was present, bulbar redness was higher compared to other visits ($p=0.022$). **Conclusion.** Subjects with CS exhibited poorer wetting and more deposited lenses and were more likely to experience associated bulbar redness in the first few hours after lens insertion in SiH DW. Previous higher levels of palpebral redness may also be associated with CS.
[This work was supported by the Australian Government through the Cooperative Research Centres programme, Institute for Eye Research and CIBA Vision.]

Dry Eye In Congenital Aniridia. Gonzalo Carracedo [1], Assumpta Peral [1] Jesús Pintor [2]. Dept. Optica II (Optometría y Visión) [1], Dept. Bioquímica y Biología Molecular IV [2], E.U. Optica (Universidad Complutense), Madrid, Spain.

Purpose. To analyze the progression of dry eye in patients with congenital aniridia. **Methods.** Fifteen subjects, diagnosed with congenital aniridia, participated in the present study after filling the McMonnies questionnaire. The tear secretion was measured and collected by Schirmer I test. The strips were placed in Eppendorf tubes containing 500 µL of ultrapure water. All the tear samples were processed by High Pressure Liquid Chromatography (H.P.L.C.). The mobile phase consisted of 10 mM KH_2PO_4, 2mM tetrabutyl ammonium, 15 % acetonitrile, pH, 7.5. with a NovaPak C18 column. The control group were 37 subjects without symptoms and a normal values of Schirmer test and BUT. **Results.** The values of Schirmer I test for patients with congenital aniridia under 10 years old were 20.6 ± 9.4 mm, 12.6 ± 9.3 mm for patients between 10 and 25 years old and 13.6 ± 8.3 mm for the patients between 25 and 50 years of age. Only a patient was older than 50 years old, this patient presenting Schirmer values of 1,25 mm. The values for Mcmonnies test were 2.8 ± 2.4, 3.5 ± 3.5, 10.83 ± 4.2 and 14 for the same groups respectively. The concentrations observed for diadenosine tetraphosphate were 11.8 ± 2.3 µM and 21.5 ± 1.7µM, 29.5 ± 1.8 µM and 196.1 ± 3.3 µM, for each group. Control patients presented values of diadenosine tetraphosphate of 0.107 ± 0.048 µM.
Conclusions. The present work demonstrates that the patients with aniridia develop eye dryness which severity increases with aging.
This work has been supported by Ocupharm Diagnostics S.L.

Levels Of Diadenosine Polyphosphates In Sjögren Syndrome.
Gonzalo Carracedo, [1]Assumpta Peral [1], Jesús Pintor [2]. Dept. Optica II (Optometría y Visión) [1], Dept. Bioquímica y Biología Molecular IV[2], E.U. Optica (Universidad Complutense), Madrid, Spain.

Purpose. To analyse the levels of diadenosine polyphosphates -Ap_4A and Ap_5A- in tears of subjects with Sjogren Syndrome with a control group. **Methods.** Nine subjects, diagnosed with Sjögren Syndrome, participating in the present study were invited to fill the Dry Eye Questionaire (DEQ). The tear secretion was measured and collected by Schirmer I test. The strips were placed in Eppendorf tubes containing 500 µL of ultrapure water and submitted to Sep-Pak Accell QMA chromatography. All the tear samples were then processed by High Pressure Liquid Chromatography (H.P.L.C.). The system was equilibrated with a mobile phase consisting of 10 mM KH_2PO_4, 2mM tetrabutyl ammonium, 15 % acetonitrile, pH, 7.5. with a NovaPak C18 column. The control group were 37 subjects without symptoms and a normal values of Schirmer test and BUT. **Results.** The values of The Schirmer I test for patients with Sjögren Syndrome were 4.12 ± 3.90 mm**.** DEQ shows that discomfort, dryness and light sensitivity are the most important symptoms the patients report. The patients were organised in two groups, those individuals presenting normal tear production and those presenting low tear production. When the concentrations of diadenosine polyphosphates, diadenosine tetraphosphate, Ap_4A, and diadenosine pentaphosphate, Ap_5A, were measured in normal tear production patients, the concentrations observed for Ap_4A and Ap_5A were 0.42 ± 0.01 µM and 6.21 ± 3.07 µM respectively. Those patients presenting Sjögren Syndrome and low tear production presented Ap_4A and Ap_5A concentrations of 4.57 ± 1.20 µM and 44.53 ± 8.69 µM, respectively. Control individuals presented concentrations of Ap_4A of 0.107 ± 0.048 µM and of 0.036 ± 0.005 µM for Ap_5A. **Conclusions.** As occurs with patients diagnosed with dry eye, Sjögren Syndrome patients also present abnormally elevated concentrations of diadenosine polyphosphates indicating that these compounds could be used for the diagnose of this pathology.
This work has been supported by Ocupharm Diagnostics, S.L.

Tgf-Beta Regulation Of MUC4 In Corneal Epithelial Cells. Kermit L. Carraway, Joseph Lomako, Wieslawa M. Lomako, Coralie A. Carothers Carraway. University of Miami School of Medicine, Miami, FL, USA

Purpose. The membrane mucin Muc4 is found only in the most superficial layers of the rat cornea even though its transcript is expressed in all layers. These observations suggest a post-translational mechanism for regulation of corneal Muc4. A similar mechanism in the mammary gland was found to block the proteolytic processing of precursor Muc4 to its heterodimeric mature form. One explanation for this mechanism is that the repression of the processing prevents proper folding of the Muc4 and shunts it into the proteosomal degradation pathway. **Methods.** Rat corneal epithelial stratified cell cultures were grown as previously described. Cells were treated with TGF-beta, proteosome inhibitors, and glycosylation inhibitors, then processed for confocal microscopy or solubilized for SDS-PAGE. Ubiquitination was analyzed by sequential immunoprecipitation and immunoblotting with anti-Muc4 and anti-ubiquitin. **Results.** Treatment of rat corneal epithelial layers with TGF-beta reduced their levels of Muc4, an effect which could be partially prevented by proteosomal inhibitors. Proteosome inhibitors could also increase levels of Muc4 without TGF-beta treatment. This inhibition resulted in intracellular accumulation of Muc4 in basal and medial cells of the stratified layers, which was not present in untreated cultures. The glycosylation inhibitor kifunensin, which inhibits degradation through the proteosome pathway, also increased the intracellular and total levels of Muc4. Ubiquitinated Muc4 was shown to be increased by the proteosome inhibitors, further supporting a role for proteosome degradation. **Conclusions.** These findings indicate that Muc4 is regulated in the corneal epithelium through TGF-beta via proteosome degradation to prevent its expression at the cell surface of the basal and medial cell layers. This mode of regulation is consistent with recent studies showing TGF-beta and its receptors primarily in the more basal layers of this epithelium.
No commercial relationships.
This work was supported by National Institutes of Health grant EY12343.

Clinical Experience Of Managing Ectasia After Lasik Surgery: Taiwanese Experiences. David Chaokai Chang MD PhD, Taiwan Nobel Eye Institute, 4F, 179, Section 1, Dunhua South Road, Taipei, Taiwan,10690

Purpose. To report one case experience with managing keratectasia following laser refractive surgery with RGP contact lens; we also report another case of a mistaken diagnosis of keratoconus caused by corneal warpage secondary to hydrogel lens wear. **Method**. We retrospectively reviewed the chart of the first 35 years old patient who underwent RGP contact lens fitting for LASIK induced ectasia (OD only) with at least one year follow up. The Main outcome measures were: preoperation refraction , topographic characteristics, residual stromal bed thickness, time to develop ectasia, final UCVA and BSCVA. The second 26-year-old Chinese woman who wore hydrogel lens presented with an interest in refractive surgery. Topographies and pachymetries were performed, keratoconus was diagnosed in her right eye, and suspect keratoconus was diagnosed in her left eye. **Results.** UCVA, BSCVA and topographic flattens and regularity improved in first patient's right eye. Minimal 12 months clinical data follow up will be shown. As to second

patient, eight weeks after initial presentation and without lens wear, corneal maps were again performed. The maps showed no evidence of keratoconus. **Conclusion.** Our first patient experience indicates that RGP contact lens fitting may be an effective and safe procedure for Keratectasia following laser refractive surgery. However, second case gives clinicians reasons for pause when dealing with contact lens wearers presenting with corneal curvature irregularities such as keratoconus or ectasia, because of the possibility of lens-induced warpage.

The Role Of Rab27b In The Regulation Of Intracellular Trafficking In The Lacrimal Gland. Lilian Chiang,[1] Kaijin Wu,[1] Francie Yarber,[1] Serhan Karver,[2] Sarah F. Hamm-Alvarez.[1,2] School of Pharmacy,[1] Keck School of Medicine,[2] University of Southern California, Los Angeles CA, USA.

Purpose. We have shown previously that Rab27, a small GTPase implicated in exocytosis in other cells, is expressed in the lacrimal gland. The purpose of this study is to elucidate the mechanism of Rab27b's participation in the formation and release of secretory vesicles (SVs) which supply tear proteins into ocular surface fluid. **Methods.** Rab27b function was examined by adenoviral-mediated expression in cultured rabbit lacrimal gland acinar cells (LGAC) of wild-type (WT) and mutant Rab27b (constitutively active, CA; dominant negative DN) with Xpress-epitope tags, to study release of secretory proteins, or YFP, to track by live-cell fluorescent LSCM. Some analyses were performed in LGAC co-transduced with SV marker, syncollin-GFP (sync), and stimulated with carbachol (CCH). **Results.** Transduction with either Xp- or YFP-tagged Rab27b confirmed previous data showing its co-localization with SV markers such as Rab3D and MyosinVc. Secretion data measuring release in LGAC expressing Xp-tagged Rab27b constructs and co-transduced with sync suggested that WT did not significantly influence basal or CCH-stim secretion (n=5). Although CCH-stim sync release was not altered in either CA or DN, CA significantly ($p \leq 0.05$) increased basal release 62±9% by 5min after CCH, suggesting constitutive activity of Rab27b mediated SV exocytosis. To verify this, effects of CCH on YFP-tagged WT and CA Rab27b in live LGAC were characterized by LSCM: subapical WT SV diameter averaged 0.75±0.03μm before and 0.82±0.05μm after CCH (n=3), consistent with some stimulated compound fusion prior to exocytosis. Expression of CA however yielded marked increases to 1.23±0.10μm before and 1.38±0.12μm after CCH (n=3), suggesting enhanced fusion in resting as well as CCH-stim LGAC. **Conclusions.** Expression of CA Rab27b enhances CCH-independent exocytosis while promoting increases in vesicle diameter in resting acini consistent with compound fusion. Altogether, these data implicate Rab27b in the regulation of SV fusion in response to secretagogue stimulation.
[Support: NIH EY011386]

Corneal Stroma And The Innate Immune Response To Adenovirus Infection. James Chodosh, Ashish V. Chintakuntlawar. Molecular Pathogenesis of Eye Infection Research Center, Dean McGee Eye Institute, Departments of Ophthalmology, Cell Biology, Microbiology & Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA.

Purpose. Resident tissue fibroblast capacity to contribute to innate immune responses remains poorly characterized. Keratocytes within the corneal stroma express fibroblast markers during inflammation. The C57BL/6J mouse model of human adenovirus type 37 (Ad37) keratitis permits the study of innate immune responses by fibroblasts in response to infection. **Methods.** C57BL/6J wild type or toll-like receptor 9 knock out (TLR9-/-) mice were infected by corneal injection with wild-type, uv-inactivated, or heat-inactivated Ad37, or virus-free buffer, or injected with chemical inhibitors of Src (PP2) or ERK1/2 kinase (U0126) prior to infection. Clinical signs of infection, expression of chemokines CXCL1 (KC) and CCL2 (MCP-1), myeloperoxidase content, and histopathology were analyzed to 4 days post-infection. **Results.** Ad37 and uv-inactivated Ad37 induced comparable levels of corneal inflammation in C57BL/6J mice as determined by chemokine expression, immune cell infiltration, and clinical opacity. In comparison, neither virus-free buffer or heat-inactivated Ad37 induced keratitis. Ad37 infection induced an inflammatory response in TLR9 -/- mice similar to that of wild type mice. Increased phosphorylation of the tyrosine kinases c-Src and ERK1/2 were evident within 2 hours of infection in Ad37-infected corneas as compared to buffer-injected corneas. The kinase inhibitors PP2 and U0126 decreased the expression of CXCL1, and PP2 reduced inflammation in Ad37-infected corneas when compared to DMSO-treated controls. **Conclusion.** The innate immune response to Ad37 infection in the corneal stroma occurs independently of viral replication, and results from activation of host cell kinases. TLR9 does not appear essential to the development of adenovirus keratitis.
Supported by NIH grants EY13124, EY12190, P20 RR017703, and a Physician Scientist Merit Award from Research to Prevent Blindness (to J.C.)

The Glucocorticoid Receptor: One Gene, Many Proteins - New Mechanisms For Tissue Specific Anti-Inflammatory Actions Of Glucocorticoids In Health And Disease. John Cidlowski, Nick Lu, Christine Jewell, Onard Schoneveld, Danielle Duma, Kathy Gross, Javier Revollo, Robert Oakley. LST, NIEHS, 111 TW Alexander Dr., Research Triangle Park, NC, 27709.

Glucocorticoids are necessary for life after birth and regulate numerous homeostatic functions in man, including glucose homeostasis, protein catabolism, skeletal growth, respiratory function, inflammation, development, behavior and apoptosis. They are also one of the most prescribed classes of anti-inflammatory drugs in the world. Our understanding of how one hormone or drug regulates all of these diverse processes is limited, although most of these actions are thought to be mediated via the glucocorticoid receptor, which is a product of a single gene. However, recent studies in our laboratory have shown that multiple glucocorticoid receptor isoforms are produced from one gene via combinations of alternative mRNA splicing and alternative translation initiation. In addition these glucocorticoid receptor isoforms are subject to several post-translational modifications including ubiquitination, phosphorylation and sumoylation which also modulate receptor function. In this lecture, we will show that these GR receptor isoforms regulate specific subsets of genes and selectively regulate distinct cellular functions such as apoptosis. Finally, we will also describe new studies on the human glucocorticoid receptor _ protein whose expression is associated with various states of glucocorticoid resistance in human inflammatory disease.

Boston Type 1 Keratoprosthesis Retention Rates For 3 Ocular Groups (Autoimmune Disease, Chemical Injury, Other): Results From The Boston Type 1 Multicenter Study Group. Joseph B. Ciolino [1,2], Brian L. Zerbe[1], Michael W. Belin[1],[3]. Albany Medical College, [1]Albany, NY, Massachusetts Eye and Ear Infirmary, Boston, MA, and Cornea Consultants of Albany [2], Albany, NY, USA.

Purpose. To report the Boston Type 1 keratoprosthesis retention rates from three different patient populations: underlying cicatricial

autoimmune disease, history of chemical injury, and the remaining eyes. **Methods.** Prospective, noncomparative, interventional case series from the Boston Type 1 Keratoprosthesis Study Group. We analyzed 221 Boston Type 1 keratoprosthesis implanted in 215 eyes from 213 patients from 17 surgical sites over a 3 _ year period. The eyes were separated into one of three groups: chemical injury, autoimmune disease, and other (e.g immunologic graft rejection). Retention rates between the three groups were compared. **Results.** Encompassing all groups, 96.3% of the keratoprosthesis implanted survived at their most recent follow-up (average follow-up of 9.3 months). Of the 221 eyes, 22 (10%) had suffered a chemical injury, 33 (15%) had an underlying cicatricial autoimmune disease, and the remaining 166 (75%) eyes did not have either predisposing condition. Eyes lacking chemical injury or an autoimmune state had the highest retention rate (99.4%), followed by eyes in the autoimmune disease group (85%), and finally by eyes which suffered chemical injuries (50%). The former group had a statistically significantly higher retention rate (chi-square test, $P<0.0001$, with 2 degrees of freedom). **Conclusions.** The Boston Type 1 keratoprosthesis has a very high retention rate amongst eyes that do not have autoimmune disease states or that have not suffered chemical injuries; eyes with the latter conditions may have a higher rate of keratoprosthesis failure.
The authors have no financial interest in the study material.

Conjunctival Morphology With Daily Wear Of Silicone-Hydrogel Lenses. Chantal M-L Coles, Noel A Brennan, Heather RM Connor, Robert G McIlroy. Brennan Consultants, Melbourne, Australia.

Purpose. Contact lens wear can impact the morphology of conjunctival epithelial cells; however, the impact of daily wear, silicone-hydrogel lenses on conjunctival morphology has not previously been studied. **Method**. Conjunctival impressions were obtained from 4 sites on on eye of 114 subjects and stained with either PAS or PAP. A total of 15 subjects had not previously worn contact lenses, while 26 had worn balafilcon A, 28 lotrafilcon B, 25 senofilcon A and 20 had worn hydrogel lenses from a range a mid-water content brands for a minimum of at least one month. Conjunctival lissamine green and NaFl staining was also monitored on the fellow eye. **Results**. The various markers of conjunctival squamous metaplasia- goblet cell concentration, cell size and nucleus to cytoplasm ration- all correlated highly. Goblet cell percentages, cell sizes and nucleus to cytoplasm ratios all varied significantly ($p < 0.05$) with lens type. Hydrogel lens wear alone induced a decrease in goblet cell percentage and increase in cell size, whereas all lenses caused a decrease in nucleus to cytoplasm ratio. The inferior conjunctiva also showed significantly larger cell sizes and reduced nucleus to cytoplasm ration compared to the superior conjunctiva ($p < 0.05$). Neither lissamine green nor NaFl staining could predict the impression cytological results. **Conclusion**. Silicone-hydrogel lenses worn in daily wear do not seem to produce as great an impact on conjunctival morphology as traditional hydrogel materials from the perspective of goblet cell density and overall cell size. Length of wear may impact the degree of effect.

Do Socio-Cultural Factors Have An Impact On Vision? Paul Courtright. Kilimanjaro Centre for Community Ophthalmology, Tumaini University, Moshi, Tanzania.

Vision loss is estimated to affect over 161 million people globally, with the highest proportion living in the least developed countries. Anterior segment disease, primarily cataract, accounts for the vast bulk of these figures. Risk factors for the major blinding conditions have been well researched, however, this understanding has not translated into strategies to prevent many of these conditions. Thus, efforts have generally focused on addressing the socio-cultural factors that either limit or enhance use of appropriate eye care services.

Age and gender either contribute to the incidence of most eye diseases or they contribute to how people respond to these diseases. Over 82% of the blind are 50 years of age and women are 1.5 to 2.2 times more likely to be blind compared to men. There is considerable disparity in vision loss in the elderly, even in industrialized countries. In developing countries, the elderly are becoming more marginalized, primarily because of population shifts due to economic opportunities in urban areas. Evidence from throughout the developing world strongly suggests that women have both an excess burden of eye disease compared to men as well as less access to available services. Besides age and gender, the other important socio-cultural factor that impacts on vision is the decision-making characteristics of households; size of household, educational attainment of adult members, and economic status (and competing priorities) all contribute to decision making.

Eye care service delivery in most non-industrialized settings is not provided in such a way as to facilitate access and the absence of planning has meant that there is often little consideration given to the population needing the service. Evidence from a number of settings suggests that addressing the socio-cultural factors will have a significant impact on improved vision of a population, although progress is likely to be slow.

Comparison Of Hand-Held And Slit-Lamp Mounted Techniques For Assessing Non-Invasive Tear Film Stability. Jennifer P Craig, [1,2,4] Kenneth J Blades,[2] James Farrell,[2] Paul J Murphy,[2,3] Simon J Dean.[4] Ophthalmic Research Group, Aston University, Birmingham, UK[1], Department of Vision Sciences, Glasgow Caledonian University, UK[2], School of Optometry and Vision Science, Cardiff University, UK[3] and Department of Ophthalmology, University of Auckland, NZ[4]

Purpose. Several techniques assess tear film stability non-invasively. This study compared three slit-lamp/keratometer mounted (high magnification) techniques and two hand-held (low magnification) techniques in a prospective, randomised, masked, cross-over study. **Methods.** Non-invasive tear film stability of 22 normal subjects (12M, 10F, aged 21-68) was assessed independently by 5 trained observers, using 5 different techniques. High magnification measurements included non-invasive break up time (NIBUT) with the Keeler Tearscope™, and tear thinning time with the HIRCAL grid (HTTT), and keratometer mires (KTTT). Hand-held measurements included tear thinning time with the Keeler Tearscope Plus™ with fine grid insert (TPTTT) and the Loveridge Grid™ (LGTTT). Stability data were log transformed prior to parametric statistical analysis. **Results.** NIBUT, HTTT, KTTT, TPTTT and LGTTT ranges (and median values) were 6.0 - 94.8 (15.8), 3.3 - 90.0 (18.7), 8.3 - 28.7 (16.8), 4.1 – 21.6 (10.1) and 6.3 - 26.3 (11.2) seconds, respectively. Repeated measures ANOVA highlighted significant variation between subjects ($p=0.002$) and between tests ($p<0.001$). Post hoc testing demonstrated that TPTTT and LGTTT stability results were significantly different from those of all other tests. No significant differences were observed between the NIBUT, HTTT and KTTT data. **Conclusions.** Stability measures with all the high magnification techniques tested were comparable for the same group of subjects. Hand-held techniques produced significantly different results, however, relating perhaps to difficulty in maintaining a focussed grid image, and/or confusion created by crowded grid-lines at low magnification. Stability results from slit-lamp/keratometer mounted techniques and hand-held techniques are therefore not interchangeable.
[Disclosure: none]

Effect Of A Liposomal Spray On The Preocular Tear Film. Jennifer P. Craig[1], Christine Purslow[2], Paul J. Murphy[2], James S. Wolffsohn[1]. Ophthalmic Research Group, Aston University, Birmingham[1] and School of Optometry and Vision Sciences, University of Cardiff[2], UK

Purpose. Until recently, few over-the-counter topical dry eye therapies have been formulated specifically to supplement the tear film lipid layer. Clarymist™ represents a novel delivery system in which liposomes are sprayed onto the surface of the closed eye, and are purported to migrate to the superficial tear film to supplement the existing lipid layer. This study evaluated changes to the preocular tear film following application of this liposomal spray. **Methods**. Eight subjects (6M, 2F) with a mean age (±SD) of 35.9±9.7 years participated in this prospective, randomised, double-masked investigation in which Clarymist™ spray (CS) was applied to one eye, and an equal volume of saline spray (SS) applied to the contralateral eye. Lipid layer quality and non-invasive tear film stability (NIBUT) were evaluated before application, and then at 15, 30, 45 and 90 minutes post application. Subjective reports of comfort were also compared. **Results**. Log-transformation of NIBUT data enabled parametric statistical analysis. Mean NIBUT at baseline was not significantly different between the eye undergoing CS application (14.7±15.3s) and that undergoing SS application (15.4±16.3s) ($p>0.05$). Differences were significant at 15 minutes (CS: 25.7±26.5s, SS: 15.7±19.5s, $p=0.042$ and 30 minutes (CS: 27.2±31.4s, SS: 18.4±19.9s, $p=0.049$) post instillation, but not at 45 minutes (CS: 18.5±21.5, SS: 13.6±12.9) or 90 minutes (CS: 14.2±13.7, SS: 13.7±13.5). Lipid grade increased by an average of 1.7 and 1.2 grades at 15 and 30 minutes, respectively but returned to baseline levels thereafter. All subjects who reported a subjective improvement in comfort (62.5%) found the CS eye more comfortable. Most subjective differences were reported only at 15 minutes post-instillation with occasional reports at 30 minutes. **Conclusions**. Consistent with subjective reports of improved comfort, statistically and clinically significant differences in lipid layer quality and tear film stability were observed up to 30 minutes after application of a liposomal spray.*[Disclosure: none]*

The Impact Of Fluorescein Quantity On Tear Film Break Up. Jennifer P Craig[1,2], Andreas Müller[2], Jennifer Mooi[2]. Ophthalmic Research Group, Aston University, Birmingham, UK[1] and Department of Ophthalmology, University of Auckland, New Zealand[2]

Purpose. Non-invasive tear film stability measurements with reflected mires are generally considered to be superior to fluorescein break-up time (FBUT) measurements, due to the absence of the presumed destabilising action of fluorescein. However, the FBUT test remains popular, clinically, due to facilitated visualisation of break-up. This study investigated whether reducing the volume of fluorescein from the 25 – 40µl typically instilled with fluorescein paper strips or minims, to approximately 1µl, significantly altered the test results. **Methods**. Tear film stability was measured, non-invasively, in 41 subjects with the Tearscope Plus™ and fine grid insert. Measurements were made in each subject before fluorescein was instilled, then following application of 1µl of fluorescein (DET, Akorn, Inc), and finally following application of fluorescein from a conventional fluorescein strip (Smith & Nephew). Fifteen minutes between each set of measurements ensured recovery from any reflex tearing, and a mean of three readings was recorded each time. **Results**. Log transformation of the stability data allowed parametric statistical analysis. The traditional standard fluorescein test gave significantly shorter tear film stability measurements than either the non-invasive measure without fluorescein, or that with 1µl fluorescein (ANOVA, $p<0.05$ in both cases). However, there was no significant difference between the non-invasive measure without fluorescein and that with just 1µl of fluorescein (ANOVA, $p>0.05$). **Conclusions**. Instillation of fluorescein has been believed to be the cause of reduced tear film stability measures in clinical practice. The results of this study show the reduced values obtained with the traditional FBUT test may be a function of the volume of fluid instilled, rather than the fluorescein itself, since 1µl volumes give comparable stability values to non-invasive tests. Clinicians wishing to conduct the FBUT test can obtain similar results to a non-invasive test by significantly reducing the volume of fluorescein instilled.
[Disclosure: none]

A Comparison Of Two Marketed Artificial Tears In Improvement Of Tear Film Stability As Measured By Tear Film Break-Up Time (TFBUT) And Ocular Protection Index (OPI). [1]D'Arienzo P, [2]Ousler III GW, [2]Schindelar MR. [1]New York Medical College, Valhalla, NY; [2]ORA Clinical Research and Development, North Andover, MA

Purpose. Ocular surface protection is a vital function of the tear film. In dry eye patients, tear film instability can lead to rapid tear break-up and inadequate surface protection. An exposed ocular epithelium is prone to desiccation, yielding symptoms of dry eye and impacting the adherence of the tear film to the ocular surface. A PG/PEG tear substitute containing HP-Guar as a gelling agent and a CMC/glycerin formulation were tested for their respective abilities to improve diagnostic measures of tear film stability. **Methods.** This was a single site, randomized, double-masked, crossover study involving 2 visits over 1 week. Forty-two dry eye subjects (N=42; 84 eyes) were randomized to receive either the PG/PEG or CMC/glycerin drop at visit 1, and the other drop at visit 2. After tear instillation, TFBUT and OPI were calculated at 0, 5, 10, 15, 20, 30, 45, 60, and 90 minutes. TFBUT was determined by a masked investigator after fluorescein instillation and OPI was calculated as the ratio of TFBUT to inter-blink interval (IBI). **Results.** Both formulations yielded significant changes from baseline OPI–the CMC/glycerin drop at 5, 10, 15 and 20 minutes, and the PG/PEG drop at 5, 10, 20, 60 and 90 minutes ($p<0.05$). The PG/PEG drop yielded a significantly greater extension in TFBUT from baseline at 45, 60, and 90 minutes post-instillation than was seen in eyes dosed with the CMC/glycerin drop ($p<0.05$). These changes were also significant versus baseline, while CMC/glycerin-treated eyes saw no significant changes from baseline TFBUT. **Conclusions.** Both drops exhibited an immediate impact on tear film stability that lasted through the 20 minute time point. The PG/PEG drop saw significant extensions in TFBUT at 45, 60 and 90 minutes, whereas the CMC/glycerin drop did not. The data demonstrate that PG/PEG provides a greater duration of tear film stabilization, and subsequently ocular surface protection, than that provided by CMC/glycerin.

Protectve Effect Of Permanent Soft Contact Lens Wear With The Boston Keratoprosthesis. Mona Harissi-Dagher, MD, Claes H. Dohlman, MD, PhD. Department of Cornea and External Disease, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA, USA.

Purpose. To document the effects of soft contact lenses (SCL) use following Boston keratoprosthesis (KPro) type I implantation. **Methods.** A retrospective chart review of 157 eyes that underwent a Boston KPro type I surgery between 1990 and 2004 by one surgeon (CHD) was conducted. Data on ophthalmic diagnosis, SCL fitting, retention rate, retention duration, and adverse effects of SCL was collected. Retention was defined as the percentage of patients who did not lose their SCL between visits. **Results.** 83 eyes were fitted with

SCL intraoperatively. Retention rate was 60% and average duration of retention was 23 months. Steepening of SCL and tarsorraphies improved retention. Hydration of the cornea was superior with SCL. This prevented damage to the corneal surface and peripheral melt caused by desiccation. **Conclusion.** This study demonstrates the substantial benefit and practicality of SCL after Boston KPro type I surgery. Not only do SCL play an important protective and therapeutic role in the post-operative management of KPro patients, they can also be helpful in correcting residual refractive error, or in improving cosmesis. Around-the-clock soft contact lens wear is mandatory in autoimmune diseases and chemical burns.
Commercial relationship: Neither of the authors has any financial interest in any of the products in this manuscript.
Financial Disclosure: Alcon Research Institute and Massachusetts Eye and Ear Infirmary provided support for this study.

Current And Future Perspectives For Ocular Stem Cells: Biology And Therapeutic Potential. Julie T. Daniels. Cells for Sight Transplantation & Research Programme, UCL Institute of Ophthalmology, London, UK

Improvement of vision by therapeutic transplantation of cultured limbal epithelial stem cells in the cornea is so far one of only a few examples of the successful use of adult stem cells in regenerative medicine. In the future it is anticipated that stem cell populations could be used to treat a variety of more difficult to manage sight threatening diseases including retinal degeneration. The transparency and ready accessibility of the cornea make it an ideal model system in which to study the regulatory mechanisms involved in adult epithelial, mesenchymal and endothelial stem cell function during health and disease. The cornea therefore has the potential to address key generic basic and translational stem cell research questions including; which stem cell source to use in the clinic i.e. adult or embryonic, autologous versus allogeneic, the method of cell delivery including tissue engineered constructs, how to facilitate cell integration into the matrix, how to overcome immunogenicity and development of safe cell labelling and tracking strategies to assess therapeutic benefit in humans. The establishment of regulatory agency compliant laboratories have already been developed in a number of countries for the delivery of limbal epithelial stem cell therapy. This state-of-the-art technology is therefore already available for rapid clinical translation of new ocular and other cell type therapies as they are developed, again emphasising the broader importance of the cornea in regenerative medicine. This presentation will provide an overview of the current status of ocular stem cell biology and propose key research directions that will facilitate the future development of ocular stem cell therapies.
Commercial relationships: none
Grant support: BBSRC, Eranda Foundation, MRC, Special Trustees of Moorfields Eye Hospital.

EGF Activates Protein Kinase C*a* And -E And ERK1/2 To Stimulate Cultured Rat And Human Conjunctival Goblet Cell Proliferation. Darlene A. Dartt, Robin. R. Hodges, and Marie A. Shatos. Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School, Boston, MA.

Purpose. To determine the signaling pathway activated by EGF to induce proliferation goblet cells isolated from rat and human conjunctiva. **Methods.** For all experiments, P1 goblet cells cultured from rat or human conjunctiva were grown to 75% confluence and then serum starved for 24 hr in RPMI-1640 medium containing 0.35% BSA. To determine the role of PKC isoforms, goblet cells were preincubated for 2 hr with the PKC inhibitors calphostin C, which inhibits PKC a and e, and Gö6983, which inhibits PKC a,b,d,and z. each at 10-10 to 10-7 M. This was followed by stimulation with EGF (10-7 M) for 18-20 hr. Goblet cells were incubated with adenovirus to overexpress dominant negative (dn) PKCa and –e and then stimulated with EGF. To investigate the role of ERK1/2, goblet cells were preincubated with the ERK1/2 inhibitor U0126 (10-7 to 10-5 M) before stimulation with EGF. Cell proliferation was determined by the WST-8 assay that measures cell number. **Results.** Calphostin C and Gö6983 significantly inhibited EGF-stimulated rat goblet cell proliferation in a concentration dependent manner. Maximum inhibition of 90 ± 11 % and 60 ± 12% was obtained with calphostin C (10-8 M) and Gö6983 (10-7 M), respectively. Use of adenovirus caused overexpression of both dn PKCa and –e in rat goblet cells. With dnPKCa overexpression EGF-stimulated proliferation was significantly inhibited 65 ± 14%, with dnPKCe overexpression by 97 ± 2%, and with overexpression of both dn PKC isoforms by 95 ± 3%. U0126 significantly inhibited EGF-stimulated proliferation of goblet cells with a maximum inhibition of 40 ± 15%. In 2 experiments calphostin C and U0126 inhibited EGF stimulated human goblet cell proliferation with a maximun inhibition of 96% and 37%, respectively. **Conclusions.** We conclude that EGF stimulates rat and human goblet cell proliferation by activating protein kinase Ca and –e followed by activation of ERK1/2.
Supported by NIH grant EY009057

Tear Cytokine Profiles In Dry Eye And Effect Of Cyclosporine Emulsion Therapy. Cintia S. De Paiva[1]; Lauren S. Blieden[1], Helen Y. Lam[1], William J. Farley[1], Frank Bucci[2], Michael E. Stern[3], Stephen C. Pflugfelder[1]. [1]Ocular Surface Center, Cullen Eye Institute, Baylor College of Medicine, Houston, TX. [2] Bucci Laser Vision Institute, Wilkes-Barre, PA. [3]Allergan, Irvine, CA

Purpose. To evaluate tear cytokine profiles in patients with ocular rosacea (n=8), non-Sjögren's syndrome (non-SS, n=15) aqueous tear deficiency (ATD), SSATD (n=6) and Stevens-Johnson Syndrome (n=2) with age-matched controls (n=14) and in dry eye patients after treatment with cyclosporine for 6 weeks. **Methods.** Luminex immunobead assay was performed in tears to evaluate levels of IL-6, IL-8, EGF and IL-13. **Results**. Patients with ocular rosacea and SSATD had higher mean IL-6 (259.4±266 pg/mL and 504±402 pg/mL, $P<0.05$ and $P<0.01$ respectively) concentration and SSATD had higher IL-8 (2583±2188 pg/mL) than controls (35.54±41.81 pg/mL for IL-6 and 108.8±102.9 pg/mL for IL-8). EGF was reduced in SSATD and SJS (73.84±43.43 pg/mL and 101.4±84.35 pg/mL, respectively, $P=0.07$). IL-13 showed a 50% reduction in SSATD tears compared to normals (73.84±43.43 pg/mL versus 132.1 pg/mL 51.28 pg/mL) but did not reach significance. There was a significant positive correlation between IL-6 and IL-8 with corneal fluorescein staining ($R2=0.40$ and $R2=0.48$, $P=0.001$ for both, respectively). Tears of cyclosporine treated eyes showed a significant increase in IL-13 levels and reduced levels of IL-6 and IL-8 at 6 weeks compared to baseline levels (100.5±85.09 pg/mL, 76.23±63.66 pg/mL and 1642±2358 pg/mL vs. 58.32±43.28, 188.2 ±266.7 pg/mL and 2429±4778, respectively, $P<0.05$ for IL-13). **Conclusions.** Ocular rosacea differs from SSATD in tear profiles with high IL-6, IL-13 and normal EGF while SSATD has high IL-6, IL-8 and low EGF. Treatment of cyclosporine increases IL-13 levels while decreasing IL-6 and IL-8.
Support: Unrestricted research grant from Allergan
Commercial Relationship: C.S. De Paiva, none; Lauren S. Blieden, none; Helen Y. Lam, none; William J. Farley, none; Frank Bucci, none; Michael E. Stern is an employee of Allergan Inc; Stephen C. Pflugfelder, none.

Treatment With Doxycycline Preserves Cell Area After Desiccating Ocular Stress. Cintia S. De Paiva[1], Robert M. Beardsley[1], David Power, Stephen C. Pflugfelder[1]. [1]Department of Ophthalmology, Cullen Eye Institute, Baylor College of Medicine, Houston, TX, [2]Alacrity Biosciences, Inc., Laguna Hills, CA.

Hypothesis. Treatment of experimental dry eye (EDE) with the tetracycline doxycycline would preserve corneal apical epithelial cell area. **Purpose/Objectives.** To investigate the effects of desiccating stress on corneal apical epithelial cell area and subsequent percentage of epithelial loss and to evaluate the effects of doxycycline on this process. **Methods.** C57BL/6 mice, aged 6-8 weeks of mixed gender, were placed in a dehumidified room with air draft and given scopolamine subcutaneously for 4 days to induce experimental dry eye (EDE) with or without topical therapy with ALTY-0501, a proprietary formulation of 0.025% doxycycline or the drug vehicle QID. Untreated (UT) C57BL/6 mice that were not exposed to EDE were used as controls. Cell area, cell morphology, and the percentage of cell loss were evaluated in whole mount corneas stained for occludin and visualized by laser scanning confocal microscopy. Digital images were analyzed using Metavue 6.24r software and cell area, mean cells per field, and average cell loss were recorded in a database. **Results.** EDE caused a significant decrease in apical corneal cell area (1073±135.90 $\_m^2$), an increase in number of cells (44.79±5.77 cells per field) and these findings were accompanied by a greater percent loss (20.61±12.47%) than UT controls (1341±95.28 $\_m^2$, 35.72±2.78 cells per field, 2.81±3.34%, P<0.001 for all, respectively). Treatment with doxycycline preserved cell area (1337±144.6 $\_m^2$) and the mean number of cells (39.96±3.12 cells per field) and prevented cell loss (8.51±7.1%) compared to the vehicle control group (1154±88.10 $\_m^2$, 41.51±2.49 cells per field, 21.43±69.31%, respectively). **Conclusions.** The presence of desiccating stress decreases apical corneal epithelial cell area, increases the mean number of cells per field, and intensifies epithelial cell loss. Treatment with doxycycline preserves cell area and mean cell number in addition to preventing EDE-induced corneal apical epithelial cell loss, while the vehicle alone showed no significant effect.
Grant support: NIH EY 11915 and unrestricted research grant from Alacrity Biosciences, Inc
Commercial Relationship: C.S. De Paiva, None; Robert M. Beardsley, None; David Power: president of Alacrity Biosciences, Inc.; S.C. Pflugfelder, None.

Alteration Of Mucin Expression In The Ocular Surface Epithelium In Patients With Dry Eye. Seika Den[1], Murat Dogru[1,2], Jun Shimazaki[1], [2]Department of Ophthalmology, Tokyo Dental College, Chiba, Japan, [1] Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan [2]

Purpose. To determine the alteration in expression of mucin proteins and mRNAs in patients with dry eye. **Methods.** Twenty eyes of 12 females (mean age; 56 years) who were diagnosed as dry eye according to tear function (Schirmer's value < 5 mm and/or tear-film break-up time < 5 seconds) and vital staining scores were studied in their cytology and mucin expression. The results were compared with healthy subjects consisting of 20 eyes of 10 subjects (9 males and 1 female, mean age; 27 years). Goblet cell density in bulbar conjunctival epithelia obtained by nitrocellulose filter-paper stripping (impression cytology) was studied using PAS staining. Keratinization of conjunctiva was assessed using Nelson's squamous metaplasia grading of bulbar conjunctival epithelium. In immunohistochemistry, MUC5AC in epithelia of bulbar conjunctiva and MUC4 in peripheral corneas obtained by impression cytology were studied. Real-time PCR was used to determine the mRNA expression of MUC5AC and MUC4 in the bulbar conjunctival epithelia obtained by brush cytology. **Results.** Goblet cell density was significantly lower and squamous metaplasia grading was higher in dry eye patients than in healthy eyes. Immunohistochemical staining showed decreased expression of MUC5AC and MUC4 in dry eye patients compared with healrthy subjects. The mRNA expression of MUC5AC and MUC4 were significantly reduced in patients with dry eye compared with healthy subjects. **Conclusions.** The goblet cell density, expression of MUC5AC and MUC4 ocular surface epithelia of dry eye subjects were decreased compared with normal subjects. These alterations may be responsible for the tear-film instability and account for the damage of ocular surface epithelia in dry eye patients.

Influence Of Insulin Treatment On Lacrimal Gland And Ocular Surface Of Diabetic Rats. Ana Carolina Dias, Carolina Maria Módulo, Angélica Gobbi Jorge, Alexandre Martins Braz, Rubens Bertazolli Filho, Jayter Silva de Paula, Alceu A. Jordão Jr., J. Sérgio Marchini, Eduardo M. Rocha. Departamento de Oftalmologia, Otorrinolaringologia e Cirurgia de Cabeça e Pescoço e Departamento de Clínica Médica da Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brazil.

Purpose. Previous works have observed structural, functional and molecular alterations in lacrimal gland (LG) and ocular surface (OS) after chronic diabetes mellitus in rodents and humans. The aim of this study is a) to compare the expression of markers of oxidative stress and histological markers of disease and b) to evaluate whether insulin treatment inhibit LG and OS alterations. **Methods.** Diabetes was induced in male Wistar rats with a single intravenous streptozotocin or vehicle and a sub group was treated with insulin. After 10 weeks, LG and OS of the three groups (n=5/group) had the structure and morphology compared, and analyzed for expression of malonaldehyde (MDA) (a lipid oxidation marker). Impression cytology (IC) graded and compared the ocular surface epithelia. **Results**. After 10 weeks, no significant difference was found in ocular surface epithelia morphology (IC). LG morphology alterations and increased number of lipofucsin-like inclusions was observed in diabetic, however similar to controls in insulin treated diabetic rats. It was observed MDA levels were significantly higher in diabetic but similar to controls in insulin treated diabetic rats (P= 0.0025). **Conclusions.** Diabetes induces significant alterations in rat LG suggesting that hyperglycemia-related oxidative stress may take a part in diabetic dry eye syndrome, as indicated by differences in MDA levels. Those events were reverted by insulin replacement, which may be a direct effect or secondary to glycemia control.
Financial Support: CAPEs, CNPq, FAPESP, FAEPA

Intralobular Duct Cells Of Rabbit Lacrimal Gland Are Actively Involved In Lacrimal Fluid And Electrolyte Production. Chuanqing Ding,[1], [2], Janos Peti-Peterdi[2], Austin K. Mircheff,[2] Joel E. Schechter.[1] Cell & Neurobiology,[1] Physiology & Biophysics,[2] University of Southern California, Los Angeles, CA, USA.

Purpose. Lacrimal fluid has been assumed to be produced in two stages: primary fluid that is initially secreted by acinar cells, and then modified by duct cells as it is transported through the ducts. However, little direct evidence exists regarding duct cells' involvement in lacrimal fluid production and modification, in contrast to an extensive literature regarding the functions of acinar cells. The purpose of the present study was to study lacrimal duct cells' role in lacrimal function by live cell imaging using multiphoton confocal fluorescence microscope. **Methods.** Intralobular duct segments were microdissected

from rabbit lacrimal gland, cannulated at one end with micropipettes, and microperfused with Ringer's medium. Appropriate fluorescent dyes were added to the perfusate. The ducts were imaged with a Leica TCS SP2 multiphoton confocal fluorescence microscope.
Results. Intracellular fluorescence increased with time during perfusion with Rhodamine-dextran, indicating apical fluid phase endocytosis presumably associated with constitutive plasma recycling. Addition of pilocarpine to the bath medium caused a dramatic increase of intracellular [Ca^{2+}], as indicated by Fluo-4 and Fura Red fluorescence changes. Use of calcein as a cell volume marker indicated that pilocarpine also caused cell shrinkage, consistent with net solute efflux following opening of K^+ and Cl^- channels, followed by an apparent regulatory volume increase. Switching to a hypotonic bath medium caused cell swelling followed by a regulatory volume decrease. Intracellular [Cl^-], measured with MQAE, decreased when perfusate [Cl^-] was decreased; Cl^- efflux was abolished by addition of DIDS at concentrations associated with Cl^- channel inhibition. **Conclusions.** Intralobular duct epithelial cells in rabbit lacrimal gland respond to cholinergic stimulation by activating ion- and water fluxes consistent with a significant role in fluid production and modification.
CR: None. Support: EY010550 (JES), EY005801 (AKM), Zumberge Faculty Research Fund (CD), DK64324 (JP)

Evaluation Of Conjunctival Inflammatory Status By Confocal Laser Microscopy And Conjunctival Brush Cytology In Patients With Atopic Keratoconjunctivitis (AKC). Murat Dogru, MD,1,2 ; Osama Ibrahim, MD,1 ; Yukihiro Matsumoto, MD,3; Yoji Takano,MD,4; Mari Tanaka, MD,5; Yoshiyuki Satake, MD2; Kazumi Fukagawa, MD3; Hiroshi Fujishima4; Kazuo Tsubota3. 1) Johnson & Johnson Department of Ocular Surface and Visual Optics, Keio University School of Medicine, Tokyo, Japan.2) Department of Ophthalmology, Tokyo Dental College, Chiba, Japan.3) Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan. 4) Department of Ophthalmology, International Welfare University, Mita Hospital, Tokyo, Japan5) Ajisai Eye Clinic, Funabashi, Japan.

Purpose. To investigate and compare the conjunctival inflammatory status by confocal laser scan microscopy and conjunctival brush cytology. **Methods.** Sixteen eyes of 8 AKC patients (8 males, mean age: 20.3±5.9 years) as well as 12 eyes of 6 healthy normal subjects (6 males, average age 25.4±6.8 years) were studied. All subjects underwent slit lamp examinations, Schirmer test, tear film break-up time (BUT), fluorescein and Rose Bengal staining of the ocular surface, brush cytology and confocal laser scanning microscopy of the tarsal palpebral conjunctiva by using the Heidelberg Rostock Corneal Module (HRT-RCM). The density of conjunctival inflammatory infiltrates was calculated from confocal microscopy scans and also from brush cytology specimens after Diff Quik staining. **Results.** The inflammatory cell infiltrate counts were significantly higher in AKC subjects than controls in both confocal laser scans and brush cytology specimens. We also observed a significant correlation between the inflammatory cell counts calculated from confocal laser scans and brush cytology specimens. **Conclusion.** HRT-RCM was both noninvasive and efficient in evaluating the conjunctival inflammatory status compared to brush cytology in AKC.
Disclosure: None

Mechanisms Of Compound Exocytosis. J. Michael Edwardson. Department of Pharmacology, University of Cambridge, United Kingdom.

Purpose. My colleagues and I are interested in the process of compound exocytosis, specifically in the acinar cell of the exocrine pancreas. I will review our recent results and try to draw parallels with exocytosis in the lacrimal acinar cell. **Methods**. We have used a number of techniques to study exocytosis at the single vesicle level; these include brightfield imaging, and fluorescent imaging of extracellular dyes, which enter exocytosing zymogen granules through the open fusion pores. Exocytosis has been quantified both by video imaging in single cells and through measurement of amylase release from populations of permeabilised acini. SNAREs present in the pancreatic acinar cell have been detected both by immunoblotting and immunofluorescence, and their roles in exocytosis have been assessed through the functional effects of anti-SNARE antibodies and Clostridial neurotoxins. **Results**. The pancreatic acinar cell exhibits compound exocytosis, which involves both primary fusion of zymogen granules with the apical plasma membrane and secondary fusion of deeper-lying granules with granules that have already undergone exocytosis. Exocytotic membrane fusion primarily involves the SNAREs syntaxin 2 and SNAP-23 on the apical plasma membrane and VAMP 8 on the zymogen granule membrane. Exocytosis in permeabilised acini is inhibited by antibodies against these SNAREs and by treatment of the cells with botulinum neurotoxin C, which cleaves syntaxin 2. Syntaxin 2 enters the membranes of fused granules, and this translocation may initiate secondary granule fusion events. The fusion pore is extraordinarily large (at least 50 nm in diameter). It is also very stable; for example, some granules remain open to the exterior of the cell for several minutes. The stability of fused granules might depend on the coating of their cytoplasmic surfaces with filamentous actin. Despite its large diameter, the fusion pore can on some occasions be seen to close, an event that might initiate the recovery of membrane via endocytosis. **Conclusions**. The exocytotic fusion event in the pancreatic acinar cell is now well characterised. Perhaps the most interesting current question relates to the dynamics of the fusion pore.

An Investigation Of The Realtionships Between Tear Ferning, Tear Film Stability And Ocular Comfort. Katharine S. E. Evans, Christine Purslow, Rachel V. North. Contact Lens and Anterior Eye Research Unit, School of Optometry and Vision Sciences, Cardiff University, UK

Purpose. Tear ferning (TF) has been shown to be sensitive in the diagnosis of dry eye, but is a relatively uncommon test, especially in contact lens wearers. The aim of this study was to investigate the relationship between TF and tear film stability amongst healthy contact lens (CL) wearers and non-contact lens (NCL) wearers, expressing varying degrees of ocular comfort. **Methods.** All subjects (36 NCL, 24 CL; mean age 23.2±4.8yrs) underwent assessment of non-invasive tear break up time (NIBUT), fluorescein tear break up time (FBUT) and completed the Ocular Comfort Index (OCI) questionnaire. A glass capillary was used to collect non-stimulated tears from the inferior fornix. 1.5µl dried tear samples were observed with a light microscope (x20) and the TF pattern quantified according to Rolando's grading scale. **Results.** Significantly higher grades of TF pattern and discomfort (higher OCI scores) were observed in CL wearers compared to NCL wearers (Mann-Whitney; $p<0.005$ and $p<0.05$ respectively). Differences in tear film stability were insignificant between groups ($0.194<p<0.369$). Even when asymptomatic (low OCI scores) CL and NCL subjects were compared, TF remained significantly different ($p<0.005$). In both CL and NCL subjects, TF displayed poor correlation with tear film stability tests and OCI scores ($0.267<p<0.989$). **Conclusions.** CL wearers exhibit higher grades of TF pattern, compared to NCL wearers. TF appears to be more sensitive than traditional tear film stability tests in the discrimination between CL and NCL wearers, even when comparing subjects with relatively high

degrees of ocular comfort. Abnormal TF in dry eye is thought to be caused by tear film hyperosmolarity, a feature which has also been observed in CL wearers. TF may offer a simple, indirect method to assess tear film osmolarity in CL wearers.
Commercial relationships: none. Grant support: Cardiff University.

Dry Eye In Patients Treated With Continuous Positive Airway Pressure (CPAP). Fabiani C.,[1] Roma R.,[2] Spinelli S.,[2] Fabiani M.,[2] Pivetti Pezzi P.[1] Department of Ophthalmology[1] and Center for the Study of Sleep Apnea, Department of Neurology & Otolaryngology[2], Università di Roma "La Sapienza", Italy

Purpose. Continuous positive airway pressure (CPAP) is now the treatment of choice for patients with Obstructive Sleep Apnea Syndrome (OSAS). Ocular side effects and adverse reactions, such as conjunctival irritation, are commonly reported with this device. The aim of this study was to determine the presence of signs of dry eye in OSAS patients treated chronically with CPAP. **Methods**. We studied 24 patients (48 eyes; mean age: 51±12 years) who had been using CPAP for a period of 28 days for moderate to severe OSAS (BMI: 31±5 kg/m^2; RDI = 54±19/h). Airway pressure was set at 16 cm/H_2O and ventilation volume was 0.5L/min; environmental Temperature and Relative Humidity were monitored (T: 20±2.6°C and RH: 49±3.5 %). Each patient answered a dry eye questionnaire and underwent a complete ocular examination. Clinical measurements of tear function (BUT, Schirmer 1) were completed. Corneal surface integrity was determined by means of fluorescein staining. Patients were evaluated before starting the treatment at day 0 (baseline), and after 7 days and 28 days of treatment. Individuals with ocular surface diseases, contact lens or ocular drug users were excluded from the study. **Results**. No symptoms and signs of dry eye were detected in OSAS patients before starting CPAP treatment. A significant increase in mean corneal fluorescein staining score was observed in the nasal area at day 7 and 28, as compared to baseline (P<.001). At day 28, the pattern of fluorescein staining was either diffuse (48%) or punctate (52%). Furthermore, the Schirmer test value decreased during the treatment, reaching significance at day 28 (3.4±1.8 mm/5min, P<.001). There was a concomitant significant decrease in BUT score. As determined by the questionnaire, patients reported dry eye symptoms after CPAP application. **Conclusions**. Our results indicate that exposure to a contiunuous positive airway pressure may lead to symptomatic dry eye. Continuous airflow in those patients results in damage to the ocular surface epithelia and decreased tear volume. The use of a humidifier, to moisten the air, and customized masks, to avoid air leaking, is needed to prevent the appearance of dry eye in patients treated with CPAP.

Simplified Technique Of Fluorophotometry For Corneal Permeability Evaluation In Humans. Vincent C. Fan, MD, Elvin Yildiz, MD, Karuna Bitra, MD, D.Chen, MD, PhD, T.T. Du, MD, P.A. Asbell, MD, Department of Ophthalmology, Mount Sinai School of Medicine, New York, NY, USA.

Purpose. Fluorophotometry was first used 1963 to study permeability in animal studies. In 1997, McNamara et al. measured corneal epithelial permeability using two in vivo methods - a single drop fluorescein to quantify the coefficient of corneal permeability and the eye bath method. The first method demonstrated substantial variability between measurements. The second method had several clinical limitations. In lieu given past difficulties, we have modified the Fluorophotometry technique to develop a simple non-invasive method to measure corneal permeability and present our repeatability findings. **Methods.** After obtaining IRB approval, we varied all elements of the technique (fluorescein concentration, time to irrigation, timing of scans, etc.) to determine the simplest, most repeatable technique. Ten healthy adult, right eyes were scanned with the Fluorotron Master (Ocumetrics Inc, Mountain View, CA). On the first day, two baseline fluorometric scans of the right eye were taken using the Fluorotron Master. 15 µL 2.0% fluorescein was then instilled using a calibrated pipette in the lower conjunctival sac. After thirty seconds, topical 0.5% proparacaine ophthalmic solution was given followed by 10 ml rinse using a balanced saline washout. Two immediate washout Fluorophotometry scans were taken. Fifteen minutes after the instillation of fluorescein, two more fluorphotometry scans were taken. Peak corneal florescence values (PCFV) was measured by fluorophotometry, which is an indicator of corneal epithelial permeability.
Results. The baseline mean corneal readings in 10 eyes of patients was 15.86 (SD=2.98) on day 1, compared to 15.34 (SD=1.46) on day 2 in healthy volunteers. The immediate post-rinse mean corneal reading was 405.02 (SD=260.32) on day 1, compared to 426.90 (SD=301.29) on day 2. The 15 minute post-rinse mean corneal readings was 59.43 (SD=35.99) on day 1, compared to 57.17 (SD=20.16) on day 2. Using an ANOVA paired, 2-sided t-test, the differences were determined not to be statistically significant (P-value > 0.05). **Conclusion.** Utilizing a new, simplified technique, results were found repeatable to measure fluorescein penetration into the cornea. Further research with a larger sample size and increased number of clinical visits will help us validate the repeatability of our results and technique. A simplified technique to measure corneal permeability will prove useful to quantifying ocular surface disease such as dry eyes and follow changes in new treatments and provide an objective metric for end points in clinical trials.
Author Disclosure Block: V.C. Fan, None; E. Yildiz, None; K. Bitra, None.; D. Chen, None; T.T. Du, None; P.A. Asbell, None.

Reversal Of Sjögren's-Like Syndrome In Non-Obese Diabetic Mice. Denise Faustman[1], Simon Tran[2], Shohta Kodama[3], Beatrijs Lodde[4], Ildiko Szalayova[5], Sharon Key[5], Saeed Khalili[2], Eva Mezey[5]. Massachusetts General Hospital and Harvard Medical School, Boston, MA, McGill University, Montreal, Canada, Brigham and Women's Hospital, Boston, MA, National Institutes of Health, USA and Division of Rheumatology, University of Amsterdam, Netherlands, National Institutes of Health, NIDCR, CSDB, Bethesda, MD.

Purpose. Non-obese diabetic (NOD) mice exhibit autoimmune diabetes and Sjögren's-like syndrome. We tested if a therapy that reverses end-stage diabetes in the NOD mouse would affect their Sjögren's-like syndrome. **Methods.** NOD mice have LMP2 subunit proteasome defect. Improperly selected naïve T cells escape but can be killed by re-introducing MHC class I-self peptides on matched normal splenocytes. The proteasome defect also impairs NF-κB, a transcription factor in pathogenic memory T cells, increasing their susceptibility to TNF induced apoptosis stimulated via complete Freund's adjuvant (CFA). We studied the impact of this two-limb therapy (injections of matched normal splenocytes and CFA) on the autoimmune salivary gland disease of the NOD mice. **Results.** All NOD mice receiving the above therapy have a complete recovery of salivary flow and were protected from diabetes. Restoration of salivary flow could be a result of a combination of rescue and regeneration of the gland, as confirmed with immunohistochemistry. All untreated NOD mice showed a continuous decline in salivary flow, followed by hyperglycemia and death. **Conclusion.** This study establishes that a brief intervention into NOD mice with Sjögren's-like syndrome can reverse salivary gland dysfunction. With the recent identical LMP2 proteasome defects in humans with Sjögren's, similar therapeutic approaches might be considered for clinical development.

Key Words. Sjögren's syndrome, salivary gland regeneration, MHC class I, proteasome, Y chromosome lineage tracking.

The Development Of A Composite Score That Incorporates Both Clinical And Patient Reported Outcomes For The Assessment Of Disease Severity In Dry Eye Patients. Figueiredo FC[1], Steeds CS[2], Figueiredo MS[1], Irwin DE[3], Buchholz P[4]. [1]Royal Victoria Infirmary, Newcastle, UK; [2]CS Consulting, UK; [3]University of North Carolina; [4]Allergan Europe

Purpose. To develop a composite score of both clinical and patient measures of disease severity for the assessment of dry eye disease severity. **Methods.** Various clinical tests and patient reported outcome were collected from patients attending a tertiary referral centre at the Royal Victoria Infirmary in Newcastle, UK between March 02 and June 05. The clinical tests included Tear Function Index (TFI), Tear Break Up Time (TBUT) and Oxford Grading (OG). The Ocular Surface Disease Index (OSDI) and a subjective assessment of dry eye disease severity by the physician and patients on a scale of 0-9 were also included. A composite score was derived by adding a severity score for the OG, TFI and OSDI for each patient. The results of each of these tests were pre-assigned by the physician a level and an equivalent score as follows: normal (1), mild (2), moderate (3) or severe (4). **Results.** A composite score was calculated for 75 patients at baseline, 58 at visit 2 (8 months) and 44 at visit 4 (16 months). At baseline, 42% of patients were rated as moderate by the composite score and 44% were severe. This compares with 46%, 61% moderate, and 43%, 36% severe by the patient and physician respectively. At visit 2, 40% of patients improved, 28% stayed the same and 32% deteriorated by the composite score. This compares to; 30%, 50% improved; 38%, 53% stayed same; 32%, 6% deteriorated by the patient and physician assessment respectively. At visit 2, significant correlations were found between composite score and: oxford grading (0.55,$p<0.001$), TFI (0.76,$p<0.001$) and OSDI score (0.32,$p<0.05$). **Conclusions.** There is a need to develop a robust measure to assess patient dry eye that includes both clinical and patient reported outcomes. The composite score developed here closely reflects the patient's assessment of their disease status and therefore can be used to assess patient's dry eye disease severity.
[Sponsored by Allergan]

Ocular Surface Repair And Regeneration. M. Elizabeth Fini. Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, FL, USA

The ocular surface is composed of a layer of mucosal epithelium, which is constantly self-renewing. Corneal stem cells reside in the basal cell layer of the epithelium at the limbus, which circumscribes the cornea at its periphery, at the transitional region between cornea and sclera. They divide infrequently to yield transit amplifying cells that migrate centripetally toward the corneal center as they proliferate. The conjunctiva is similarly self-renewing, and recent studies have identified stem cells specialized for this tissue. The ocular surface epithelium can completely regenerate after injury, migrating across a provisional matrix of fibrin and fibronectin deposited in the wound bed. Once restored, the epithelium then removes this matrix and resynthesizes adhesion complexes mediating attachment to the underlying stroma. If the injury penetrates through the epithelial basement membrane, the corneal epithelium resynthesizes it, interacting with the underlying stromal cells. The epithelium also controls the quality of the stromal repair tissue and the epithelial basement membrane acts as a gatekeeper, controlling epithelial-stromal interactions. Epithelial regeneration recapitulates many aspects of self-renewal, but at a faster rate. The various processes of migration, proliferation, and differentiation must be temporally coordinated with one another, and with the protective inflammatory response. This presentation will provide a concise overview of our current knowledge of repair and regeneration of the ocular surface including the role of matrix metalloproteinases, transcription factor Pax-6, cytokine TGF-beta2, and stem cells, and will identify areas of gaps in our knowledge.
Commercial relationships: none
Grant support: EY09828, EY14801, Research to Prevent Blindness. MEF holds the Walter G. Ross Chair in Ophthalmic Research

A Non-Apoptotic Model Of Exocrine Gland Hypofunction. Philip C. Fox. Department of Oral Medicine, Carolinas Medical Center, Charlotte, NC, USA and PC Fox Consulting, LLC, Spello, Italy.

The causes and mechanisms of exocrine damage in Sjögren's syndrome remain unclear. Glandular hypofunction in Sjögren's syndrome has been explained as a result of exocrine tissue loss secondary to immune attack mediated by a combination of apoptosis and cytotoxic cell death. The salivary glands are viewed as reacting to infiltrating mononuclear cells and the immunologically active products of these cells. It is a reduction of the water-transporting acinar cells that is felt to be of greatest importance. Secretory dysfunction is seen as a direct consequence of this tissue loss. However, the following four observations call this view into question: 1. apoptosis of exocrine epithelial cells in Sjögren's syndrome is a relatively rare event; 2. the relationship between the amount of remaining normal-appearing acinar tissue and glandular function is weak. Patients with abundant exocrine tissue have severe hypofunction, while, conversely, some with minimal normal-appearing tissue have adequate function; 3. *in vitro* studies of exocrine tissues in Sjögren's syndrome patients demonstrate good function, albeit with reduced sensitivity to muscarinic stimulation; and 4. *in vivo,* patients with marked secretory hypofunction respond well to stimulation with systemic sialogogues. Taken together, these observations suggest that exocrine hypofunction in Sjögren's syndrome cannot be explained fully by immune-mediated tissue loss. Recent observations suggest a new, non-apoptotic model to explain exocrine hypofunction in Sjögren's syndrome. In this model, glandular atrophy follows chronic immune-mediated inhibition of acinar secretory function. That is, the atrophy is a consequence of salivary gland hypofunction and not the cause of it. Significantly, this raises the possibility that glandular hypofunction is not an irreversible process. Identification of mechanisms or molecules responsible for inhibition of exocrine secretory process (such as recently described anti-muscarinic functional autoantibodies and BAFF) may lead to new approaches to therapy and true disease-modifying treatments. (Ref: Dawson, Fox & Smith, Rheumatol. 45(7):792-8, 2006.)

Stem Cells Of Epithelium And Stroma: Proximity Is Not Identity. J Funderburgh, Y Du, S Harvey, M Funderburgh. Department of Ophthalmology, University of Pittsburgh, Pittsburgh, PA

Purpose. Corneal epithelium is maintained by a population of stem cells localized in basal regions of limbus. Previously we identified ABCG2-positive cells in limbal stroma subjacent to the basal lamina near epithelial stem cells (Stem Cells 23:1266, 2005). Proximity of the two types of stem cells suggests a biological relationship or possibly a single population. Here we examine gene expression profiles of the two stem cell types to define phenotypic commonalities and distinctions between epithelial and stromal stem cells. **Methods.** Human stromal cells were isolated by collagenase after removal of epithelium with

Dispase. Side population (stem) cells were isolated from limbal stroma by cell sorting in Hoechst 33342. Clonal cultures were expanded in reduced-serum stem cell growth medium for 8-10 passages before analysis. Primary keratocytes were isolated similarly from central stroma and cultured without sorting, in serum-free media without subculture. 35-40 ng of poly-A containing mRNA was subjected to two-cycle cDNA amplification and analysis on HG-U133 Plus 2.0 GeneChips. Gene expression was confirmed by RT-PCR. **Results.** Recent reports identified upregulated transcripts in limbal basal epithelium for CK15, CK14, CDH2, CDH3, CDH4, CTNNA2, WNT4, BMP3, BMP4, and EREG (IOVS 2007 v48, p144, J Biol Chem 2006 v281 p19600). Our gene array analysis reported absence all these transcripts in both stromal stem cells and keratocytes. All stromal cells strongly expressed CDH10, CDH11, and TUBB. RT-PCR showed upregulation of OSIL, KIT, SIX2, BMI1, FLH1, NOTCH1, and B4GalT1 in stem cells compared to keratocytes, suggesting a neural-crest progenitor phenotype. **Conclusions.** Comparison of gene expression profiles of stromal and epithelial stem cells shows a clearly divergent phenotype for the two populations in spite of their close spatial localization in the corneal limbus. Anterior keratocytes undergo apoptosis and repopulation after epithelial damage. We hypothesize that stromal stem cells serve as progenitors for this regeneration process.

Supported by NIH Grants EY013806, 30-EY08098, Research to Prevent Blindness.

EMMPRIN/CD147 Promotes Myofibroblasts Differentiation By Inducing aSMA Expression And Collagen Gel Contraction: Implications In Tissue Remodelling. Eric E Gabison[1,5], Eric Huet[1], Benoit Vallée[1], Dominika Szul[1], Franck Verrecchia[2], Samia Mourah[3], James V Jester [4], Than Hoang-Xuan[5], Suzanne Menashi[1]. [1]CRRET laboratory, CNRS UMR 7149, University Paris XII, 94010 Créteil, France. [2]INSERM U 697, Hôpital Saint-Louis, Paris, France. [3]INSERM U716, Laboratoire de Pharmacologie, Hôpital Saint-Louis, Paris, France. [4]Department of Ophthalmology, University of California at Irvine, Irvine, California. [5]Department of Ophthalmology at Fondation Ophtalmologique A. de Rothschild and Bichat Hospital, Paris, France.

Purpose. EMMPRIN is a cell surface glycoprotein enriched on tumor cells and normal epithelia. It is mainly known for its ability to induce MMPs production in fibroblasts following epithelial stromal interaction. We sought to examine whether EMMPRIN has a broader role promoting fibroblasts to myofibroblasts differentiation. **Methods.** Since aSMA is considered a marker of this differentiation process, we analysed the effect of EMMPRIN on its expression in corneal and skin fibroblasts by western blots, immunocytochemistry and by a functional assay of collagen lattice contraction. **Results.** Increasing EMMPRIN expression by cDNA transfection or by treatment with exogenously added recombinant EMMPRIN resulted in an upregulation of aSMA expression. EMMPRIN also increased the contractile properties of the treated fibroblasts as demonstrated by the immunohistochemical appearance of stress fibres and by the accelerated contraction of fibroblasts embedded collagen lattices. Blocking EMMPRIN expression by siRNA inhibited aSMA and collagen gel contraction induced not only by EMMPRIN but also by TGFb, a major mediator of myofibroblast differentiation that also regulated EMMPRIN expression. **Conclusions.** These findings, and the fact that EMMPRIN and aSMA colocalised to the same cells in the stroma of pathological corneas, expand on the mechanism by which EMMPRIN remodels extracellular matrix during wound healing.

Modelling Tear Volume And Osmolarity In The Normal And The Dry Eye. Eamonn Gaffney,[1] John M. Tiffany [2] Anthony J. Bron, [2] Mathematical Institute[1] and Nuffield Laboratory of Ophthalmology, [2] University of Oxford, UK.

Purpose. To develop a mathematical model of tear volume and osmolarity in relation to the mechanism of damage and clinical features of dry eye disease. **Methods.** Considering the conservation of tear solute and fluid mass for each of the tear compartments (tear film, menisci and conjunctival sacs) a mathematical model can be formulated using parameters derived from the literature. **Results.** Modelling of the normal eye demonstrated a differential molarity between the tear meniscus and the tear film, with a higher molarity in the film than in the meniscus, as predicted by the *compartmental hypothesis* (Bron et al. 2002). This differential was increased in dry eye and substantially more so in evaporative dry eye (EDE) than in aqueous-deficient dry eye (ADDE). **Conclusions.** These findings suggest that in dry eye states, nanolitre samples from the tear meniscus may not truly reflect the level of hyperosmolarity to which the ocular surface is exposed. This could explain clinical features such as the distribution of ocular surface damage and the stimulation of pain. Thus sensory responses to hyperosmolar solution occur at molarities well above the diagnostic cutoff (Chen et al ARVO 2007, abstract 427). Also, for a given level of meniscus hyperosmolarity, the higher tear film molarity in EDE may expose the ocular surface to a greater risk of damage than in ADDE.

Disclosure: E. Gaffney, N; JM Tiffany, N; AJ Bron, I, P

Human Mast Cell Chemokine Production: Effects Of Anti-Allergic Drugs. Grazyna Galatowicz[1], Samantha W.-Y.Chan[1], Michael E. Stern[2] & Virginia L. Calder[1]. [1]UCL Institute of Ophthalmology, London, UK; [2]Allergan Inc., CA.

Purpose. Conjunctival mast cells (MC) are important effector cells in ocular allergy and secrete a range of mediators. Human cord blood-derived mast cells (CBMC) are a useful in vitro model as they are phenotypically and functionally similar to conjunctival MC in their response to IgE stimulation. The aim of this study was to characterise CBMC chemokine profiles and the effects of anti-allergic drugs on chemokine profiles. **Methods.** Human cord blood CD34+stem cells (105) were cultured in 200ul Stemspan™ medium containing SCF [100ng/ml], IL-6 [50ng/ml] and IL-3 [1ng/ml], adding 10% fetal calf serum on week 9. On week 11, differentiated CBMC were confirmed by coexpression of CD117 (c-kit) and Fc_R1. For cross-linking, CBMC were incubated with 4_g/ml IgE for 16h before 25_g/ml anti-IgE Ab was added and culture supernatants harvested at 24h. Multiplex bead arrays were performed to detect CCL5 (RANTES), CXCL10 (IP-10), CCL2 (MCP-1), CCL3 (MIP-1_), CCL4 (MIP-1_), CCL11 (eotaxin-1), CXCL9 (Mig) and CXCL8 (IL-8) by flow cytometry. Anti-allergic drugs (azelastine, epinastine, ketotifen, nedocromil, olopatadine) were added to cultures 30 min prior to anti-IgE Ab. **Results.** CBMC were immunophenotyped as 100% c-kit+ and 90% Fc_R1+. Following Fc_R1 stimulation, secretion of CXCL8 (5.5 ± 0.2), CCL3 (0.2 ± 0.01), CCL4 (7.6 ± 0.5) ng/ml were detected in comparison with unstimulated cells [$p<0.001$], whereas CCL2 (9.5 ± 0.02 ng/ml) was present in unstimulated cells. CCL5, CCL11, CXCL9 and CXCL10 were undetectable. In comparison with controls, azelastine and epinastine pre-treatment decreased levels of CXCL8, CCL2, CCL3 and CCL4 ($P<0.01$); ketotifen and olopatidine decreased levels of CXCL8, CCL2 and CCL4 ($P<0.01$); nedocromil had no effect on chemokine levels. **Conclusions.** The CBMC secreted CCL2, CCL3, CCL4 and CXCL8 following IgE cross-linking, and the anti-allergic drugs all inhibited CCL2, CCL4 and CXCL8.

Ocular Surface Expression And Regulation Of β-Defensins. Fabian Garreis[1], Thomas Schlorf[1], Deike Varoga[2], Friedrich P. Paulsen[1]. [1]Department of anatomy und cell biology, Martin-Luther-Universität Halle-Wittenberg, Germany, [2]Department of anatomy, Christian-Albrechts-Universität Kiel, Germany

Purpose. Human β-defensins (hBD) are important antimicrobial peptides at the ocular surface. In this study the expression and inducibility of β-defensins at the ocular surface were investigated *in vitro* and *in vivo* after challenge with frequent ocular pathogens. **Methods.** The expression of human β-defensins was determined by RT-PCR and immunohistochemistry in tissues of the ocular surface and lacrimal apparatus. Three human cell lines, a sebocyte (SCL), a corneal (HCE) and a conjunctival epithelial cell line (HCjE) were treated with different concentrations of supernatants of heat-inactivated bacteria as well as cytokines (TNFα, IL-1β). Real-time PCR and ELISA experiments were performed to study the effect on the inducibility of hBD2 and 3. The expression and inducibility of mouse β-defensins-2, -3 and -4 (mBD2 – 4) were tested in a mouse ocular surface scratch model with and without treatment of supernatants of *Pseudomonas aeruginosa* (*PA*) by means of immunohistochemistry. **Results.** Results revealed constitutive expression of hBD1, -2 and -4 in conjunctiva and nasolacrimal ducts, but not of hBD3. Cornea and lacrimal gland only expressed hBD1. TNFα and IL-1β induced hBD2 and -3 expression and secretion in HCjE and HCE. Supernatants of *Staphylococcus aureus* (*SA*) and *Haemophilus influenzae* (*HI*) increased the relative expression of hBD2 and -3 mRNA but only led to an induction of hBD3 in HCE cells on the protein level. Data obtained in the *in vivo* scratch model revealed induction of mBD3 and -4 in corneal and conjunctivial epithelial cells but not of mBD2, if the ocular surface was scratched and got into direct contact with supernatant of *PA*. *PA* alone (without scratch) or scratching alone (without *PA*) did not significantly induce mBD3 and -4 production. **Conclusions.** Our results indicate specific expression and regulation of β-defensins against frequent ocular surface pathogens. Induction of β-defensins against *PA* only occurs if the ocular surface is damaged. In all other cases the content of antimicrobial substances in tear fluid seems to be highly effective in protecting against *PA* infection.

Expression Of The Carnitine Transporter OCTN2 In Ocular Epithelium. Qian Garrett[1], Shunjiang Xu[1], Peter Simmons [2], Joseph Vehige[2], Mark Willcox[1]. Institute for Eye Research, Sydney, Australia[1], Allergan Inc, Irvine, USA[2].

Purpose. OCTN2, the organic cation transporter novel type II, is a high affinity uptake system for carnitine and has been identified as a carnitine transporter. This organic cation transport process may exist in the ocular surfaces to facilitate the absorption of topically applied ophthalmic compounds that are positively charged at physiological pH, such as carnitine. The study was to examine ocular carnitine transport in relation to expression of OCTN2 in ocular surfaces using human ocular cell lines and rabbit corneal tissues. **Methods.** Human limbal-corneal epithelial (HCLE) and human conjunctival epithelial (HCjE) cell lines (kind gifts of Prof Gipson) were cultured to 70% confluence in GIBCO Keratinocyte SFM medium on chamber slides pre-coated with collagen I. Rabbit corneal tissues obtained from Garrett and her co-worker's other study using NZ white rabbits (Garrett 2007) were fixed and paraffin sections of 2µm were generated. Expression of OCTN2 protein was investigated by immunocytochemistry and immunohistochemistry using polyclonal antibodies from rabbits raised against the 15 C-terminal amino acids of human OCTN2 (a kind gift of Dr Kroemer, Kroemer 2005). Preimmune rabbit serum was used for negative controls. **Results.** Significant expression of OCTN2 in both human croneal or conjunctival epithelial cells and rabbit corneal epithelium was observed using the antibody against the carboxy terminus of OCTN2 protein. The specificity of this staining was demonstrated by the comparison of the cells or tissue sections incubated with the antibody and the preimmune serum. OCTN2 also appeared to localize predominately in the cell membranes. **Conclusions.** The presence of OCTN2 in ocular epithelium was detected, suggesting it might be involved in transport of carnitine on ocular surfaces. *[This research was sponsored by Allergan Inc, USA] (1Garrett et al. Invest Ophthalmol Vis Sci 2007;48(4):1559-67. 2 Grube et al. Drug Metabolism and Disposition 2005:33:31-37)*

Impact Of Administration Angle On The Cost Of Artificial Tear Solutions. Bruce I. Gaynes, Ramesh M. Singa, Gabriel Schaab, Yevgeniva Sorokin. Regenstein Eye Center of Rush University Medical Center, Chicago, IL, USA.

Purpose. Due to chronic dry eye therapy, drop size has an important role in the economics of tear replacements. We aim to measure drop volume differences of artificial tear products based on angle of administration and elucidate its impact in cost of dry eye therapy. **Methods.** Retail drug prices for study products were randomly sampled. The drop mass to 0.1mg was determined by holding a bottle at 45 and 90 degrees from horizontal and administering 10 drops into a dish. This process was duplicated on five bottles of each product. The density was then determined after measuring the total mass of a 100uL sample. **Results.** Differences in drop volume and drops per bottle as a result of dispensing solutions at 45 and 90 degrees were statistically significant for all products with the exception of CMC/hypromellose combination solution. The correlation between drop volume and density was however not significant (Spearman correlation, P=0.4500, alpha<0.05). All products, with the exception of the PEG/propylene glycol based solution demonstrated smaller drop volume when dispensed at a 45 degree angle. Only the PEG/propylene gylcol based product demonstrated a greater drop volume at 45 vs. 90 degrees ($p < 0.05$). Overall, drop size ranged from a high of 65.9 uL for the 1% CMC solution dispensed at 90 degrees to a nadir of 30.8 uL for the mineral oil product dispensed at 45 degrees. **Conclusions.** The results of this study are consistent with previous studies examining the relationship between drop volume and angle of administration that demonstrated larger drop masses of various artificial tear solutions when administered at 90 vs. 45 degrees. Predicted cumulative cost savings based on consistent drop administration at 45 rather 90 degrees over a 12-month interval of assumed four times-a-day bilateral administration resulted in savings of from $4.81 to $24.49 for the CMC/hypromellose and CMC 1% products respectively. The use of PEG/propylene glycol product at 45 rather than 90 degrees over the same time interval resulted in a $2.05 loss.
Research funded in part by Alimera Sciences, Alpharetta, GA, USA.

Time-Kill Assay Results For A Linalool-Based Eyelid Cleanser. Jeffrey P. Gilbard, MD, Department of Ophthalmology, Harvard Medical School, Advanced Vision Research, Woburn, Massachusetts, USA.

Purpose. Patients with dry eye have bacterial overgrowth on their eyelids, and lid hygiene has been recommended for these patients. Time-kill testing was performed for a linalool-based eyelid cleanser (PL) (SteriLid® Eyelid Cleanser, Advanced Vision Research, Woburn, MA) against pseudomonas aeruginosa (PA), moraxella catarrhalis (MC), e. coli (EC), serratia marcescens (SM), staph. aureus (SA), MRSA, staph. warneri (SW)and staph. epidermidis (SE) to evaluate its

antibacterial efficacy. **Methods.** 10% povidone iodine (PI), proprietary cleanser without linalool (P), and a commercially available eyelid cleanser were used as controls (CA). Stock cultures of each test organism were grown. An inoculum of 1 mL of each organism was added to 49 mL of each test cleanser and swirled to mix. At each exposure period (30 sec, 1 min. 5 min, 15min) 1 mL of sample was removed from each test mixture and added to 9 mL of neutralizer. 5 mL aliquots of this neutralized inoculated test mixture, as well as serial dilutions, were filter concentrated, plated out, incubated for 48 hr. and counted for colony forming units.
Results. At 1 minute, PL produced a 100% reduction in PA, MC, EC and SM, a 99% reduction in MRSA and SE, a 90% reduction in SA, and a 52% reduction in SW; PI produced 100% reduction in PA, MC, SM and SE, a 94% reduction in EC, 83% reduction in SW, a 78% reduction in SA, 55% reduction in MRSA. At 1 minute P and CA did not reduce SA. P had no effect on PA, CA and reduced PA by 40%.
Conclusions. The linalool-based eyelid cleanser showed a broad-spectrum of anti-bacterial activity, and at 1 minute this cleanser, was more effective than PI (10% povidone iodine) at killing MRSA, staph. aureus and e. coli. PL may prove to be a useful adjunct in dry eye management, as well as in blepharitis and other conditions where bacterial colonization and overgrowth on the eyelids is a concern.
Dr. Gilbard is Founder, CEO and Chief Scientific Officer of Advanced Vision Research.

Old Bugs And New: Classical And Emerging Pathogens. Michael S. Gilmore,[1,2] Susan Heimer, [1,2] Ai Yamada, [1,2] Irmgard Behlau, [1] and Keeta S. Gilmore.[1] Schepens Eye Research Institute,[1] Harvard Medical School,[2] Boston, MA, USA.

Purpose. As the most exposed wet mucosal surface on the human body, the eye is particularly vulnerable to physical insults from the environment as well as to bacterial pathogens. Not surprisingly, then, a large portion of clinical ophthalmology is focused on treating overt infections as well as infection-induced inflammatory conditions of the ocular surface. The purpose of this presentation is to re-examine the spectrum of bacterial agents causing ocular surface infection, and to review the evidence for association between pathogenic or commensal flora and inflammation of the ocular surface. **Methods.** In addition to standard surveillance methods, the ability of microbes to induce proinflammatory responses from the host was tested in vitro using a microarray of host response genes, and by ELISA for direct detection of inflammation mediators. Further, select strains were examined for the ability to produce biofilms. **Results.** The problem of ocular surface infection is becoming more complex because of the occurrence of new pathogens, and by the spread of new types of antibiotic resistance. Community acquired infection with methicillin resistant strains of *Staphylococcus aureus*, for example, is becoming common in the US and Europe. We are now also appreciating the importance of antibiotic resistant, non-typeable strains of *Streptococcus pneumoniae* in causing community conjunctivitis. **Conclusions.** As antibiotic resistance continues to spread from hospitals into the community, it will be increasingly imperative to emphasize surveillance and accurate diagnostics to insure successful treatment of ocular surface infection.
This research was supported by NIH grants EY08289 and EY017381.

Functions Of The Membrane-Associated Mucin MUC16 At The Corneal Surface. Ilene K. Gipson, Timothy D. Blalock. Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School, Boston, MA, USA.

Purpose. We have previously shown that MUC16, an ovarian tumor cell marker previously designated CA125, is highly expressed by the ocular surface epithelium with greatest expression by the corneal epithelium. We have also shown that MUC16's ectodomain is shed into the tears. The purpose of recent research in our laboratory has been to determine specific functions of the membrane-associated mucin on corneal epithelial cells and to determine factors that induce it's shedding into tears. **Methods.** To test functions of the mucin in human corneal epithelia, Retroviral delivery of siRNA's was used to stably knock down MUC16 in an immortalized human corneal-limbal cell line designated HCLE. Surface microplicae structure, rose Bengal dye penetrance and bacterial adherence was assessed in cells with MUC16 knockdown as well as in vector transfected and untreated control cells. To determine factors involved in MUC16 ectodomain shedding, a battery of potential sheddases was applied to cultured HCLE cells expressing the mucin at their apices. After sheddases were identified, the time required to replenish the surface mucin was assessed by biotinylation of HCLE cell surfaces cultured 1, 4, 6 and 24 hours after sheddase treatment, followed by densitometry of western blots to assess surface mucin content. **Results.** Knockdown of MUC16 expression in HCLE cells did not alter surface microplicae structure. The 80-90% reduction of MUC16 expression did, however, allow rose bengal penetrance into differentiated HCLE cells, and significantly increased bacterial (*Staphylococcus aureus*) adherence. Neutrophil elastase induced MUC16 ectodomain shedding without affecting the two other membrane-spanning mucins expressed by HCLE cells, MUC1 and MUC4. Twenty-four hours were required to replenish surface MUC16 levels to pretreatment levels. **Conclusions**. MUC16 provides a barrier at the corneal surface, preventing dye penetrance and bacterial adherence. Induction of MUC16 shedding by inflammatory cells may induce corneal surface damage.
Supported by an R01 grant from NIH-NEI to IKG and an NRSA fellowship grant from NIH-NEI to TB.

Evidence For Two Binding Sites In Tear Lipocalin. Ben J. Glasgow,[1,2] Oktay K. Gasymov,[2] Adil R. Abduragimov,[1] Jules Stein Eye Institute,[1] Departments of Ophthalmology,[1] and Pathology and Laboratory Medicine,[2] UCLA School of Medicine, Los Angeles, CA.

Purpose. To characterize binding sites and modes of binding for tear lipocalin (TL). **Methods**. ANS (8-anilino-1-naphthalenesulfonic acid) binding to apo-TL was studied by steady state and time-resolved fluorescence. Fluorescence decays of samples with various ANS/protein ratios were measured. Competitive displacement assays were performed with lauric acid and its analogs that carry double negative, double positive (one at each end of the acyl chain) and single positive charges. Resonance energy transfer from single Trp (donor) mutant proteins of TL to ANS was used to establish proximity of the ligand to amino acid residues. **Results.** Deconvolution of ANS binding into lifetime components reveals two binding modes. The binding affinities for the two modes differ greatly from each other. At pH 7.3, 16.99 ns and 2.76 ns lifetime species of ANS bind to apoTL with dissociation constants of 0.58 _M and 5.7 _M, respectively. At pH 3, despite the increased steady state intensities, the 16.04 ns and 6.30 ns lifetime components show decreased affinities, dissociation constants of 2.42 _M and ~21_M, respectively. The selective displacement of ANS molecules representing the long lifetime component from ANS-apo-TL complex by stearic acid discriminates the internal binding site. Low pH and high ionic strength affect differently the two ANS binding sites. Site-directed mutagenesis and ANS binding data demonstrate that the sulfonate group of ANS interacts with neighboring residues Lys114, His84 and Glu34. Resonance energy transfer from Trp to ANS indicates that the naphthalene group of ANS is proximal to Leu105 in the cavity. **Conclusions**. The Lys 114, His84, Glu 34 cluster of

residues may play a role in the ligand recognition site for some negatively charged ligands. TL can also interact, albeit relatively weakly, with fatty acids oriented in the opposite direction, so that the negatively charged carboxyl group points toward the cavity. This interaction may fit a model in which TL stabilizes the surface lipid layer of tears.
Supported by EY11224 and the Edith and Lew Wasserman Professorship.
Commercial Relationships- None

Ocular Surface Sensitivity And Symptoms In Contact Lens Wear.
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Purpose. This study aimed to investigate whether objective measurement of ocular surface sensitivity is a predictor of subjectively reported ocular discomfort symptoms such as those experienced during contact lens wear. **Methods.** 27 long term (13M:14F, age 40.1±6.9yrs, wear exp. 13.5±4.7yrs) extended wearers of low Dk/t soft contact lenses ceased lens wear for 1 week prior to transfer into high Dk/t silicone hydrogel lenses. Corneal and conjunctival sensitivity was measured using the CRCERT-Belmonte aesthesiometer during low Dk/t wear, following 1wk of no lens wear, and after 1, 3, 6 and 12 months of high Dk/t wear. Subjects completed CLDEQ and DEQ symptom questionnaires at each timepoint. Symptom scores were evaluated overall and for specific symptoms and examined with respect to frequency, intensity and diurnal effects. Repeated measures and Friedman ANOVA were used to examine sensitivity and symptoms respectively. Associations were examined using Spearman's rho correlation. **Results**. Corneal sensitivity reduced significantly following 1wk of no lens wear ($p=0.03$) and remained diminished after transfer into high Dk/t lenses. Conjunctival sensitivity did not change but was significantly lower than corneal sensitivity ($p= 0.001$). Overall symptoms scores were reduced after 1wk of no lens wear ($p<0.01$) but were not significantly different between low and high Dk/t wear. Significant differences between lens types were evident in the symptoms of eye discomfort, dryness and foreign body sensation and in the diurnal pattern of symptom occurrence. Although the reduction in corneal sensitivity after a week of no lens wear was positively associated with a reduction in dryness ($p<0.02$), inverse correlations were shown between sensitivity and other symptoms and only at sporadic timepoints. **Conclusions**. Contact lens wear induces concurrent changes in ocular sensitivity and in subjective symptoms, however a clear relationship between these factors was not evident in this study.

Rheological Effects On Tear Film Rupture.
Madhu S.R.Gorla[1] and Rama S.R.Gorla.[2] Rush University Medical Center, Chicago, IL, USA[1], Cleveland State University, Cleveland, OH, USA[2]

Purpose. The rupture of the pre-corneal tear film is an important phenomenon in various pathological states. Although tear films are thought to consist of shear thinning non-Newtonian fluids, previously stated investigations are confined to the kinematics and dynamics of a Newtonian fluid. The present work has been undertaken in order to investigate the hydrodynamics of the non-Newtonian mucus layer and its influences on the rupture of the tear film. **Methods.** A fluid dynamic model for the drainage of the aqueous layer is developed that includes rheological effects. The Ostwald de Waele type power law model is employed to model the tear film. The nonlinear evolution equation for the film is formulated using the balance equations including a body force term due to van der Waals molecular attractions, lubrication theory and perturbation expansion method. The governing equation was solved by the finite difference method as part of an initial value problem for spatial periodic boundary conditions. A long wave theory is formulated for the nonlinear dynamic instabilities of the thin film. **Results.** The tear film viscosity was modeled by a power law model using pseudoplastic, Newtonian and dilatant fluids. The film thickness is highest in pseudoplastic fluids and thinnest in dilatant fluids. The rupture time decreases as the disturbance amplitude increases. The results indicate that the rheological properties, interfacial tension and the initial disturbance amplitude have significant effects on tear film rupture. Tear film rupture may be delayed by using anti-surfactant which increases the aqueous-mucus interfacial tension. **Conclusions.** We have investigated the rupture of a thin tear film assuming that evaporation is minimal. We plan to explore scenarios when evaporation is suspected of contributing to dry eye. The methodology presented could be used to examine tear film evolution in the presence of a contact lens. Research into the effect of slip on the tear film rupture time will be the subject of future work as recent experimental evidence suggests that the no slip condition may not be suitable for hydrophilic flows over hydrophobic boundaries at the micro and nano scales.

Pathogen Or Commensal: A PCR Based Study Of Ocular Surface Bacterial Flora In Normal And Dry Eyes.
Joanna E. Graham,[1] Jonathan E. Moore,[1,2] Xu Jiru,[3] John E. Moore,[1,2] Edward Goodall,[1] James Dooley,[1] Darlene A. Dartt,[3] Stephen C. Downes,[1] Tara CB. Moore.[1] Centre for Molecular Biosciences, University of Ulster, Northern Ireland,[1] Royal Group Hospitals, Belfast, Northern Ireland,[2] Schepens Eye Research Institute, Boston, USA[4].

Purpose. To determine the ocular surface bacteria in normal and dry eye subjects and investigate whether there was any association between the bacterial population and goblet cell density (GCD). **Methods.** Ninety-one subjects, (n=57 normal and n=34 dry eye) were recruited. Conventional bacterial culture, 16S rDNA PCR and DNA sequencing were used for bacterial identification. The association between reduced GCD and bacterial numbers in a sub-group of 27 subjects was assessed. Conjunctival impression cytology (IC) samples were stained with PAS and GCD determined and graded as follows: Grade 1, >30 goblet cells/4 high power fields; Grade 2, 15-30 GC/4HPF; Grade 3, 5-15 GC/4HPF; Grade 4, <5 GC/4HPF. Grades 3 and 4 indicated reduced GCD. **Results.** The majority of cultured bacteria were coagulase-negative Staphylococci, while molecular methods demonstrated a number of additional atypical bacterial species. Bacterial levels were higher overall in subjects displaying reduced GCD compared to normal controls. A statistically significant difference ($P = 0.005$) was noted between mean bacterial counts in IC Grade 4 samples of dry eye subjects (35 cfu/swab) and normal controls (5 cfu/swab). **Conclusions.** Molecular analysis revealed a diverse ocular surface bacterial population with identification of various potentially pathogenic bacteria presenting a diagnostic dilemma. A trend of increasing bacterial numbers with reduced GCD was observed and studies are ongoing to investigate whether bacterial colonisation of the ocular surface may alter the number and function of goblet cells. The clinical relevance of such results is not yet fully determined and it is unknown whether they should prompt intervention with therapy. A fuller definition of the normal ocular flora is needed to determine bacteria implicated in ocular surface disease.
[Supported by the Department for Employment and Learning, Northern Ireland].

The Human Tear Film Proteome And Its Potential Application To Disease Biomarker Discovery. Kari B. Green-Church[§], Kelly K. Nichols, Paul Eichenseer[§], Richard Sessler[§], Nan M. Kleinholz[§], Jason J. Nichols. [§]Mass Spectrometry and Proteomics Facility, College of Optometry, The Ohio State University, Columbus, OH, USA.

Purpose. The purpose of this work is to examine the tear film proteome of healthy individuals and link changes of the proteome to disease with a focus on post-translational modifications related to various forms of dry eye disease. **Methods.** A variety of proteomic methods are employed including 1D-GE, 2D-GE, Differential Gel Electrophoresis (DIGE), multiplex analysis, LC-MS/MS. Proteins were extracted from tear samples collect by capillary and Schirmer strips to determine differences in the observed tear film proteins between the two collection methods. Glycosylation of the tear film was analyzed by treating the tear film with stepwise with PNGase, O-Glycosidase, _-2(3,6,8,9) neuraminidase, _(1-4)Galactosidase and _-N-acetylglucosaminidasean enzymes. Use of multiplex protein stains and DIGE labeling was used to elucidate changes in glycosylation patterns. **Results.** Analysis of proteins from tear film collected by capillary and Schirmer strip reveal significant differences. Forty-three proteins were identified by capillary collected tears while Schirmer strip identified 84 proteins (30 proteins overlap between the two collection methods). Deglycosylation of the N-linked sugars has a dramatic effect on the 2D GE pattern while removing O-linked sugars has less of an effect indicating that the majority of proteins in the tear contain N-linked glycans. Preliminary identification of these regions suggests that lactoferrin, Zn-a2-glycoprotein, albumin and the Ig proteins are heavily glycoslyated. These are similar findings in the analysis of serum. **Conclusions.** Proteomic methods were utilized to examine the proteins and post translational modifications found in the tear and differences are observed based on the method of collection. The proteomic methods utilized in this study are effective in examining protein profiles and each method provides a different insight regarding protein identification and modification analysis for disease biomarker discovery.
Commercial Relationships. None Support. None

Prevention of Exposure Keratopathy in Intensive Care Units. Darren G. Gregory. Rocky Mountain Lions Eye Institute. Denver, CO, USA

Purpose. Describe a comprehensive ocular surface evaluation and treatment algorithm developed to aid nursing staff in recognition of lagophthalmos and exposure keratopathy in the intensive care setting. **Methods.** An informational handout, illustrated evaluation flowchart with recommended interventions, and a photographic guide for application of a polyethylene moisture chambers is provided. **Results.** Development of this user-friendly, systematic protocol for ocular surface evaluation, combined with educational seminars, has increased nursing vigilance for potential ocular surface problems in intensive care patients at our institution. **Conclusion.** An educated nursing staff is the first line of defense against potentially severe ocular surface disease in the intensive care setting, particularly in ventilated patients. Appropriate consultation by the ophthalmology service is sought by following the guidelines on the evaluation flowchart. Our simple but effective protocol has been well received by the nursing staff and is a key piece in our total care of critically ill patients. [No financial interest or grant support to declare.
The author thanks the Rocky Mountain Lions Eye Bank for logistical support of this project]

Proteomics Study Of The Influence Of Contact-Lens Cleaning Solutions On The Protein Profiles In Tear Film. F. Grus, S Beyer, N Bozkurt, S. Haeder, N. Pfeiffer. Experimental Ophthalmology, Dept. of Ophthalmology, University of Mainz, Germany.

Purpose. To analyze the tear protein profiles of non-contact lens wearers, and of contact lens wearers using different multipurpose solutions (MPS) for cleaning and storage. **Methods.** _Wearers of soft contact lenses were recruited and allocated to use either Optifree Express, Optifree Replenish, or AMO Complete Plus for 4 weeks (n = 20 in each group). Tears were collected and analyzed before starting use of solutions, and at 1, 2, and 4 weeks after starting use. Tears were also collected and analyzed from 20 control patients (non-contact lens wearers), who were not exposed to either MPS. Specific protein biomarkers were found by means of ProteinChips (SELDI-TOF) and LC-MALDI with subsequent multivariate statistics and artificial neural networks, and identified using tandem mass spectrometry (LC-MSMS). **Results.** _Before starting use of solutions, tear protein composition in all contact lens wearers deviated from tear composition in normal controls (non-contact lens wearers). After 4 weeks using the different care regimens, tear protein composition of patients using OptiFree Express and OptiFree Replenish were further deviated from normal. In contrast, tear protein composition of patients using Complete Plus returned towards normal. In fact, the tear composition of over 50% of Complete Plus users was classified as "normal" rather than "contact lens wearer" at 4 weeks. Using Complete Plus, a decrease of inflammatory markers and an increase of potentially protective markers could be clearly demonstrated. **Conclusions.** _Contact lens wear alters tear protein profiles in a complex manner. The use of multipurpose solutions such as Complete can return the tear profile towards normal.
Supported in part by AMO (Advanced Medical Optics)

Tear Film Dynamics During Contact Lens Wear. Michel Guillon, Cécile Maissa. OTG Research & Consultancy, London, UK.

Purpose. The incidence of dry eyes amongst contact lens wearers is significantly higher than amongst non-wearers. The tear film stability (break up time) has been shown to be shorter in the presence of a contact lens than for the bare eye. Recently it has been suggested that tear film dynamics (e.g. the manner in which the tear film breaks up) may be different in dry eye sufferers than in normal. The aim of the paper was to measure the tear film stability and dynamics of a large population of contact lens wearers and non wearers. The hypothesis tested was that the tear film dynamics in the presence contact lenses is different than the tear film dynamics of the bare eye. **Methods.** Tear film stability (Non Invasive Break Up Time) and dynamics (Lipid and aqueous layer structures, Tear break up type & position) were evaluated using the Tearscope lighting system and biomicroscope. The subjects were divided into three groups: i) Contact lens wearers wearing contact lenses (CLW n=184 subjects) ii) Contact lens wearers having not worn contact lenses on the day of the visit (CLW no CL n=189 subjects) and iii) Non contact lens wearers (NW n=221 subjects). **Results.** In the presence of a contact lens both the lipid layer ($p<0.001$) (Thin: CLW 12.2% CLW no CL 0.5% NW 1.4%; Average: CLW 76.3% CLW no CL 44.4% NW 37.6%; Thick: CLW 11.5% CLW no CL 55.1% NW 61.0%) and the aqueous layer ($p<0.001$) (Thick: CLW 56.1%: CLW no CL 93.9%: NW 93.4%.); were significantly thinner than for the bare eye. The break observed was significantly ($p< 0.001$) more severe in the presence of a contact lens (Minimal break: CLW 26.1% CLW no CL 80.2% NW 79.5%; Total surface break: CLW 17.4% CLW no CL 0.5% NW 0.9%). The stability of the tear film in front of a contact lens (CLW NIBUT = 5.7s) is a third of that of the cornea ($p<0.001$) (CLW no CL = 14.9s; NW = 18.1s). **Conclusions.** The investigation revealed that the presence of a contact lens has more unwanted effects on the

tear film than reducing the break up time. The pre contact lens tear film is thinner and more destabilised once a break occurs than the pre-ocular tear film. The current findings have significant implications to solve the problem of contact lens related dry eyes.

Tear Volume Assessment Techniques – Repeatability In Normal Patients. Michel Guillon, Cécile Maissa, Aurélie Briffault. OTG Research & Consultancy, London, UK.

Purpose. The objectives of the investigation were: i. to optimise the experimental set up routine of two tear volume assessment techniques ii. to establish the precision of determining the tear volume of a normal population. **Methods.** Two techniques to assess the tear film volume were: i. Tear Prism Height (TPH) characteristics by digital photography using the Tearscope lighting system and subsequent analysis; ii. Phenol Red Thread Test (PRT)). Ten normal subjects were enrolled in this repeated measures investigation. Each subject was measured on two different days within a one-week period at the same time of the day (V1 & V2). From the digital photographs, several parameters were recorded to characterise the tear volume: Central height of tear prism, average height, minimum and maximum height, total area covered by tear reservoir. **Results.** The assessment of the tear volume was more repeatable by the characterisation of the TPH than by the PRT test. The repeatability of the TPH was, however, dependent upon the parameters measured: the poorest repeatability was recorded for the parameter usually reported in clinical study, Central Height (mm) (V1 vs.V2: Mean Difference 0.027[15.9%], SEM 0.009, 95% Confidence 0.009 to 0.046 p=0.006). The repeatability of the Mean Height (mm) of measurements every millimeter (V1 s.V2: Mean difference 0.008 [4.9%], SEM 0.007, 95% Confidence -0.007 to 0.024 p=0.265) and the Total tear prism area (mm2) (V1 vs.V2: Mean Difference 0.047 [5.9%], SEM 0.050, 95% Confidence -0.060 to 0.153 p=0.364) were good. The PRT test (mm) was poorly repeatable (V1 vs. V2: Mean Difference 4.9 [18.9%], SEM 1.5, 95% Confidence 1.9 to 7.9 p=0.003). **Conclusion.** The study showed that the improved measurement of the prism height using digital photography is a reliable technique to assess tear volume; the findings also indicate that average values rather than single point values are necessary to optimise the technique. The Phenol Red Thread Test revealed a high variability, possibly due to the invasive nature of the test. The data obtained permits the calculation of required sample sizes for future studies for all the tests evaluated.

Occult Thyroid Eye Disease In Patients Presenting With Dry Eye Symptoms. Anita Gupta[1], Pooyan B. Sadeghi[2], Esen K. Akpek.[1] Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD[1], University of Cincinnati, College of Medicine, Cincinnati, OH[2].

Purpose. To describe the clinical presentation, laboratory features, and orbital echographic findings in a series of patients with underlying occult thyroid eye disease (TED) who presented for evaluation of dry eye symptoms. **Methods.** A total of 539 new consecutive patients were evaluated, at a single referral-based dry eye center, over a period of 2 years. Twenty-one were diagnosed with occult thyroid eye disease based on typical findings of standardized orbital echography. Medical records of these patients were reviewed retrospectively to collect information on their demographics, clinical findings, thyroid function tests, antibody panel, and treatment results. **Results.** All patients presented with the symptom complex of dryness, foreign body sensation, redness, discomfort and excessive tearing. The median age of patients was 57 years (range 24 to 78 years), with the majority being female (86%). No patients carried a prior diagnosis of TED or had typical findings of TED such as proptosis, dysmotility, and diplopia at the time of presentation. Suspicion of TED was based on generalized chemosis with conjunctival/episcleral hyperemia mainly localized to the extraocular muscles. A subtle widening of the interpalpebral fissure was present only in 24% of the patients. Other clinical findings included corneal flourescein staining (57%), abnormal tear film break up time (31%), and abnormal Schirmer test (19%). Patients were treated topically using cyclosporine 0.05% bid to qid initially, with or without topical steroid. Other treatments were also employed as necessary including warm compresses, artificial tears, and puntal plugs. The majority of patients (76%) had significant improvement of their symptoms and were able to discontinue topical steroid treatment after a 4 to 6 week course. **Conclusion.** Occult thyroid eye disease is a potential cause of an inflammatory ocular surface disease with dry eye symptomatology and should be considered in the differential diagnosis when evaluating dry eye patients.
This research was supported by the Wilmer Residents Research Award.

Evaluation Of The Ocular Surface In Health And Disease - Based On Confocal In-Vivo Microscopy On The Way From Image Interpretation To Quantification. Rudolf F. Guthoff, Robert Kraak, Oliver Stachs, Joachim Stave, Andrey Zhivov, Universitats-Augenklinik, Rostock, Germany, Univ. Eye Hospital, Rostock Germany

Over the past two decades, the applications of in vivo confocal microscopy to the investigation of ocular surface diseases in the living eye have been greatly extended. Confocal microscopy enables detailed investigation of tarsal and palpebral conjunctiva, central and peripheral cornea, tear film and lids, and it allows evaluation of the ocular surface at the cellular level. High-quality imaging in both contact and non-contact modes has allowed new understanding of the functions of the ocular surface system, and in the coming years, such knowledge will become increasingly comprehensive and specific. Confocal microscopy may provide a link between well-established ex vivo histology and in vivo study of ocular pathology, not only in clinical science, but also in clinical practice. Especially antigen presenting cells, their density and localisation enable us to evaluate some aspects of immunological activity of ocular surface tissues. The purpose of the presentation is to summarize the current knowledge about in vivo confocal microscopy of the ocular surface.

A Correlation Between Fluorescein Corneal Staining And Ocular Discomfort In Patients Diagnosed With Dry Eye. Juan Guzman[1], Gary Foulks[2], Peter Zhang[1], Satoshi Nakatsu[1], Chau Whatley[1], Ayako Tano[1]. [1]Otsuka Pharmaceutical Development & Commercialization, Inc., University of Louisville, KY[2].

Purpose. To explore the relationship between categorical change in fluorescein corneal staining (FCS) and dry eye symptoms in patients diagnosed with dry eye. **Method.** Approximately 1500 subjects in 2 randomized, controlled, double-masked studies diagnosed with dry eye were evaluated for FCS Total Score (utilizing the NEI scale 0-15), primary ocular discomfort (POD) (0-4), lissamine green conjunctival staining (LGCS) and Tear break-up time at baseline and 12 weeks. Subjects identified their POD, which was the most bothersome symptom at screening and continued to rate the symptoms daily for 12 weeks with a 7 day average POD score 2 and more at baseline. **Results.** ITT analysis of subjects that had a reduction of ≥5 points from baseline in FCS Total Score (responders) had a statistically significantly lower POD score than those who did not achieve a decrease of at least 5 points in their FCS score (non-responders). 34% of subjects in the responder group had a POD score ≤1 compared to 19% in the non-

responder group (p=0.0001) in the first trial and 28% in the responder group and 16% in the non-responder group (p=0.0023) in the second trial. Additionally, responders had a statistically significant lower score in the LGCS compared to non-responders. **Conclusions.** The data in the dry eye population studied demonstrates a clinically meaningful correlation between a reduction in corneal staining and improvement in patient symptoms. A reduction of ≥5 points from baseline in FCS total score is associated with an improvement in both signs and symptoms of dry eye and may be considered a valid endpoint for clinical trials of dry eye.

Development And Application Of Tear Lipidomics In Mass Spectrometry. Bryan M. Ham Pacific Northwest National Laboratory, Richland, WA USA

Abstract. Lipidomics, the study of the lipid profile (or lipidome) of a biological system and the processes involved in the organization of the lipid species present including the signaling processes and metabolism of the lipids is increasingly being applied to the ocular system. The thin film lipid layer that is the outer layer of the tear film covering the eye primary function is to reduce the amount of evaporation of the inner aqueous layer. Early studies of tear lipid were typically conducted using chromatographic methods (i.e., thin layer chromatography, gas chromatography, high performance liquid chromatography). The major classes of lipids present (wax esters, cholesterol, acylglycerols, and phospholipids) were characterized and quantitated, however, since then advances in more contemporary analytic methods such as electrospray mass spectrometry are now adding new insight into the lipids associated with the tear film. Some advantages of mass spectrometry include the ability to structurally identify the individual lipid species present giving specific information of the tear film lipidome makeup, and the ability to qualitatively and quantitatively compare expressions of the tear film under conditions of a normal state to that of a diseased state such as dry eye syndrome. A primary future direction of lipidomics is to apply mass spectrometry for lipid expression studies of the eye under normal and stressed states. This next step entails the profiling and fingerprinting of the normal state of the eye in conjunction with the search for early onset and detection of an abnormal state to advanced diseased state biomarkers using the highly efficient separation methodology of nano-liquid chromatography coupled with high resolution/high mass accuracy mass spectrometry.

Relaxin, Relaxin-Like Factor And Relaxin-Like Receptors LGR7 And LGR8 At The Ocular Surface. Ulrike Hampel[1], Thomas Klonisch[2], Friedrich Paulsen[1]. [1]Department of Anatomy and Cell Biology, Martin Luther University Halle-Wittenberg, Germany, [2]Department of Human Anatomy and Cell Science, University of Manitoba, Winnipeg, Manitoba, Canada

Purpose. To analyse the relaxin-like ligand-receptor system, i.e. human relaxin 2 (RLN2), relaxin-like factor INSL3 and their cognate relaxin-like receptors LGR7 (RXFP1) and LGR8 (RXFP2), at the ocular surface, the lacrimal apparatus, and in ocular surface-derived epithelial cell lines. **Methods.** Human tissue samples were obtained from surgical and postmortem tissues and processed for RT-PCR and immunohistochemistry. Transcriptional gene activity for human RLN2, INSL3, LGR7 and LGR8 was monitored by RT-PCR in human ocular surface and lacrimal apparatus tissues and in human corneal (HCE) and conjunctival (IOBA-NHC) epithelial cell lines. Specific antisera were employed to determine the presence and tissue distribution of immunoreactive RLN2, LGR7 and LGR8. **Results.** Transcripts for RLN2 and INSL3 were observed only sporadically in some lacrimal and Meibomian glands, as well as in some conjunctivae, corneae and nasolacrimal ducts. Immunohistochemistry revealed production of RLN2 protein in all investigated tissues. Transcripts and protein for RLN2 receptor LGR7 were detected in Meibomian gland and nasolacrimal ducts. Transcripts and protein for LGR7 were not observed in lacrimal gland, cornea and conjunctiva. RT-PCR demonstrated expression of INSL3 receptor LGR8 in lacrimal gland, Meibomian gland, conjunctiva, cornea and nasolacrimal ducts. Moreover, specific transcripts were detected for all, RLN2, INSL3, LGR7 and LGR8 in the human conjunctival and corneal epithelial cell lines. **Conclusion.** mRNA expression of RLN2 and INSL3 occurs only in a few samples of ocular surface and lacrimal apparatus tissues. In contrast, detection of their respective proteins in these tissues may be explained by the transport of RLN2 and INSL3 via the blood stream. Presence of LGR7 in nasolacrimal ducts and Meibomian glands as well as LGR8 in all tissues of the ocular surface and lacrimal apparatus suggests a role for RLN2 and INSL3 in ocular surface homeostasis. Both, human conjunctival and corneal epithelial cell lines investigated may serve as models to elucidate possible roles of the relaxin-like ligand-receptor system at the ocular surface.

Mucin Structure And Function – State Of The Art. Gunnar C. Hansson, M.D., Ph.D. Department of Medical Biochemistry, Göteborg University, Medicinaregatan 9A, 413 90 Gothenburg, Sweden

Mucus and their main constituent, the mucins, are instrumental for the lubrication and clearing of the ocular surface as well as all other mucosal surfaces of the body. The mucins have common structures characterized by their heavily O-glycosylated mucin domains that hold water at the same time as it traps bacteria and other components. Other domains of mucins, both the membrane bound and the secreted gel-forming mucins, are also typical for this class of molecules and can be traced back to early days of metazoan evolution. The gel-forming mucins, including the MUC5AC mucin, are assembled into large complex polymers within the goblet cells. Recent understanding of the gel forming mucins as obtained from the intestinal tract can further illustrate the function of these mucins. In addition to the discussed typical roles of mucins, the transmembrane ones probably also have regulatory roles as suggested from structural studies of the SEA domain of the MUC1 mucin.

Surface Tension Reducing And Host Defense Functions Of Lung Surfactant. Sam Hawgood, University of California San Francisco, San Francisco, California, USA.

In the gas-exchange regions of the lung a large, wet surface area is exposed to more than 10,000 liters air a day including all the particulates, allergens, and microbes in ambient air. The surface forces associated with the extensive air-fluid interface play a major role in the compliance or pressure-volume characteristics of the lung. Pulmonary surfactant forms an insoluble film at the surface of the alveolar lining fluid and reduces the surface tension to allow normal breathing. The reduction of surface tension at the alveolar surface promotes lung expansion on inspiration and prevents lung collapse on expiration. The importance of a functioning surfactant system to lung function is perhaps best demonstrated by the acute respiratory failure secondary to the atelectasis that often accompanies premature birth prior to the maturation of the surfactant system and the dramatic response of some of these infants to exogenous surfactant. Surfactant is composed of structurally heterogeneous phospholipid-rich lipoproteins secreted by specialized alveolar epithelial cells. In the alveolus surfactant structures include the contents of the lamellar inclusions in pulmonary

type II epithelial cells, an unusually ordered vesicular structure called tubular myelin, the surface film itself and other vesicular and lamellar structures with quite varied dimensions and structures. These surfactant particles are components of a complex metabolic cycle that includes alveolar epithelial cells and the alveolar macrophage. Four apoproteins associated with the lipids of pulmonary surfactant regulate the surfactant metabolism and function and assist in maintaining the alveolus free of infection and inflammation. Two surfactant proteins (SP) are small hydrophobic proteins (SP-B and SP-C) that modulate surfactant lipid packing and catalyze the critical step of alveolar surface film formation. Mutations in these genes lead to severe forms of acute and chronic lung disease. For optimal gas exchange function the fragile membranes of the alveolus must also remain free of inflammation and infection. Two apoproteins, SP-A and SP-D, members of the collectin subgroup of the C-type lectin super-family participate in the innate immune defense of the lung as well as other mucosal surfaces exposed to the external environment including the eye.

New Formulation Based On Liposomes For Dry Eye Treatment. Tolerance Studies. Rocio Herrero-Vanrell[1], Marta Vicario[1], Beatriz de las Heras[2], Natalia Girón[2], Assumpta Peral[3], Irene T. Molina-Martínez[1]. [1]Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad Complutense, Madrid, Spain. [2]Departamento de Farmacología, Facultad de Farmacia, Universidad Complutense, Madrid, Spain. [3] Departamento de Bioquímica y Biología Molecular IV, E.U. de Optica, Universidad Complutense, Madrid, Spain.

Purpose. Characterisation and tolerance studies of a new formulation able to replace a disturbed preocular tear film. **Materials and Methods.** Phospholipon 90G containing > 95% of phosphatidilcholine (PC) purified from soy lecithin was purchased from Phospholipid GmbH (Cologne, Germany). Cholesterol, Vit E (α-tocopherol) and Trehalose were purchased from Sigma Chemical Co. (St. Louis, Missouri), Hyaluronic acid Ophthalmic grade (Mw 400.000-800.000Da). Liposomes were prepared from PC, Cholesterol and vitamin E (8:1:0.08). The vesicles were dispersed in a hypotonic solution of hyaluronic acid and trehalose. Tolerance of the formulation was evaluated in Human Inmortalized-Limbal Epithelial Cells (HCLE) at 1 day, 1 month and 2 months after storage (5ºC). Cytotoxicity studies were assessed by the mitochondrial-dependent reduction of the (tetrazolium salt 3 (4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) [MTT] to formazan. Studies were carried out at short (15minutes) and long term (2 and 4 hours) exposures. Viability was set as 100% in untreated cells. **Results.** The formulation resulted in a surface tension value of 25.5 (±0.3)mN/m, pH 7.04 (±0.5) and osmolarity 188.5 (±0.4) mOsm/L . The size of the lipid vesicles was in the range 392nm and 478.5nm. In all cases cell viability resulted higher than 80%. **Conclusions.** The new formulation presents good properties to be employed as a preocular tear film substitute in dry eye.
Acknowledgements: Research Group UCM (CAM 920415, CCG06-UCM/BIO-1304).

Selenoprotein P Protect Production Of The Oxidative Stress In The Cornea Of The Dry Eye Model Rat. Akihiro Higuchi[1], Yuri Okubo[1], Kazuo Tsubota[1,2] 6N9 Research Park [1],Department of Ophthalmology[2], Keio University School of Medicine, Tokyo Japan.

Purpose. Since autologous serum is useful for the treatment of severe dry eye, serum components may be a potential candidate for the treatment of dry eye. We found selenoprotein P (SeP), which is a new candidate for the treatment of dry eye, from serum components. SeP contains 10 Selenocysteine (Sec) residues, which is essential amino acid of glutathione peroxidase (GPx) activity, and transport Sec to peripheral tissues. GPx catalyze hydorgen peroxide and lipid peroxides. **Methods.** Conjunctival epithelial cell line, CCL20.2 (CCL) requires 10% fetal bovine serum (FBS) in the medium to survive. When serum is removed from the culture medium, CCL undergo apoptosis. Human serum was fractionated by column chromatography. Each fraction was added to the medium in exchange for FBS to assay the ratio of apoptosis of the cells, which is an index of purification. Finally, since we identified SeP as a candidate, we tried to apply SeP to dry eye model rat. Dry eye model rat was prepared by removing both lacrimal glands from SD rat. To estimate a degree of dry eye condition, cornea of both eyes were stained with fluorescein, and stained-area was scored after 4 week's treatment. Right eye of rat was treated with SeP and the other eye was treated with PBS. **Results.** Fluoescein score of PBS-treated cornea was 7.6 $\pm$ 3.6. Fluoescein score treated with SeP was significantly lowed (2.4 $\pm$ 2.2). SeP suppressed production of 8-hydroxy-2'-deoxyguanosine, which is the oxidative stress marker, in the cornea. Treatment with fragment of C-terminal of SeP, which contains 9 Sec residues, also suggest same effect as treatment with whole SeP. SeP treatment suppressed increment of fluorescein score induced by removing lacrimal glands, which may be involving in oxidative stress. **Conclusions.** It was suggested that SeP was a useful candidate for the treatment of dry eye.

$P2x_7$ Purinergic Receptors Active P42/P44 Mapk And Stimulate Protein Secretion From Rat Lacrimal Glands. Robin R. Hodges, Marie A. Shatos, Joanna Vrouvlianis, and Darlene A Dartt. Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School. Boston, MA.

Purpose. To determine the effects of agonists of $P2X_7$ purinergic receptors on p42/p44 MAPK activity and protein secretion from rat lacrimal gland. **Methods.** Acini were isolated by collagenase digestion and preincubated with the specific P2X7 receptor inhibitor, brilliant blue G (BBG, 10^{-5}-10^{-7} M) or the MAPK inhibitor U0126 (10^{-6} M) for 15 min. Acini were then stimulated with the specific $P2X_7$ receptor agonist benzoylbenzoyl-ATP (BzATP (10^{-4} M) for 5 min to measure activation of p42/p44 mitogen-activated protein kinase (MAPK) or 40 min for protein secretion in buffer without Mg^{2+}. Western blot analysis was performed using antibodies to phosphorylated (active) and total p42/p44 MAPK. Peroxidase, a marker of protein secretion was measured after stimulation using Amplex Red. Results after incubation with no additions (basal) was set to 1. **Results.** BzATP increased phosphorylation of MAPK by 1.4 ± 0.1 fold above basal. This increase was inhibited by BBG to 1.2 ± 0.2 fold at 10^{-7} M and 1.1 ± 0.1 fold at 10^{-6} M, and significantly decreased to 1.0 ± 0.2 fold at 10^{-5} M, when compared to basal. BzATP increased peroxidase secretion 1.2 ± 0.06 fold above basal. This increase was not inhibited by BBG at 10^{-7} M, but was decreased to 1.1 ± 0.01 at 10^{-6} M and was significantly decreased to 1.0 ± 0.02 fold above basal at 10^{-5} M. BzATP-stimulated peroxidase secretion was significantly increased from 1.2 ± 0.04 to 1.6 ± 0.2 fold above basal after preincubation with U0126. **Conclusions.** We conclude that the lacrimal gland contains functional $P2X_7$ receptors that stimulate an increase in p42/p44 MAPK activation and protein secretion. Activation of MAPK negatively modulates BzATP-stimulated secretion.
Supported by NIH EY06177.

Comparison Of Membrane-Associated Mucins Expression In The Human Ocular Surface And Oral Mucosal Epithelium. Yuichi Hori[1], Kohji Nishida[2], Hiroaki Sugiyama[1], Takeshi Soma[1], Shizuka Koh[1], Tomoyuki Inoue[1], Naoyuki Maeda[1], and Yasuo Tano.[1] Department of Ophthalmology, Osaka University Medical School, Suita, Osaka,[1] Department of Ophthalmology, Tohoku University Medical School, Sendai, Miyagi, Japan[2]

Purpose. Recent reports have described the use of cultivated oral mucosal epithelial sheet transplantation for the reconstruction of the ocular surface. We investigated the expression of membrane-associated mucins in human oral mucosal epithelial cells and compared with that in human ocular surface epithelial cells. **Methods.** Specimens (3mm x 3-mm) of oral mucosal tissue were harvested from healthy volunteers. The oral mucosal epithelial cells obtained from the specimens, corneal epithelial cells, and oral mucosal cell line (KB cells) were cultured together with mitomycin-C-treated 3T3 feeder cells on temperature-responsive culture surfaces for 2 weeks with the aim of producing stratified cell sheets. RT-PCR was used to determine the expression of membrane-associated mucins (MUC1, -3, -4, -12, -13, -15, -16, and -17) in these cell sheets. Sections containing the oral mucosal cells were subjected to immunohistochemical examination using MUC16 antibody (OC125) to determine the distribution of MUC16 protein in the cultivated oral mucosal epithelial sheets and human oral mucosal epithelium. **Results.** MUC1, -4, and -16, but not -3, -12, -13, -15, or -17 mRNA was detected in the oral mucosal epithelial as well as in the corneal epithelial sheet. KB cells could not produce a stratified cell sheet. MUC16 protein was localized in the apical cell layers of the cultivated oral mucosal and corneal epithelial sheets, but the human oral mucosal epithelium did not express MUC16 proteins in any cell layers. **Conclusions.** The membrane-associated mucins of the ocular surface, MUC1, -4, and -16, are also expressed in the human oral mucosal epithelial cell sheet. These membrane-associated mucins may thus contribute to the ocular surface reconstruction after oral mucosal epithelial sheet transplantation for patients with severe ocular surface disorders.
CR: none Support: Grant #18591920 from the Japanese Ministry of Education, Culture, Sports Science and Technology, Osaka Eye Bank Society Research Grant to YH.

Long Term Evaluation Of FCI Silicone Punctal Plugs In Dry Eye. Jutta Horwath-Winter, Eva-Maria Haller-Schober, Anna Gruber, Ingrid Boldin, Department of Ophthalmology, Medical University of Graz, Austria, _Auenbruggerplatz 4_, 8036 Graz_Austria _

Purpose. To evaluate long term retention rates, complications and efficacy of silicone punctal plugs among dry eye patients. **Methods.** Ninety five FCI silicone punctal plugs were placed in 93 eyes of 47 dry eye patients. Up to 8 years (median two years) of survey following these patients included recording of the retention rate and complications, as well as subjective dry eye symptoms, frequency of tear substitute application, tear film and ocular surface parameters. **Results.** The retention rate of the plugs was 84.2% after 3 months, 69.5% after one year and 55.8% after a median of two years. No infection was observed but granulomatous formation occurred in 3 eyes (1 with extrusion, 2 with intrusion). Three plugs had to be removed for local discomfort or epiphora, and one piece of a broken plug intruded. Subjective symptoms and frequency of tear substitute application were reduced, Schirmer test values without local anaesthesia increased and corneal and conjunctival staining were found reduced after 3 months, one year and after a median of two years. Impression cytology of the conjunctiva was found improved after a median of two years. **Conclusions.** The retention rate after a median of two years of FCI silicone punctal plugs was satisfactory with quite few complications. FCI silicone punctal plugs proved to be an efficient option in the treatment of dry eye.

Reduced Corneal Sensitivity In Patients With Primary Sjögren's Syndrome. Joon Young Hyon,[1,2,5] Yun Jong Lee,[1,3,5] Pil-Young Yun.[1,4,5] Seoul National University Bundang Hospital,[1] Department of Ophthalmology,[2] Department of Internal Medicine,[3] Department of Oral and Maxillofacial Surgery Section of Dentistry,[4] and Seoul National University College of Medicine,[5] Seongnam, Korea

Purpose. To compare the objective clinical signs, subjective symptom scores and the corneal sensitivity of primary Sjögren's syndrome patients with those of non-Sjögren dry eye patients. **Methods.** The objective clinical signs and subjective visual analog scale for dry eye symptoms were assessed in 26 eyes of 13 patients with primary Sjögren's syndrome and 26 eyes of 13 patients with non-Sjögren dry eye patients. The corneal sensitivity was measured using Cochet-Bonnet esthesiometer. **Results.** The rose bengal staining score was significantly higher in Sjögren's syndrome patients than non-Sjögren dry eye patients (2.0±1.7 vs. 0.5±0.6, p=0.001). Paradoxically, the visual analog scale for dry eye symptoms was worse with non-Sjögren dry patients (6.8±8.1 vs. 13.4±8.2, p=0.007). Corneal sensitivity was significantly reduced in Sjögren's syndrome patients (5.3±1.0 vs. 5.8±0.4, p=0.03). **Conclusions.** Better visual analog scale in the patients with Sjögren's syndrome in spite of more severe ocular surface change may be attributed to reduced corneal sensitivity. These findings suggest that the ocular surface of Sjögren's syndrome is under the neurotrophic state.
Commercial Relationships: None

MUC1 Gene Polymorphism In Dry Eye Patients. Yoannis Imbert, Gary N. Foulks, Mark D. Brennan, Marcia M. Jumblatt, George John, Hassan A. Shah, Catherine Newton, William W. Young, Jr. Schools of Dentistry and Medicine, University of Louisville, Louisville, KY, USA

Purpose. We reported that the frequency of non-Sjogren's aqueous deficient dry eye patients expressing only the MUC1/A splice variant may be lower than a normal control group (Imbert *et al* Exp. Eye Res. 83, 493-501, 2006). In the present study we determined the statistical significance of that observation and whether that difference reflected a difference in the MUC1 variable number of tandem repeat (VNTR) size class between normal control and dry eye patients. **Methods.** Non-Sjogren's aqueous deficient dry eye patients were identified as having both tear deficiency and ocular surface staining. Normal control subjects were age and gender matched. Genomic DNA was harvested from buccal swabs for single nucleotide polymorphism (SNP) analysis or from peripheral blood for Southern blotting, total RNA was extracted from conjunctival brush swabs for splice variant analysis by PCR, and tear samples were used for western blotting. A real time PCR SNP assay was used to determine the frequency of the SNP that controls the MUC1/A and MUC1/B splicing event. **Results.** Southern and western blotting indicated that the correlation between the MUC1/A or B splice pattern and VNTR size class held in less than 80% of the cases tested. Thus, the results did not support a difference of VNTR size class between dry eye patients and normal controls. In contrast there was a perfect correlation between splice pattern and SNP choice, and therefore, all subsequent patient samples were assayed by SNP analysis. There was a significant difference between non-Sjogren's aqueous deficient dry eye patients and normal controls in the frequency of the MUC1 SNP genotypes ($P= 0.03$; $x^2= 4.65$). **Conclusions.** The results suggest that individuals of the MUC1/A only genotype may be less

susceptible to developing non-Sjogren's aqueous deficient dry eye with ocular surface damage. The mechanism for this apparent protection from dry eye is under investigation.
Supported by NIH F31 EY017275 (to YI), RO1 EY017094 (to GNF), and RO3 EY015134 (WY).

The Impact Of A Near Task On Tear Stability. Meredith Jansen, Monica Bedroya, Carolyn Begley, Robin Chalmers. Indiana University.

Purpose. The purpose of this study was to determine whether near work destabilizes the tear film. **Methods.** Two µl of 2% sodium fluorescein were instilled into the left eye of 15 control (C) and 15 dry eye (DE) subjects, who were seated behind a slit lamp biomicroscope. Subjects were asked to keep one eye open as long as possible while the tear film was videotaped to assess tear break-up dynamics (TBUD) and tear break-up time (TBUT). After the initial measurement, the subject played a computer game requiring intense concentration for 20 minutes and then the procedure was repeated. Subjects completed current symptom questionnaires before and after testing. A custom MATLAB program was used to quantify the changing area of breakup (AB) over the maximum blink interval (MBI) for computation of TBUD. A masked observer measured TBUT from digital movies. **Results.** TBUD decreased in 20 (67%) and TBUT in 22 (70%) of subjects after the near task. Individual measures that increased significantly after the near task included the AB after 5 sec, the final AB (DE only), and the symptoms of discomfort and burning ($p<0.05$, Wilcoxon Signed Ranks test). However, DE subjects were significantly more symptomatic than C both before and after performing the near task ($p<0.003$, Mann-Whitney). TBUT correlated with TBUD measures (AB, slope, MBI), and the AB at 5 sec correlated with the symptom of discomfort (Spearman's rho $r<0.52$, $p<0.003$). **Conclusion.** Both TBUT and TBUD measures show that only 20 minutes of continuous near work led to a significantly destabilized tear film and increased symptoms. After performing the near task, subjects showed substantially accelerated tear breakup in only 5 sec, which may increase the risk for ocular surface exposure if the average blink rate is 12/min (once every 5 sec). This marked change in tear stability after a relatively brief near task may account for the worsening of symptoms of ocular irritation commonly noted later in the day among dry eye patients.

A Comparison Of The Effectiveness Of Eyedrops Containing Carbomer And Sodium Hyaluronate In The Treatment Of Moderate Dry Eye. Michael E Johnson,[1,2] Paul J Murphy,[1] Mike Boulton.[1,3] School of Optometry, Cardiff University, UK;[1] Bristol Eye Hospital, Bristol, UK;[2] University of Texas, Galveston, USA.[3]

Purpose. This study compared the effectiveness of two commercially available eyedrops containing either 0.3% Carbomer 934 or 0.18% Sodium Hyaluronate (SH) in treating dry eye. **Methods.** Sixty-five subjects with moderate dry eye were recruited, who, after a run-in period of 7–14 days, were randomly supplied with eyedrops containing either Carbomer or SH, in a double-masked manner, to use for a month. Principle outcome measures were the severity of symptoms of ocular irritation, measured with the Ocular Comfort Index (OCI), and corneal and conjunctival staining with Fluorescein and Lissamine Green, respectively. At the end of the experiment subjects were also asked, on average, how many times a day they used the treatment and the duration of any post-instillation blur. **Results.** Both Carbomer and SH reduced symptom severity and ocular surface staining. The treatment effects of Carbomer and SH were equivalent for symptoms, but, for both corneal and conjunctival staining, SH outperformed Carbomer in improving the integrity of the ocular surface. There was no difference in the average instillation frequency of the two eyedrops. Visual disturbance after treatment usage was generally short and not bothersome, however, lengthy periods of blur were significantly more common after the use of Carbomer. **Conclusions.** Both of the eyedrops trialled are suitable for patients with moderate dry eye, but of the two, the SH containing treatment has marginal benefits in therapeutic efficacy and in having less propensity to cause visual disturbance on instillation. *[This study was sponsored by TRB Chemedica, Geneva, Switzerland]*

Measurement Of Ocular Surface Irritation On A Linear Interval Scale With The Ocular Comfort Index (OCI). Michael E Johnson,[1,2] Paul J Murphy.[1] School of Optometry and Vision Sciences, Cardiff University, UK;[1] Bristol Eye Hospital, UK.[2]

Purpose. To examine the psychometric properties of the Ocular Comfort Index (OCI), a new instrument that measures ocular surface irritation. **Methods.** The OCI was designed using the methods of Rasch analysis. These are a group of probabilistic techniques that excel in producing linear estimates on an interval scale from ordinal observations of category responses. Here, the probability of an observed response is modelled as a function of the 'difficulty' of an item and the 'ability' of the respondent. A 15-item pilot questionnaire was self-completed by 452 subjects, from which poorly performing items were eliminated and the number of categories was optimized. In subsequent analyses, data from deleted items was ignored. A number of these individuals repeated the questionnaire to determine its reliability and test-retest repeatability. Additionally, three construct hypotheses were tested to verify that the questionnaire was measuring what was intended; concordance with the Ocular Surface Disease Index (OSDI), relationship with tear break-up time (TBUT) and its change after the use of ocular lubricants in individuals with moderate dry eye. **Results.** A 12-item OCI was developed with well functioning items and categories: 95% confidence interval for the intraclass correlation coefficient = 0.81 to 0.91; person separation = 2.66; item separation = 11.12; 95% repeatability coefficient = 13.1 units (0–100 scale). The OCI measures exhibited a positive correlation with the OSDI score ($P < 0.0001$) and a negative correlation with TBUT ($P < 0.0001$); and was able to detect the improvement in symptoms of individuals with dry eye before and after treatment ($P < 0.0001$). **Conclusions.** The OCI was shown to have favourable psychometric properties that make it suitable for assessing the impact of ocular surface disease on patient wellbeing. Its major benefits over existing instruments are that it produces estimates on a linear interval scale rather than ordinal ranks, so is better able to quantify change, and through statistical methods more satisfactorily accounts for missing data.

Establishment Of Primary Acinar Culture Cells Of Lacrimal Gland. Angélica Gobbi Jorge, Ana Carolina Dias, Leticia P. Roma, Carolina Maria Módulo, Rubens Bertazolli Filho, Eduardo M. Rocha. Departamento de Oftalmologia, Otorrinolaringologia e Cirurgia de Cabeça e Pescoço e Departamento de Clínica Médica da Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brazil

Purpose: Previous studies have shown that systemic diseases affect lacrimal gland function. Moreover, hormones and inflammatory mediators have been implicated in the pathogenesis; despite their direct action over acinar cells it is not well defined. To further understand the physiopathological steps of those diseases, our aim is to establish a lacrimal gland acinar cell culture line and to promote changes in culture conditions to evaluate the impact on secretory mechanisms. **Methods**.

Acinar cells of lacrimal gland of Wistar male rats were isolated and seed in culture plastic plates, pr treated or not with fibronectin 5 μg/cm^2 and maintained with culture medium DMEM. The adherence and presence of peroxidase and insulin in the supernatant, with or without Carbachol (100μM) stimulation was evaluated along of a culture period of 7 days. **Results.** There was no adherence in neither plastic or fibronectin basis. In both conditions the supernatant levels of peroxidase or insulin was not affected by Carbachol stimulation. **Conclusions.** Our work confirms that extra cellular matrix is a key element in maintenance and function of lacrimal gland cells. Further studies are necessary to correlate culture conditions with biomarkers expression in order to follow lacrimal gland acinar cells function in culture.
CAPEs, CNPq, FAPESP

Quantitative Analsyis Of Conjunctival Goblet Cells After Exposure To Latanoprost With 0.02% Benzalkonium Chloride, Travoprost With Sofzia, Or Preservative Free Artificial Tears. Malik Y. Kahook MD[1], Robert J. Noecker MD MBA[2]. University of Colorado Health Sciences Center[1], University of Pittsburgh Medical Center[2]

Purpose. To compare changes in the number of goblet cells after exposure to latanoprost preserved with 0.02% benzalkonium chloride (BAK), travoprost preserved with Sofzia, and preservative free artificial tears. **Methods.** Fifteen New Zealand white rabbits were randomized into groups of five and treated with once daily topical application of one of the three drops for thirty days. Enucleation was performed at the end of the study followed by histologic analysis using mucin stains to identify goblet cells. Goblet cells were quantified and analyzed using student t-tests to compare means between groups. **Results.** Goblet cells per high power field were 2.21 (+/-0.40) in the latanoprost with BAK group, 6.02 (+/-1.20) in the travoprost with Sofzia group, and 7.03 (+/-1.33) in the preservative free artificial tear group. The number of goblet cells in the latanoprost with BAK group was significantly lower than the other two groups (p=0.0001). There was no statistically significant difference in goblet cell number between the travoprost with Sofzia and preservative free artificial tear group (p=0.24). **Conclusions.** Once daily dosing of latanoprost with 0.02% BAK produced more goblet cell loss compared to either travoprost with Sofzia or a preservative free artificial tear.
Financial support was received from ALCON for this study.

Quantitative Analsyis Of Corneal Epithelial Desmosomes After Exposure To Latanoprost With 0.02% Benzalkonium Chloride, Travoprost With Sofzia, Or Preservative Free Artificial Tears. Malik Y. Kahook MD[1], Robert J. Noecker MD MBA[2]. University of Colorado Health Sciences Center[1], University of Pittsburgh Medical Center[2]

Purpose. To compare changes in the number of corneal epithelial desmosomes after exposure to latanoprost preserved with 0.02% benzalkonium chloride (BAK), travoprost preserved with Sofzia, and preservative free artificial tears. **Methods.** Fifteen New Zealand white rabbits were randomized into groups of five and treated with once daily topical application of one of the three drops for thirty days. After harvesting the central cornea of each rabbit, transmission electron microscopy was performed. The number of desmosomal attachments per photomicrograph (X3400) was quantified and compared using students t-test to compare means between groups. **Results.** The average desmosome number was 0.73 (+/-0.39) in the latanoprost with BAK group, 5.22 (+/-1.26) in the travoprost with Sofzia group, and 6.45 (+/-1.72) in the preservative free artificial tear group. The number of desmosomes in the latanoprost with BAK group was significantly lower than the other two groups (p=0.0001). There was no significant difference in desmosome number between the travoprost with Sofzia and preservative free artificial tear group (p=0.23). **Conclusions.** Once daily dosing of latanoprost with 0.02% BAK produced more desmosomal loss compared to either travoprost with Sofzia or preservative free artificial tears.
Financial support was received from ALCON for this study.

Membrane-Associated Mucins Are Affected By Inflammatory Mediators On The Ocular Surface Epithelial Cells. Vinodh Kakkassery, Sandra Spurr-Michaud, Beatrice Perez, Timothy Blalock, Ilene K. Gipson. Schepens Eye Research Institute, Harvard Medical School, Boston, MA, USA

Purpose. Membrane-associated mucins are altered on the ocular surface of non-Sjögren's dry eye patients. The objective of this study was to evaluate the effect of inflammatory mediators present in tears of dry eye patients on ocular membrane-associated mucins (MAMs) at the level of gene expression, protein biosynthesis or shedding using an immortalized corneal epithelial cell line (HCLE). **Methods.** Human corneal limbal epithelial cell line **(**HCLE) cells were grown to confluence, cultured for seven days in serum-containing media to induce differentiation, serum starved for 24 hours, then treated with interleukin (IL)-1α, 6 and 8, and tumor necrosis factor-α (TNF-α) for 1, 6 or 24 hours. Receptor mRNAs of these mediators were verified in HCLE cells by reverse transcriptional polymerase chain reaction. Effect of the mediators on membrane-associated mucin MUC1 and MUC16 gene transcription was evaluated by real-time polymerase chain reaction using TaqMan primers. Effects on protein translation and shedding of the mucins were detected by immunoblot analysis of MUC1 and MUC16 in cell lysates and culture media. **Results.** Expression of Interleukin-1 receptor I, Interleukin-6 receptor, Glycoprotein 130, Interleukin-8 receptor A and B, and Tumor necrosis factor receptor I and II was demonstrated in HCLE RNA by conventional RT-PCR. No significant alterations in MUC1 and MUC16 gene transcription or protein translation were seen after treatment of cells with IL-1α, IL-6, IL-8, or TNF-α in HCLE. After 24 hours of treatment, MUC1 protein shedding was significantly increased after treatment with IL-6 and both MUC1 and MUC16 protein shedding were significantly increased after treatment with TNF-α. **Conclusion.** The shedding rate of MUC1 and MUC16 is affected by IL-6 and TNF-α in HCLE cells. These results suggest a mechanism for the decreased H185-epitope (MUC16) amount in non-Sjögren's dry eye patients.
(This research was supported by a Sponsored Research Agreement from Alcon Laboratories)

Bone Marrow Cells Can Differentiate And Assume Keratocyte Characteristics Of Keratocan Expression In Mouse Corneas. Winston W.-Y. Kao[1,2], Hongshan Liu[1], Yasuhito Hayashi[1], Chia-yang Liu[1], Eric Carlson[3], Ophthalmology[1] and Cell and Cancer Biology[2], University of Cincinnati, Cincinnati Ohio, Ophthalmology[3], Case Western Reserve University, Cleveland, Ohio.

Purpose. Bone marrow stem cells (BMSC) are multipotent and capable of differentiating to various cell types, e.g., myocardial cells, hepatocytes, neurons, epidermal and gastrointestinal epithelial cells. In present studies, we examined whether BMSC could differentiate to keratocan expressing cells: a characteristic of corneal keratocytes. **Methods.** Bone marrow cells obtained from green fluorescent, Kera-Cre/ZEG, Kera-Cre/DTRred bitransgenic mice were intrastromally injected into corneas of Kera-/- knockout, and Kera-/-/DTRred, and

Kera-Cre/DTRred bitransgenic mice mice. Immunohistochemistry and western blot analysis with anti-keratocan antibodies were used to detect the expression of keratocan in the recipient Kera-/- mice. **Results.** Immunostaining confirmed that freshly isolated BMC were keratocan negative. Green BMC injected into corneal stroma began to change shape and assumed dendritic and polygonal morphology at one week of intrastromal injection. Keratocan expression was detected in stroma and around green BMC in the recipient corneas. To make sure that BMC can indeed differentiate to corneal keratocytes rather than fusion with recipient keratocytes, EGFP negative BMC isolated from Kera-Cre/ZEG mice were intrastromally injected to corneas of Kera-/-, Kera-/-/DTRred and Kera-Cre/DTRred mice. In 6 days, injected BMC became EGFP positive and displayed a dendritic and polygonal morphology, suggesting the modification of ZEG transgene by Cre recombinase driven by keratocan promoter in Kera-Cre/ZEG BMC injected. Image analysis with confocal microscope showed the segregation of red fluorescence from the green, indicating that the donor BMC cells underwent differentiation and exhibited keratocyte phenotype instead of fusion with host keratocytes. **Conclusion.** Corneal stroma provides an environment for BMSC differentiation to keratocyte.
Supported in part by grants NEI EY011845, Research to prevent Blindness, Ohio Lions Eye Research Foundation.

Enhanced Wound Healing Properties Of Serum Diluted With Platelet Releasate. Karsten Kasper,[1] Dirk Hartwig,[2] Thilo Wedel,[3] Stefan Schrader,[2] Tim Menke,[2] Gerd Geerling.[1] University Wuerzburg,[1] University Luebeck,[2] University Kiel,[3] Germany

Purpose. Serum eye drops (SED) are beneficial for the ocular surface because they support proliferation and migration of corneal epithelial cells (CEC). The positive effect is thought to be due to nutrient factors. Platelet releasate (PR) is a cell free solution produced from platelet concentrates. In-vitro, PR has an even higher effect on proliferation of CEC than SED but serum are superior in supporting migration. We investigated whether mixtures of PR and SED exhibit superior wound healing properties than PR or serum alone. **Methods.** PR and serum (n = 10) were prepared according to a standardised protocol. Serum was diluted with PR to different concentrations. SV-40 immortalised human corneal epithelial cells were cultured at 37°C, 5% CO_2. Proliferation was quantified by means of a luminescence-based ATP-assay. Cell migration was assessed in a colony dispersion assay. Differentiation was documented by scanning electron microscopy. Growth factors (e.g. EGF, PDGF, fibronectin) were quantified by routine ELISA technology. In a 61 year old female with a persistent epithelial defect due to 2° Sjoegren syndrome saline-diluted serum eyedrops were exchanged for PR-diluted serum. **Results.** After 6 hrs time of incubation PR-Serum supported proliferation better than 100% PR or 100% serum alone. Dilution of serum to 25% with PR did not reduce the positive effects on migration and differentiation of CECs. The epithelial defect in the patient that was recalcitrant to treatment with saline-diluted serum healed with PR-diluted serum. **Conclusions.** PR-diluted serum has a superior growth supporting effect on CECs than serum or PR alone. The beneficial effect of pure serum on cell migration was not reduced by dilution with PR. The use of PR to dilute serum to the concentration of choice may be advantagous for eyes in which surface disease persists despite treatment with conventional, i.e. saline-diluted serum eyedrops.

Endoscopy-Guided Vitreoretinal Surgery Following Penetrating Corneal Injury. Motoko Kawashima, M.D.[1], Shinichi Kawashima, M.D.[2], Jun Shimazaki, M.D[1]. From [1] Department of Ophthalmology, Tokyo Dental College, Chiba, Japan and [2] Department of Ophthalmology, International University of Health and Welfare, Tokyo, Japan.

Purpose. To report a case with penetrating corneal injury with vitreoretinal complications treated by endoscopy-guided vitreous surgery without simultaneous keratoplasty. **Case presentation.** Fifty-five year old woman had a perforating corneal injury with an intraocular ferrous foreign body. She underwent primary corneal sutures and lensectomy and referred to us. Vitreoretinal surgery was needed because of endophthalmitis, vitreous hemorrhage, retinal detachment, dialysis, and necrosis of the peripheral retina. However, conventional vitrectomy was not indicated due to the corneal opacity, and no donor corneas were available. Endoscopy-guided vitreous surgery was performed using (Solid Fiber Catheter AS-611 (FiberTech, Tokyo, Japan)) and retina was successfully attached with the aid of silicon oil tamponade. Following silicon oil removal at 3 months postoperatively, penetrating keratoplasty and intraocular lens suture was performed. With a postkeratoplasty follow-up period of 6 months, the graft remains clear and visual acuity was 0.5. Endothelial cell density keeps1968/mm^2. **Conclusions.** Endoscopic surgery for vitreoretinal complications in eyes with severe corneal damage can obviate keratoprosthesis and simultaneous keratoplasty. Primary endoscopic vitreoretinal surgery might be beneficial for minimizing damage to the corneal endothelium compared with the simultaneous keratoplasty.
[CR: none]

Effective Of Tests Of Tear Dynamics In Differentiating Between Dry Eye Subtypes. Santosh Khanal,[1] Alan Tomlinson,[1] Angus McFadden,[2] Charles Diaper.[3] Departments of Vision Sciences[1] and Mathematics,[2] Glasgow Caledonian University; South Glasgow University Hospital Trust, Southern General Hospital,[3] Glasgow, Scotland, UK

Purpose. To determine the effectiveness {sensitivity (SENS), specificity (SPEC), positive predictive value (PPV), negative predictive value (NPV) and overall accuracy (OA)} of tests of tear dynamics in differentiating between dry eye subtypes. **Methods.** Tear turnover rate (TTR), evaporation (EVAP) and osmolarity (OSM) were measured in 17 patients with Sjögren's Syndrome (SS), 12 with Graft-vs-Host Disease (GVHD) and 12 with general dry eye (aqueous deficient/evaporative). A previously derived discriminant function to diagnose dry eye was applied to all of the dry eye subtypes, and a new series of discriminant functions were also derived. The effectiveness of the cut-off value of tear dynamics measures, singly and in combinations, and the discriminant functions in differentially diagnosing the dry eye subtypes were determined on the study sample. **Results.** For all dry eye subtypes, the previously derived dry eye discriminant function produced high SPEC but low SENS and OA; the new series of discriminant functions left only one parameter in the function, making the functions redundant as diagnostic tools; tear osmolarity did not produce distinct cut-offs. For SS subtype, TTR yielded SENS 82%, SPEC 65%, PPV 78%, NPV 75% and OA 74%; EVAP gave SENS 76%, SPEC 63%, PPV 76%, NPV 67% and OA 69%. For GVHD subtype, TTR yielded SENS 75%, SPEC 65.5%, PPV 47%, NPV 86% and OA 68%; no distinct cut-off was seen for EVAP. Tests in parallel and series combinations were not any more effective for both subtypes. As a single measure, TTR offered the best differentiating tool for SS and GVHD from general dry eye, with SENS (75-82%) and OA (68-74%). EVAP gave SENS 87% and OA 81% in the differential diagnosis of evaporative dry eye. **Conclusion**. TTR and EVAP can be used in conjunction to differentiate between aqueous

deficient and evaporative dry eye, the two primary subtypes of dry eye outlined by the recently published DEWS report.
[No grant support; Commercial relationships: A Tomlinson with Allergan and Pfizer]

The Inhibitory Effects Of Corneal Inflammation By Green Tea Polyphenol. J. C. Kim[1] and Y. H. Ryu[1]. [1]Department of Ophthalmology, Chung-Ang University Medical Center

Purpose. To identify whether the green tea polyphenol (GTP) can have anti-inflammatory activities in human cornea by inactivation of T cells. **Methods.** 20-200 _g/ml GTP was treated in human CD4+ T cells. In order to investigate the effects of GTP on proliferation or cytotoxicity of human CD4+ T cells, MTT assay was performed in GTP-treated human CD4+ T cells, and mRNA expressions of inflammatory cytokines were also investigated by RT-PCR. Inactivation mechanism of human CD4+ T cells by GTP was investigated by Western blot. Moreover, in-vitro corneal autoimmune disease model was established by coculture of human corneal cells with human CD4+ T cells, 20-200 _g/ml GTP was treated. The proliferation and inflammatory cytokines were assessed by MTT assay and RT-PCR. **Results.** GTP-treated CD4+ T cells showed inhibited cell proliferation, but did not showed any evidence of apoptosis. GTP-treated CD4+ T cells revealed significantly decreased expressions of inflammatory cytokines mRNA (IFN-_ and –_), and their molecular mechanism of GTP was related with MAPK signaling, especially in p38 downstream molecules. Coculture of human corneal cells and CD4+ T cells showed the similar proliferative aspects with direct treatment of GTP in CD4+ T cells. Moreover, GTP-treated corneal cells very resistance to inflammatory conditions. **Conclusion.** These results suggest that GTP can act as a not only anti-oxidant but also anti-inflammatory factor in human corneal cells, and their signaling mechanism is related with MAPK signaling. Thus the applications are expected in corneal inflammation and autoimmune diseases.

Contributions Of Evaporation And "Tangential Flow" To The Mean Thinning Rate Of The Tear Film. P. Ewen King-Smith,[1] Jason J. Nichols,[1] Richard J. Braun,[2] College of Optometry, Ohio State University, Columbus, Ohio,[1] Department of Mathematical Sciences, University of Delaware, Newark, Delaware[2]

Purpose. Nichols et al. (IOVS, 46, 2353) found that, after a blink, the mean thinning rate of the central pre-corneal tear film was 3.79 um/min. No evidence was found for flow into the epithelium, so thinning could have contributions from evaporation and "tangential flow" along the corneal surface. Tangential flow typically should cause a *redistribution* of the tears, thus causing random thickening and thinning, and making little contribution to mean thinning rate. However, corneal curvature is greater at the center of the cornea than at the periphery. Surface tension will therefore generate a greater pressure at the center of the cornea, causing an outward flow of tears and thinning of central corneal tears. This contribution to mean tear thinning rate is analyzed here. **Methods.** An analytical expression was derived for the thinning rate caused by this pressure gradient. The corneal surface was assumed to be an oblate spheroid with asphericity, Q=-0.019 and apical radius 7.77 mm (Read et al., IOVS, 47, 1404). Tear film thickness was assumed to be 3.98 um (Nichols et al., IOVS, 46, 2353) and typical values were assumed for the surface tension and viscosity of the tears. **Results.** The contribution of this pressure-gradient flow to tear film thinning rate over central cornea was found to be 0.14 um/min. **Conclusions.** The calculated contribution of this tangential flow is only 4% of the observed mean thinning rate. Other sources of tangential flow, e.g., surface tension gradients and corneal surface irregularities, are expected to contribute to the variability in thinning rate but not to its mean value. Thus evaporation may provide the main contribution to the mean thinning rate. Because measured evaporation rates are much less than the observed mean thinning rate, we suggest that the pre-ocular chambers used in evaporation rate measurements restrict air flow over the cornea, and hence reduce evaporation compared to the conditions for thinning rate measurements.
Commercial Relationships. None. Support. None

Does The Water Permeability Of The Corneal Epithelium Help Prevent Excessive Evaporative Thinning Of The Tear Film? P. Ewen King-Smith,[1] Jason J. Nichols,[1] Kelly K. Nichols,[1] Barbara A. Fink,[1] Kari B. Green-Church,[2] Richard J. Braun,[3] College of Optometry,[1] Mass Spectrometry and Proteomics Facility,[2] Ohio State University, Columbus, Ohio; Mathematical Sciences Dept., University of Delaware, Newark, Delaware[3]

Purpose. Evaporation causes increased osmolarity of the tear film. Osmotic flow through the corneal surface in turn raises epithelial osmolarity, stimulating inflammatory processes (Luo et al., Eye & Contact Lens, 31, 186). Water permeability of the corneal surface might thus seem to be counter-productive. We argue that this water permeability may help to prevent excessive dehydration of the tear film, which could cause damage from exposure of the ocular surface and/or denaturation of tear film mucins and proteins. **Methods.** Osmolarity changes in the tear film and epithelium between blinks were modeled using reported values of water permeability of the corneal surface (e.g., Levin & Verkman, IOVS, 45, 4423). We considered evaporation rates which are higher than most reported in the literature, in which we think that evaporation was limited by shielding from pre-ocular chambers. Without such shielding, much higher evaporation rates have been indicated (J Nichols, King-Smith presentations at this meeting; Aurich et al., Cornea 25, 182). **Results.** Without osmotic flow, and with these assumed high evaporation rates, the tear film could thin sufficiently between blinks that it became effectively desiccated with the corneal surface exposed. The water permeability of the corneal surface prevents this extreme desiccation. **Conclusions.** The water permeability of the corneal surface may involve a trade-off between the health of the tear film and that of the epithelium. High permeability may exacerbate osmotic stress on the epithelium. Low permeability may reduce this osmotic stress, but permit excessive tear film thinning due to evaporation. An analogy may be the "airway surface dehydration" which is thought to be a cause of mucus adhesion, inflammation and chronic infection in the lungs of cystic fibrosis patients (Boucher, J Internal Med. 261, 5).
Commercial Relationships. None. Support. None

The Thickness Of The Tear Film As A Function Of Space And Time. P. Ewen King-Smith,[1] Barbara A. Fink,[1] Jason J. Nichols,[1] Kelly K. Nichols,[1] Kim L. Boyer,[2] College of Optometry,[1] Electrical Engineering Dept.,[2] Ohio State University, Columbus, Ohio

Purpose. We describe a method of deriving the thickness of the tear film, h(x,y,t) as a function of position, x,y, and time, t. The pre-lens tear film was used, because the contrast of interference fringes is much greater than for the pre-corneal tear film. **Methods.** Interference images were obtained using narrow-band, 850 nm, illumination covering an area of about 9 x 7 mm at a rate of 30 per second (King-Smith et al., J Opt Soc Am A, 23, 2097). To eliminate the effect of eye movements, video data were first processed by "stabilizing" the tear film image, based on cross-correlation between successive pairs of

images. At any position, as the tear film thinned, intensity, I(t), showed oscillations due to interference between reflections from the front and back of the tear film. The phase of these oscillations (range -180° to 180°), p(t), was derived by fitting a single cycle of a harmonic wave to I(t). Phase was then "unwrapped" by subtracting 360° whenever a step increase of over 180° was observed (e.g., from -170° to 170°). The phase functions for all positions were then combined using least squares integration (Ghiglia & Romero, J Opt Soc Am A, 11, 107). Finally, phase was converted to thickness (360° of phase corresponds to 1/2 wavelength), using a "seed" thickness for one position, derived at a time when this thickness could be measured by counting fringes from a dry spot. **Results.** Results will be shown as contour/mesh plots from individual images and as a video of such plots. The difference between thickness distributions at two times gives the distribution of average thinning rate between those two times. Thinning rates vary considerably as a function of position; preliminary analysis supports the proposal that evaporation, rather than "tangential flow", provides the main contribution to thinning rates. **Conclusions.** Ongoing analysis of the results can provide unique information about the fluid dynamics of the tear film. The effects of tear film distribution on optical quality will also be analyzed.
Commercial Relationships. None. Support. None

Automated Identification And Quantification Of Tear Proteins From Sjögren's Syndrome By Lc/Ms On A Hybrid Linear Ion Trap Mass Spectrometer. Kazuko Kitagawa[1], Naohisa Tomosugi[2], Hideyuki Tuchida[2], Hiroshi Sasaki[1]. 1: Department of Ophthalmology. 2: Division of Advanced Medicine, Medical Research Institute, Kanazawa Medical University, Ishikawa, Japan

Purpose. Histological and functional changes of lacrimal gland might be reflected in proteomic patterns in tear fluids. In the previous study, we reported the potential of proteomic pattern technology in tear fluids as the noninvasive diagnostic test for primary Sjögren's syndrome (SS) using SELDI. In this study, we carried out an identification of the disease biomarkers in tear fluid for SS using LC-MS/MS. **Methods.** Tears pooled from 20 patients with primary SS and 4 control subjects were used in the analysis. Low molecular tear proteins were extracted after precipitating proteins by 4 fold volume acetone. The extracts were then reduced and tryptic-digested. For tear proteomic analysis, iTRAQ combined with nanoLC-MS/MS was performed to identify tear proteins and determine quantitative changes of tear proteins from SSs and controls. **Results.** In total, 22 tear proteins were identified with 95% confidence and 7 of them were down-regulated in SS, including tear-specific proteins such as lipocalin, lacritin and proline-rich protein. There were no differences in a large number of plasma proteins which were also observed in tear fluid. **Conclusions.** The results showed that coupling of this methodology with a stable isotope N-terminal labeling strategy using iTRAQ reagents used in this study was successfully applied to analyze tear proteome in the same LC-MS/MS run.
[This research was supported by the grant from Ishikawa Sunrise Industries Creation Organization.]

Eye-Associated Lymphoid Tissue (EALT) - Are We Using The Right Animal Models For Inflammatory Ocular Surface Disease? Erich Knop[1] and Nadja Knop[2]. [1]Research Lab of the Eye Clinic CVK, Charite – Universitätsmedizin Berlin; [2]Dept. for Cell Biology in Anatomy, Hannover Medical School

Purpose.The normal human ocular surface and its mucosal appendage, i.e. the conjunctiva, lacrimal gland and lacrimal drainage system have an Eye-Associated Lymphoid Tissue (EALT). As part of the mucosal immune system it maintains the immunological homeostasis and integrity. Rat and mouse however, which are, due to their convenience, common animal models for basic immunological and inflammatory mechanisms lack EALT. It is discussed whether they represent suitable models. **Methods**. Own investigations on the histology, ultrastructure, molecular biology and function of EALT in human and rabbit are analysed together with data from a Medline based literature search. **Results**. EALT in normal man and rabbit consists of diffuse lymphoid tissue continuous from the lacrimal gland throughout the conjunctiva (as CALT) and along the lacrimal drainage system (as LDALT). The lymphocytes are effector B-cells (mainly IgA-positive plasma cells for secretory immunity) and T-cells (activated T-effectors including Treg, and naïve ones) together with accessory cells. Lymphoid follicles with antigen-transporting M-cells, germinal centers and T-cell zones in CALT and LDALT can serve for local antigen uptake and effector cell production. CALT is found in many other species as e.g. swine, cattle, guinea pig, turkey but normal rat and mouse lack it. Their ocular surface immunity appears to be governed by secretory immunity via the lacrimal gland and by cellular immunity maintained by the central immune system via regional lymph nodes resulting in inflammatory immigration of lymphocytes rather than mucosal tolerant immune reactions. **Conclusions**. The central and the mucosal immune system have different functions and favour inflammation or tolerant immune reactions, respectively. In end stages of ocular inflammation rat and mouse may be suitable to resemble the immune process at the human ocular surface. However, they can probably not reflect the fine tuning of immunity that occurs in human EALT prior to disease outbreak.
Grant support: DFG KN317/11
Financial interest: None

Eye-Associated Lymphoid Tissue (EALT) Of The Rabbit Ocular Surface Contains M-Cells In Calt And LDALT. Nadja Knop[1] and Erich Knop[2] . [1]Dept. for Cell Biology in Anatomy, Hannover Medical School, Germany; [2]Research Laboratory of the Eye Clinic CVK, Charité – Universitätsmedizin Berlin, Germany

Purpose. Eye-associated lymphoid tissue (EALT) is a part of the mucosal immune system which is located at the ocular surface and its mucosal appendage (in conjunctiva as CALT, lacrimal gland, and in lacrimal drainage system as LDALT). After first description in the human its occurrence, morphology and presence of antigen-transporting M-cells in the rabbit is the topic of the present study. **Methods.** Tissues from 28 young adult rabbits were investigated as whole-mounts of the conjunctiva, lacrimal drainage system and lacrimal glands by illumination in a stereo magnifier, in cryo- and paraffin-histology, confocal microscopy, and electron microscopy (TEM and SEM). **Results**. Apart from production of secretory IgA (SIgA) by the lacrimal gland, its diffuse lymphoid tissue was continued in the conjunctiva and in the lacrimal drainage system. Similar to the gland, local production of SIgA was observed in CALT and LDALT. Both tissues also had secondary lymphoid follicles indicating antigen contact and proliferation of the lymphocytes. The follicles were covered by a follicle-associated epithelium (FAE) with antigen-transporting M-cells in the luminal layer. They stained positive for Vimentin and contained groups of lymphoid cells inside cytoplasmic pockets. Underneath, the basal cells were disarranged and the basement membrane discontinuous due to transmigrating cells. Topical application of 0,5µm FITC coated microspheres resulted in cellular uptake by the M-cells not only in the conjunctiva but also in the lacrimal drainage system. M-cells in the conjunctiva could also be detected by in-vivo confocal microscopy. **Conclusions.** The rabbit has an EALT similar to the human for immune protection and may hence be a more suitable animal model for experimental studies of ocular surface immune reactions than the

frequently used mouse or rat. M-cells in rabbit CALT could be investigated by confocal microscopy which may allow functional investigation of rabbit EALT.
Grant support: DFG KN317/11
Financial interest: None

Morphology Of The Lid Wiper Region Of The Human Lid Margin In Histology And In-Vivo Confocal Microscopy. Erich Knop[1], Nadja Knop[2], Andrey Zhivov[3], Donald Korb[4], Jack V. Greiner[5], Rudolf Guthoff[3]. [1]Research Lab. of the Eye Clinic CVK, Charite – Universitätsmedizin Berlin; [2]Dept. for Cell Biology in Anatomy, Hannover Medical School; [3]University Eye Hospital Rostock; [4]Korb Associates, Boston; [5]The Schepens Eye Research Institute and Dept. Ophthalmology, Harvard Medical School, Boston.

Purpose.The conjunctival marginal zone of the upper eyelid which serves for spreading the pre-ocular tear film is termed the "lid wiper". In order to define its morphological structure we compared histology with in-vivo confocal microscopy. **Methods**. Histology in serial sections of different zones of the upper lid margin in whole-mount specimens from ten human body donors was compared to in-vivo confocal microscopy of four eyes with a Heidelberg retina tomograph (HRT II) and attached Rostock cornea module. **Results**. At the meibomian gland orifices of the inner lid margin, the stratified squamous keratinized epithelium of the outer skin, about 5-6 layers thick, was replaced by the non-keratinized epithelium of the muco-cutaneous junction. In HRT, this zone occurred as a distinct line and appears to correspond to the line of Marx. The interdigitations of the epithelium with dermal papillae were seen in HRT as rounded protrusions, containing blood vessels with floating bloodstream cells. Then the epithelium became higher (ca. 8-12 layers) and formed a cushion-like structure of about 50µm thickness as measured in optical HT sections. Dermal papillae were gradually lost and the basement membrane level became flat. This zone continued for about 1-2mm and transformed into the epithelium of the subtarsal fold which had less cell layers (6-8) and cuboidal to columnar morphology until it was replaced by the usual 2-3 layered cuboidal tarsal conjunctival epithelium. **Conclusions**. At the marginal conjunctiva apposed the cornea a multi-layered epithelial cushion-like structure occurs that appears to correspond to the lid wiper. It is clinically detectable by in-vivo confocal microscopy, a technique that offers the possibility to detect alterations in dry eye syndromes in a clinical setting.
Grant support: DFG KN317/11
Financial interest: None

Long-Term Results Of Surperior Conjunctivochalasis Operation For Treating Superior Limbic Keratoconjunctivitis. Aoi Komuro,[1,2] Norihiko Yokoi ,[2] Kazuichi Maruyama,[2] Shigeru Kinoshita.[2] Department of Ophthalmology, Nishijin Hospital[1] and Department of Ophthalmology, Kyoto Prefectural University of Medicine,[2] Kyoto, Japan.

Purpose. In a previous study, we reported the possible involvement of superior conjunctivochalasis in the pathogenesis of superior limbic keratoconjunctivitis (SLK) based on relatively short-term results (Yokoi N, et al. AJO 135, 2003). The purpose of the present study is to report the long-term clinical results of the superior conjunctivochalasis operation for SLK. **Methods.** Nineteen eyes of 14 patients (4 males and 10 females; mean age: 52.5 yrs) who received our original superior conjunctivochalasis operation for SLK, which includes a crescent excision of the distal part of the diseased conjunctival area of SLK, were enrolled in this study_(postoperative follow-up period > 3 months). Postoperative complications, recurrence of SLK, and improvements of subjective symptoms of patients were evaluated. Subjective symptoms were assessed before and after the operation by questionnaires. **Results.** The average follow-up period was 3.0±1.6 (SD) yrs (0.3-5.3 yrs). All cases had no complications or recurrence of SLK. Main symptoms from 19 eyes were irritation (16 eyes; 84.2%), injection (2 eyes; 10.5%) and subconjunctival hemorrhage (1 eye; 5.3%). In those patients, symptoms 3 months after the operation were remarkably improved in 9 eyes (47.4%) which included 2 eyes with punctual occlusion by punctual plugs because of aqueous tear deficiency, improved in 10 eyes (52.6 %), and there was no deterioration. **Conclusions.** The superior conjunctivochalasis operation for SLK was considered to be effective in resolving patient complaints for long periods.

Murine Lacrimal Gland Is Capable Of Repair Following Experimentally Induced Inflammation. Claire L. Kublin[1], Liz Macari[1], and Driss Zoukhri[1,2]. Department of General Dentistry, Tufts University School of Dental Medicine[1] and Department of Neuroscience, Tufts University School of Medicine, Boston, MA[2].

Purpose. To determine if murine lacrimal gland is capable of self-repair following experimentally induced inflammation and if so, determine the mechanisms involved in this process. **Methods**. Inflammation was induced by direct injection of recombinant human interleukin-1a (IL-1a, 1 mg in 2 ml) into the exorbital lacrimal glands of anesthetized female BALB/c mice. Animals were sacrificed 1, 2, 3, 4, or 7 days following the injection. Before sacrifice, the amount of tears was measured using phenol red-impregnated cotton threads. The exorbital lacrimal glands were then removed and processed for measurement of protein secretion, histology, immunohistochemistry and western blotting. **Results**. Compared to saline injected BALB/c mice, the amount of tears was significantly reduced following IL-1a injection at 1, 2, and 3 days. Tear production returned to normal 7 days post IL-1a injection. Similarly, compared to control, protein secretion was inhibited by 91%, 67%, and 33% at 1, 2, and 3 days post IL-1a injection, respectively, but returned to normal at 7 days. Concomitant with these changes, the lacrimal gland underwent a severe inflammatory response at 1, 2, and 3 days during which the number of apoptotic acinar cells increased and the amount of Bcl-2 (an anti-apoptotic protein) decreased. Between 3 and 7 days following IL-1a injection, the lacrimal gland underwent a phase of repair during which the number of Ki67 (a marker of cell proliferation) and nestin (a marker of progenitor/stem cells) positive cells increased. During the repair phase, the TGFb/Smad signaling pathway was activated. Similar results were obtained in a different mouse strain (C57/BL6 mice). **Conclusions**. We conclude from these studies that the murine lacrimal gland is capable of self-repair following experimentally induced inflammation. Our results also suggest that the lacrimal gland contain progenitor stem/cells, which might actively participate in tissue repair.
(Supported by NIH grant R01 EY12383).

Assessment Of Cytokine Levels In The Tears Of Contact Lens Wearers And Non-Contact Lens Wearers. Carol Lakkis,[1] Shelly Ames,[1] Paul Connellan,[2] Linda Banbury,[2] Carol Morris.[2] Clinical Vision Research Australia, The University of Melbourne, Melbourne, Australia,[1] Centre for Phytochemistry and Pharmacology, Southern Cross University, Lismore, Australia.[2]

Purpose. The aim of this study was to determine and compare the concentration of cytokines in the tears of contact lens (CL) wearers and non-contact lens (NCL) wearers.
Methods. Reflex tears were collected from n=19 soft CL wearers and n=33 NCL wearers using glass microcapillary tubes. Subjects with ocular or systemic disease and/or using topical or systemic medications were excluded from participation. Cytokines were measured by flow cytometry using a Cytometric Bead Array (Becton Dickinson) with a human inflammation panel to detect IL-12p70, TNF-α, IL-10, IL-6, IL-1_ and IL-8. Duplicate testing of each sample was conducted and concentrations are presented as median (interquartile range) pg/ml.
Results. IL-12p70, IL-6 and IL-8 were present in both the CL and NCL wearer tear samples. The concentrations of each of these cytokines were: IL-12p70 CL 10.5 (3.4–14.5), NCL 6.0 (0-11.7); IL-6 CL 11.2 (8.1-21.3), NCL 12.8 (7.9-17.7); IL-8 CL 317.0 (216.4-523.7), NCL 370.0 (234.9-621.5). There were no significant differences in the concentrations of these cytokines between the CL and NCL wearer groups ($p>0.05$). TNF-α was below the detection limit in NCL wearer tears but was significantly higher in CL wearer tears (5.1 (2.7–6.5) pg/ml, $p<0.05$). IL-10 and IL-1_ were below the detection limit in the tears of both groups of subjects. **Conclusions.** A number of cytokines can be reliably detected in the tears of CL and NCL wearers using a Cytometric Bead Array. Soft CL wear may stimulate TNF-α production, a cytokine involved in acute and chronic inflammatory responses. Further investigation of the effect of CL type, wearing modality and correlation of cytokine levels with clinical signs and symptoms, will be beneficial for understanding ocular responses to CL wear.
Supported by CIBA Vision GmbH and Southern Cross University.

A New System For Grading Ocular Surface Staining. Christian Lang[1] Gary N. Foulks,[2] Janine A Smith,[3] John M.Tiffany,[4] Anthony J.Bron.[4] Dept. of Materials [1] and Nuffield Laboratory of Ophthalmology [4], University of Oxford, UK, Dept. of Ophthalmology, University of Louisville [2], National Eye Institute [3], Bethesda MD, USA.

Purpose. To create a standardised system for grading ocular staining using a mathematically-defined set of grading panels. **Methods.** In three current systems clinical staining is compared with panels of dots of increasing number density. The scale ranges from 0-9 in the van Bijsterveld system, 0-15 in the Oxford system and 0-33 in the NEI/Industry workshop system. The latter system grades over multiple corneal zones, so that different staining phenotypes may be described numerically and the effect of central staining on visual function may be addressed. We have modified this system to: i. standardise the size and location of recording zones, ii. create a set of scales which presents dots in random distribution and increasing number density, with a mathematically defined difference from panel to panel. To do this, (1) a series of 10 images was generated (with 512x512 pixels resolution) with the probability of a pixel being black given by the equation: exp(a0.9)/exp(b0.9)-k, where: a = number of the current panel; b = number of the last panel + 1; k = 0.00042. In order that the size of dots on the printed panels would approximate the size of a staining point on the surface of the eye, (2) the panels were scaled in MSWord so that dot size at the reading distance reflected the average size of an epithelial cell at the Haag-Streit slit-lamp magnification used. **Results.** The poster shows how such panels may be constructed and modified mathematically according to the expectation of staining densities encountered clinically. A standard recording template is presented here and a set of panels provides a grading range of 0-10 for each zone assessed. The scale range of the system is 0-110. Such an approach can be used to grade damage outside the exposed surface of the eye.
Conclusions. A standardised grading system is presented and will shortly be tested under field conditions.
Disclosure: C. Lang, N,; G. Foulks, N,; J Smith, N,; J.Tiffany, N,; A. Bron, N.

Tear Prosecretory Mitogen Lacritin: PONDR Predicted Ordered And Disordered Domains, And Conservation Of Predicted Structure In Putative Orthologues. Gordon W. Laurie[1], Ningning Wang[1], Ronald W. Raab[2], Robert L. McKown[2]. 1Dept. of Cell Biology, University of Virginia, Charlottesville VA, USA. 2Dept. of Integrated Science and Technology, James Madison University, Harrisonburg, VA, USA.

Purpose. Lacritin is a prosecretory mitogen apically released from human lacrimal acinar cells. Cell culture studies suggest that it promotes tear release, epithelial renewal and rapid MUC16 production. When topically added to rabbit eyes, lacritin promotes tearing. Lacritin is also expressed by basal proliferative cells of the human corneal epithelium, and has been reported in the meibomian gland proteome. Lacritin targets the N-terminus of syndecan-1 via a heparanase-dependent mechanism that may improve lacritin affinity for a G-protein coupled signaling receptor. Here we searched for ways to gain insight on its structure. **Methods.** Human lacritin and its putative non-human primate and non-primate orthologues were analyzed by PONDR, Helical Wheel and ClustralW. Models were drawn by GeneSilico.
Results. Prior deletion/CD analysis had demonstrated the presence of a C-terminal amphipathic alpha-helix with mitogenic/syndecan-1 binding activity absent in the lacritin C-25 fragment. N-24, N-45, N-55 and N-65 are also all mitogenic, but not N-71. Thus, activity is lost when the amino acids KSIVEK are removed from N-65. PONDR analysis reveals that both domains reside in a predicted 'ordered' region stretching over one-half of the secreted protein to the C-terminus. ClustralW and Helical Wheel suggest that both functional domains are maintained in other species. The other half of secreted lacritin is predicted to be disordered. Binding syndecan-1 might contribute to a disorder to order conversion. **Conclusion.** Lacritin's structural features appears to be conserved in other species, and suggest that lacritin may favor complexing with syndecan-1, and possibly other tear proteins.
Support: EY13143(GWL).

Hyaluronic Acid And *Echinacea Purpurea* Extracts Eye Drops In The Treatment Of Non-Specific Conjunctivitis. Andrea Leonardi, Velika Deligianni, Antonio Manfre', Chiara De Dominicis, Daniele Violato, Iva Fregona. Ophthalmology Unit, Department of Neuroscience, University of Padua, Italy.

Purpose. Non-specific conjunctival inflammation is commonly associated to mild but persistent discomfort signs and symptoms, tear film disfunction and inflammation not related to a definite disease, immune mechanism or pathogen. *Echinacea purpurea* extract is widely used herbal medicine for the treatment of upper respiratory infections and common inflammations. **Methods**. 24 patients affected by active and persistent non-specific conjunctival inflammation were included. Allergy, dry eye, blepharitis and infections were excluded. Patients were randomized and treated for 4 weeks with either a hyaluronic acid (HA) eye drops or HA with *Echinacea purpurea* extract eye drops (Iridium®). Signs and symptoms, subjective evaluation of treatment,

Schirmer test, BUT, lissamine green staining, and tear cytology were evaluated before and after treatment. **Results**. Both treatments improved overall signs and symptoms of conjunctivitis, BUT and Schirmer test. Conjunctival redness, and the number of inflammatory cell in tear cytology were significantly reduced in patients treated by Iridium® compared to HA alone. Both compounds were well tolerated but Iridium® received a better subjective efficacy score compared to HA eye drops. **Conclusions**. Iridium® eye drops reduced signs and symptoms of non-specific conjunctival inflammation improving the tear film function and reducing local inflammation.

Low Abundance Protein In Tears Of Vernal Keratoconjunctivitis (VKC) Patients. Andrea Leonardi, Massimo Bortolotti, Sonal Sathe and Robert Sack. [1]Ophthalmology Unit, Department of Neuroscience, University of Padua, Italy. [2]SUNY College of Optometry, New York, NY, USA

Purpose. To detect the presence and distribution of multiple mediators and growth factors in tears of active vernal keratoconjunctivitis (VKC) patients using stationary phase antibody array (MA). **Methods**. Tears were collected from 16 normal subjects (CT) and 26 active VKC patients. Tears were centrifuged and successively probed using three microwell plate arrays specific for: a) cytokines: IL-1, -3, -4, -5, -6, -7, -8, -10, -12, -13, IFNg and TNFa; b) growth factors FGF-b, PDGF, TPO, ANG-2, VEGF, HGF and HB-EGF; c) MMP-1, -2, -3, -8, -9, -10, -13, TIMP-1 and –2. Samples were also probed with a membrane array optimized to detect a larger number of inflammatory mediators and chemokines. **Results**. IL-8 signal was detected in all CT and highly detected in all VKC samples. Th2-cytokines, IL-4, IL-5 and IL-10 were detectable only in VKC tears. Signals for FGF-b, HB-EGF, VEGF and HGF were detected in 40-60% of VKC samples and in 25% of CT. Only TIMP-1 and –2 were found at high levels in normal tears, while in VKC, MMP-1, -2, -3, -9, and -10 were highly detected, **Conclusions**. Stationary phase antibody arrays are a useful tool for the screening of a large number of mediators in low volumes of tears. Analysis allowed the identification of previously undetected factors in tears of VKC patients (i.e., MMP-3 and MMP-10) and confirmed the presence of a high expression of multiple MMPs, growth factors and cytokines that contribute to the pathogenesis of conjunctival allergic inflammation.

Comparison Of MMP-9 Level In Tear Of MGD, NSDE And Normal Population. [1]Elsa L.C. Mai, [2]Yi-Yun Chou, [3]Chia-Che Chang. [1] Far Eastern Memorial Hospital, Taiwan, [2,3] Institute of Biomedical Sciences, National Chung-Hsing University. Taiwan,

Purpose. To investigate one of the dry eye inflammatory mediator matrix metalloproteinase (MMP-9), a gelatinase that hydrolyze type IV collagen and a potent activator of the inflammatory cytokine precursor IL-1. It was first found to be elevated in tear of patients with active peripheral ulcerative keratitis (PUK), infectious keratitis and Sjogren syndrome (SS). The tear level of this mediator was not established in Meibomian Gland Dysfunction (MGD) and Non-Sjogren Dry Eye (NSDE) patients. We investigate the tear level of MMP-9 in these two types of patients as compare to normal population level to better understand the composition of the tear film in dry eye pathological conditions. **Methods.** Tear samples were collected from patients of Non-Sjogren syndrome dry eye (NSDE), meibomian gland disease (MGD), Normal subjects are recruit from workers in our hospital. Tear fluid where collected with fine tipped pastettes and then later transfer to eppendoof tube. SDS polyacrylamide gel electrophoresis with zymographic gelatinase activity profiles of the MMP protein was performed. Commercial available Gelatinase-A,B was included on gels as positive control. Positive MMP-9 findings are those with noticeable band on the molecular wt position of 92kDa (proenzyme) and 84kDa (active enzyme). Protein bands are further quantified by using the Gel-PDMS software system. Statistical comparison was performed by SPSS. **Results.** Elevation of MMP-9 tear level in both MGD and NSDE patients was found as compare to normal person, however, a much larger increase was found in the MGD population compare to NSDE patients. Normal subjects without dry eye or ocular surface disease have minimum non-significant presentation of MMP-9 in tear. Statistical comparison will be presented. **Conclusions.** MGD patients are usually associated with evaporative dry eye, hinting that lipid dysfunction is the main cause of the DE condition. But from our results, the disproportional increase of MMP-9 in MGD patients tear indicated an inflammatory process, which can also lead to DE condition and symptom signs as contrary to simple evaporation.
Financial Disclosure: None

Differential Tear Film Characteristics Of Dry Eye Contact Lens Wearers. Cécile Maissa, Michel Guillon OTG Research & Consultancy, London, UK.

Purpose. The tear film characteristics in the presence of a contact lens are very different than those of the bare eye. Amongst non wearers the symptomatic population has different tear characteristics than the normal population. It is of interest, therefore, to know if the same applies to contact lens wearers. The aim of the study was to measure the tear film stability and dynamics of a population of contact lens wearers with different levels of dry eye symptomatology. The hypothesis tested was that the tear film dynamics of symptomatic wearers are different to that of normal. **Methods.** Tear film stability (Non Invasive Break Up Time) and dynamics (Lipid and aqueous layer structures) were analysed using the Tearscope lighting system and biomicroscope. Contact lens wearers who attended OTG Research and Consultancy Clinic for the first time, wearing their own contact lenses were measured. The subjects were divided into two populations, based on their McMonnies scores: i) Symptomatic (DE: n=81) ii) Asymptomatic (N: n=102). The two populations were aged and sex matched. **Results.** The lipid layer was significantly thinner (p=0.002) for the wearers complaining of dry eyes (Thinnest visible layer: DE 79% N 65%, Thick layer: DE 4% N 8%), but the aqueous layer was similar (p=0.219) (Thick: DE 51% N 61%). The stability of the tear film was overall greater for the normal population, (p=0.002) (DE = 4.9s; N = 6.4s). **Conclusions.** The investigation revealed that the tear film characteristics of contact lens wearers complaining of dry eye were marginally worse than that of asymptomatic wearers. Even though the differences were relatively small, they are highly clinically relevant as the tear film of all contact lens wearers is already highly abnormal compared to non wearers.

Differential Tear Film Characteristics Of Dry Eye Patients. Cécile Maissa, Michel Guillon OTG Research & Consultancy, London, UK.

Purpose. Patients complaining of dry eyes are classified as per their symptomatology; the aetiology of the problem is known to be different even in the presence of identical symptomatology. In order to develop efficient management strategies it is essential to identify the aetiology of the problem for each individual case. Previous reports have identified a decreased tear film stability in dry eyes patients and recently it has been suggested that tear film dynamics may be different in dry eye sufferers than in normal. The aim of the paper was to measure the tear film stability and dynamics of a population of non contact lens wearers with different levels of dry eye symptomatology.

The hypothesis tested was that the tear film dynamics of dry eye sufferers is different that of normal. **Methods.** Tear film stability (Non Invasive Break Up Time) and dynamics (Lipid and aqueous layer structures, tear break up type) were analysed using the Tearscope lighting system and biomicroscope. The subjects, all non contact lens wearers, were divided into two populations, based on their McMonnies scores: i) Symptomatic (DE: n=62) ii) Asymptomatic (N: n=158). The two populations were aged matched but with a greater incidence of female amongst DE (Female: DE 66%; N 46%). **Results.** The lipid layer was significantly thinner (p=0.001) for the dry eye population (Thinnest visible layer: DE 37% N 16%; Thick layer: DE 31% N 44%), but the aqueous layer was similar (p=0.335) (Thick: DE 95% N 93%). The incidence of cases for which a normal blink occurred before a visible break or only a minimal break at the lid margin took place was greater for the normal population (DE 59% N 75%). The stability of the tear film was overall greater for the normal population ($p<0.001$) (DE = 13.8s N = 19.9s). **Conclusions.** The investigation revealed that the tear film characteristics of dry eye sufferers were significantly different to that of normal. A key difference, in addition to lower tear film stability for the population averages, was a significant difference in the structure of the lipid layer but not in the structure of the aqueous layer. The current finding has significant implications when dealing with dry eye problems.

Inhibition Of Galectin-3 Association With Cell Surface O-Glycans Results In Corneal Epithelial Barrier Dysfunction. Flavio Mantelli[1], Zhiyi Cao[2], Noorjahan Panjwani[2], Pablo Argüeso[1]. 1 Schepens Eye Research Institute & Dept. Ophthalmology, Harvard Medical School and 2 Tufts University School of Medicine, Boston, MA, USA

Purpose. Galectin-3, a ß-galactoside-binding protein expressed by the ocular surface, interacts with mucin carbohydrates (O-glycans) on the corneal epithelial glycocalyx. We have hypothesized that this interaction creates an epithelial barrier that prevents uptake of rose bengal, a dye widely used in the diagnosis of ocular surface damage in dry eye. The purpose of this study was to determine whether inhibition of galectin-3 association with mucin O-glycans results in rose bengal uptake by corneal epithelial cells. **Methods.** Human corneal-limbal epithelial (HCLE) cells expressing mucin O-glycans were incubated for 1 hour with 0.1M ß-lactose –a competitive inhibitor of galectin-3 binding– and the non-inhibiting control disaccharides, sucrose and maltose. Barrier function was assayed by incubation with 0.1% rose bengal for 5 minutes. Galectin-3 in HCLE protein extracts and cell culture media was detected by Western blot and quantified using densitometry. Barrier function was also assayed on C57BL/6 mouse corneas treated with disaccharides. **Results.** The presence of mucin O-glycans and galectin-3 in HCLE cells protected against rose bengal uptake. Competitive inhibition of galectin-3 binding with ß-lactose resulted in increased areas of rose bengal uptake as compared to controls ($p<0.001$). By immunoblot, increased levels (~5-fold) of galectin-3 were detected in the media of HCLE cells treated with ß-lactose, indicating that galectin-3 was removed from the cell surface glycocalyx. In mice, diffuse rose bengal staining was observed through the entire epithelium of corneas treated with ß-lactose. **Conclusions.** These results indicate that association of galectin-3 with O-glycans on the corneal epithelial glycocalyx may contribute to the formation of a protective barrier that prevents rose bengal penetrance. Alteration of this association in patients with dry eye may result in disruption of the epithelial barrier and deterioration of tear film stability.
[Supported by NEI R01EY014847 (PA) and R01EY07088 (NP)]

Biopsy Of The Bulbar Conjunctiva In Contact Lens Wearers With Conjunctival Flaps. Maria Markoulli[1], Ian C. Francis[2,3], Jim Yong[4], Eric Papas[1,5]. Institute for Eye Research[1], Prince of Wales Hospital[2], University of New South Wales[3], South Western Sydney Area Pathology Service[4], Vision Cooperative Research Centre[5], Sydney, Australia.

Purpose. Conjunctival flaps have been reported in association with silicone hydrogel soft lens use (Fonn et al, 2005 Contact Lens Spectrum). Observation of their clinical characteristics and time course has indicated that resolution occurs without observable sequellae (Markoulli et al. Poster #5391 ARVO 2007), however the histopathology of these features has not been established. This study attempted to determine the nature of the tissue change constituting a conjunctival flap as a means of gauging the significance of the condition for contact lens wearers. **Methods.** Slit-lamp biomicroscopy along with sodium fluorescein , cobalt blue light and a Wratten filter was used to observe the presence, location and size of conjunctival flaps in a group of contact lens wearers that presented with conjunctival flaps. Two subjects who exhibited such flaps agreed to undergo conjunctival biopsy. Tissue samples were obtained both from the region of the flap and an adjacent unaffected area. The tissues were processed by standard methods for histopathology. **Results.** In the first subject, analysis of the flap tissue showed some focal increase in collagen and mild degeneration but no increase in elastic tissue. The second subject displayed even collagen distribution and overall normal histology. The squamous conjunctival epithelium was normal in both cases. **Conclusions.** These findings indicate that conjunctival flaps consist of grossly normal tissue. Their presence in association with contact lens wear does not imply the occurrence of pathological changes on the time scale of this study.
[This study was supported by the Australian Federal Government through the Cooperative Research Centre program and in part by CIBA Vision.] PARC # 2007-04-0491

Role Of Thrombospondin In Lacrimal Gland Inflammation. Sharmila Masli[12], Bruce Turpie[1], J. David Rios[1] and Darlene Dartt[1,2], Schepens Eye Research Institute[1], Harvard Medical School[2], Boston, MA.

Purpose. Inflammation of the lacrimal gland (LG) is associated with autoimmune Sjögren's Syndrome (SS) and resulting dry eye. An extracellular matrix protein, thrombospondin (TSP1) is important in the activation of latent TGF_ and avoidance of the inflammatory responses in the ocular environment. Lacrimal glands of TGF_ deficient mice develop severe inflammatory infiltrates and dry eye as seen in SS. We examined if the absence of TSP1 alters the LG environment promoting inflammation and subsequent loss of secretory function. **Methods.** Lacrimal glands from TSP1-/- mice and wild-type control (WT-C57BL/6) mice were analyzed for the presence of inflammation by (a) histologic examination (H&E), (b) flowcytometric analysis of infiltrating cells , (c) real-time PCR analysis for the detection of message for inflammation associated cytokines, and (d) ELISA to detect cytokine protein. Secretory function of LG was assessed using an assay for the secretory protein peroxidase (measures the ability of LG to secrete peroxidase in response to stimulation of nerves with high KCl buffer). **Results.** Several foci of inflammatory infiltrates were detectable in the LG of 12 months old TSP1-/- mice as compared to WT controls. At 4 months of age, the inflammatory cells in the LG of TSP1-/- mice included predominantly CD4+ T cells and some CD11b+ macrophages. Very few CD8+ T cells were detected. Although, electron micrographs of LG in 2 month old TSP1-/- mice revealed deterioration of acinar cells no detectable inflammatory cell infilteration was seen in these glands. Furthermore, significantly reduced secretory

function was noted in LG from 2 month old TSP1-/- mice compared to WT mice as determined by the peroxidase assay. The message as well as protein levels for the pro-inflammatory cytokines IL-1_, IL-6, TNF_, was significantly reduced in the LG of 2 months old TSP1-/- mice as compared to the controls. **Conclusions**. The absence of TSP1 correlates with the LG functional loss, which is followed by inflammatory infiltrates. The results indicate that TSP1 is likely to be involved in regulating inflammatory responses in the lacrimal gland.

Spectroradiometry As An Objective Measure Of Conjuncitval Hyperaemia. Louise C. McCann[1], E. Ian Pearce[1], Colin J. Gafan[1], Kevin Middleton[1], Alexander D. Logvinenko[1], Norman F. Button[1] Department of Vision Sciences, Glasgow Caledonian University, UK[1]

Purpose To investigate spectroradiometry as a novel, objective technique for the measurement of bulbar conjunctival hyperaemia and to compare findings to subjective grading scales (CCLRU and Efron). Although previous studies have used spectrophotometry as an objective technique, this method is inferior to spectroradiometry, measuring only one third of the visual spectrum. **Methods** Saturation and dominant wavelength (d_) of CCLRU and Efron grading scale images were measured using a spectroradiometer. Ten readings were taken for each of the images. For comparison purposes, a digital camera was used to photograph the bulbar conjunctiva of a normal population (n=15, age 21-29). Five readings of saturation and d_ were recorded for each image. **Results** An increase in saturation and d_ was observed with increasing grade of severity of hyperaemia for both grading scales. For CCLRU from lowest to highest grade; saturation levels increased by 36% from 42.87% to 78.90%, d_ increased by 5.3nm from 578.5nm to 583.8nm. For the Efron scale, saturation levels increased by 9.2% from 45.63% to 54.87%, d_ increased by 12.8nm from 580.8nm to 593.6nm. Mean values of saturation and d_ for a normal population were found to be 51.2% and 582.6nm respectively. This suggests subjective grading scales depict a lower degree of hyperaemia thus do not reflect normal levels. For saturation, increments in bulbar hyperaemia for CCLRU were quadratic indicating sensitivity to changes at the lower end of the hyperaemia scale. The Efron scale was more linear in function thus more sensitive to small changes in hyperaemia. **Conclusion** Spectroradiometry is a more consistent, reproducible and sensitive method of measuring bulbar conjunctival hyperaemia than both the CCLRU and Efron grading scales due to the precision of this objective method. As a result it is likely to be superior at detecting small changes in the level of hyperaemia.

Development Of A Low Humidity Environment (Lhe) Facility For The Clinical Study Of Dry Eye Syndrome. S McCue[1], B Barney[2], P Patel[1], AM Salapatek[1]. Allied Research-Cetero Research, CA[1], Northern Air Environmental Technologies Inc,CA[2].

Purpose. To develop a state-of-the-art LHE facility with capabilities to alter environmental factors of humidity, airborne irritants, barometric pressure shifts, and wind currents, towards the development of a sensitive clinical model for evaluation of dry eye therapeutics and devices in a controlled setting. Testing of a combination of environmental variables mimic natural dry atmospheres such as in the car, home or office (winter:hot/dry;summer:cool/dry), at altitude (airplane cabins), and in windy, arid locations. **Methods.** The LHE is designed to maintain Relative Humidity (RH) to low (1-10% RH) and moderate (10-20%) levels within a comfortable temperature range (20 to 23 ^{o}C) with a custom high capacity, dehumidification system in which low RH is achievable to tight tolerances (±5% RH) with ten subjects present in the LHE facility. Sensor feedback and control are fully automated with computerized outputs in real-time including temperature, absolute and relative humidity, carbon dioxide levels and barometric pressure. To reduce static electricity within the LHE, all surfaces are made electrostatically dissipative. **Results.** Dehumidification of the LHE occurs by titanium silica gel desiccant wheel technology that removes air moisture at 29.2 gr/lb (~20%RH at 21^{o}C) upstream to 3.9 gr/lb (3% RH, 21^{o}C) downstream of the desiccant wheel with an air recirculation rate of 600 ft^{3}air/min. Low RH equilibration of the LHE is rapid and responsive, equilibrating quickly within minutes, to maintain set point conditions upon ten patient entry. Well-sealed adjoining airlock and observation areas act to maintain low RH and allow direct monitoring of patient progress and compliance within LHE, respectively. Space for ocular assessment equipment within the LHE allows for rapid objective and subjective symptom collection towards the earliest determination of drug efficacy onset. **Conclusions.** The LHE is an ideal clinical setting for the study of patients with dry eye and the efficacy of putative therapeutics or devices for this condition.
Funding provided by Allied Research-Cetero Research, CA

Role Of Evaporation On Aqueous Tear Loss And Potential Strategies For Treatment. McCulley JP, Uchiyama E, Di Pascuale MA, Butovich IA. Ophthalmology, University of Texas Southwestern Medical Center, Dallas, TX, USA.

Purpose. To determine the impact of evaporation on aqueous tear loss and the effect of two common dry eye therapies on the aqueous tear evaporative rates in dry eye patients at different relative humidities (RH). **Methods.** Two groups of patients were evaluated. Group A consisted of 16 patients with symmetrical clinical meibomianitis including signs of inflammation. Group B consisted of 12 patients with a clinical diagnosis of Aqueous Tear Deficiency with/without Meibomian Gland Dysfunction. Aqueous tear evaporation was measured at baseline of therapy. Group A received a 3 month therapy of oral Minocycline. Evaporation was measured after this period and at a 6 month study follow up. Group B received a 40ul drop of either HP-guar with a non-classical preservative system or saline on two separate days. Evaporation was measured 30 and 60 minutes after instillation. **Results.** Group A showed no change in evaporation at 25-35%RH at any of the visits. At 35-45%RH, evaporation increased in the second visit ($p<0.05$) and returned to normal at the third visit. Group B showed a decrease in evaporation at 30 minutes post instillation of HP-guar under 25-35%RH (p=0.02, 16% reduction) and 35-45% RH (p=0.03, 12% reduction). At 60 minutes post instillation evaporation also decreased but not to a statistically significant level. Normal saline produced a mixture of small increases or decreases of evaporation. **Conclusion.** Evaporation contributes significantly to aqueous tear loss (~40%) and is RH dependent. We were unable to detect a decrease in evaporation with the systemic use of Minocycline. There was an unexpected increase in evaporation on therapy that disappeared 3 months later. At this point is not possible to assign a role for evaporation in either meibomianitis or its therapy with oral Minocycline.
An HP-guar containing solution decreased evaporation 30 minutes after application. The use of topical medication with known antievaporative effect may be beneficial in dry eye therapy.
Supported by NIH grants (EY12430, EY16664), an unrestricted grant from Alcon, and by an unrestricted grant from Research to Prevent Blindness, NY.

Role Of Antimicrobial Peptides At The Ocular Surface. Alison M. McDermott. University of Houston, College of Optometry, Houston, TX 77204

Defensins and cathelicidin are cationic antimicrobial peptides that kill pathogens and modulate mammalian cell behaviours. The human ocular surface epithelia express three beta-defensins (hBD) and the cathelicidin LL37. hBD-1 and -3 are constitutively expressed whereas hBD-2 and LL37 are up regulated in response to inflammation and infection. hBD-3 and LL37 have the most potent in vitro antibacterial activity, being effective against both Gram positive and negative ocular surface pathogens. In contrast hBD-2 only shows significant activity against Gram-negative organisms and hBD-1 is the least effective. Interestingly, we have observed that human tears markedly compromise the antibacterial activity of hBD-1 and -2 but not that of hBD-3 and LL37. Recently we have shown a role for cathelicidin in defence against Pseudomonas aeruginosa keratitis. Infection was compared among wild type mice and Cnlp knockout mice, which are deficient in CRAMP, the murine homologue of LL37. The knockout mice showed more severe clinical disease, greater numbers of infiltrating neutrophils, persistence of bacteria and higher cytokine levels. While the infection resolved in the wild type, the knockout mice showed permanent corneal damage, neovascularisation and in some cases perforation. These data are the first to highlight the importance of cathelicidin in ocular surface immunity.

To further our understanding of the role of antimicrobial peptides, contributions from the defensins need to be elucidated, as does the interplay between the peptides and how their ability to modulate epithelial and immune cell behaviour contributes to protection and resolution of infection. Antimicrobial peptides have obvious clinical potential, however none have been successfully brought to market. Careful in vitro testing under physiological conditions representative of the disease to be treated is required to identify the peptides most likely to be functional in vivo. Furthermore, delivery mechanisms taking advantage of the latest nanotechnology need to be investigated as simple application in solution is unlikely to be of value, and may induce significant toxicity.

Commercial Relationships: None; Grant support: EY 13175

Proteomic Analysis Of Conjucntival Swab By Mass Spectrometry. Vicky McGilligan,[1] Joanna E. Graham,[1] Jonathan E Moore,[1,2] Geoff McMullan,[1] Robert LJ Graham,[1] Raymond O Beirne,[1] Stephen C. Downes,[1] Tara CB. Moore[1]. Centre for Molecular Biosciences, University of Ulster, Northern Ireland,[1] Royal Group Hospitals, Belfast, Northern Ireland.[2]

Purpose. The purpose of this study was to identify proteins present in the tears and mucosal epithelium of the ocular surface. **Methods.** A cotton swab was rubbed across the anaesthetized inferior conjunctiva of a dry eye patient. Protein was extracted and subjected to 1D gel electrophoresis. After excision and trypsinisation, protein profiles from swab samples were identified using mass spectrometry carried out on a 3200 Q-TRAP Hybrid ESI Quadropole linear ion trap. Protein identification was performed using MASCOT software against a human database extracted from NCBI. Curation of the protein list was achieved using the bioinformatics tool PROVALT, which also calculated false-discovery rates. **Results.** In total 75 validated proteins were identified including the tear proteins, lactotransferrin, lysozyme, and proline rich proteins as well as a number of proteins not previously associated with the tear proteome. Proteins identified had a wide range of physio-chemical properties and included structural and functional proteins. **Conclusions.** Use of a simple swab combined with a GeLC-MS proteomic protocol led to unequivocal identification of a large range of proteins associated with the ocular surface proteome. This may allow a better characterisation of the ocular surface environment and discrimination between various eye conditions. Tear collection using capillaries can be tedious and may discourage clinicians from performing such a test. Use of a swab that can be frozen for analysis may encourage the use of this methodology. The protocol used in this study identified proteins previously unseen within tear or meibomian gland secretions. Analysis of this proteome offers huge clinical potential for investigation of ocular surface biomarkers for the development of novel diagnostic tools and monitoring of ocular disease.

[Research was supported by Invest Northern Ireland]

Identification And Mapping Of Oligosaccharide Moieties On Individual Secreted Mucins. Terence McMaster[1], Sarah Baos[1, 2], Debra Brayshaw[1], Peter Heard[1, 3], Monica Berry[2]. H.H. Wills Physics Laboratory,[1] Academic Unit of Ophthalmology,[2] and Interface Analysis Centre, [3] University of Bristol, Bristol, U.K.

Purpose. We propose a novel technique for the mapping of functional groups on single secreted mucin molecules, and describe the preliminary steps in achieving this. **Methods.** Spent medium from IOBA-NHC (conjunctival) and Araki-Sasaki (corneal) epithelial cell lines was collected, centrifuged and dialysed to about 1/10 of the original volume against 15% polyethylene glycol compound, and a denaturing agent, 4 M urea, was added. Caesium Chloride was added to achieve a density of 1.4 g/ml, and the samples ultra-centrifuged. Size fractionation of the supernatant samples was carried out on Sepharose CL2B columns. Purified and fractionated samples were then prepared for investigation in the Atomic Force Microscope (AFM). Mucin fractions were injected into the liquid cell of the AFM and immobilised onto an atomically-flat mica surface. Two types of image were recorded: (1) a conventional topographic image, and (2) a recognition image. The first data set utilises an unmodified AFM cantilever tip which records sample height, whilst the second data set utilises a molecular-functionalized cantilever tip which will form chemical recognition bonds only with specific epitopes on the mucin molecule. Correlation of these 2 data sets will allow mapping of the mucin molecule. **Results.** AFM images have been obtained for mucins in an extended but equilibrium conformation, and measurements of molecular parameters such as contour length, and persistence length ("molecular stiffness") obtained. AFM tips have been functionalized with lectins and with antibodies to non-VNTR regions of the peptide core, and force recognition maps recorded. **Conclusions.** We have correlated single molecule mucin topography and recognition forces in development of a molecular mapping technique. Different functional groups on the tip will allow more complete mapping of mucin functionality.

Efficacy Of Systane Compared To Hylocomod In The Treatment Of Dry Eye. Elisabeth M. Messmer, Department of Ophthalmology, Ludwig-Maximilians-University, Munich, Germany

Purpose. To compare the efficacy of two lubricating eye drops, one including PEG400/PG/HP-Guar (Systane), the other one including HA 1% (Hylocomod) in the treatment of dry eye. **Methods.** Thirty-six dry eye patients with frequent ocular symptoms and corneal staining >/= 2 in at least one corneal area were randomized to either Systane or Hylocomod. Eye drops were administered 4x/day after a wash out phase with saline for one week. Examinations including visual acuity, thorough slit lamp exam, corneal and conjunctival staining and Schirmer test with and without anaesthesia took place at the screening visit, at baseline, after 4 weeks, and after 3 months. A symptoms questionnaire was completed at each visit. **Results.** Patients were 25 to 82 years old (mean 53 years) with a female preponderance of 88%.

There was no significant difference in age, gender, incidence of symptoms, and frequency of eye drop application in both groups. Systane significantly increased tear film break up time (TFBUT), and significantly decreased conjunctival injection, conjunctival staining and corneal staining compared to baseline after 4 weeks and 3 months (all $p<0.003$). Moreover, burning sensation, foreign body sensation and overall dryness as well as end of the day dryness were significantly decreased compared to baseline (all $p<0.03$). No significant difference was present between Systane and Hylocomod in TFBUT, conjunctival injection and staining as well as corneal staining. Hylocomod significantly decreased end of the day dryness after 3 months compared to Systane ($p=0.03$). Burning sensation was significantly less in the Systane group on visits 1 to 3 (all <0.05). Moreover, Systane made patients forget their symptoms significantly more often after 4 weeks compared to Hylocomod ($p=0.03$). **Conclusion.** Systane was effective in reducing symptoms and signs of dry eye. Its performance was comparable to Hylocomod.
[This research was supported by grants from Alcon]

Biophysical Properties Of Lipids Spread At The Pre Ocular Tear Film. Fausto Miano SIFI S.p.A.

The lipid layer of the ocular tear film covering the cornea and conjunctiva has several essential functions that include maintaining an optically smooth surface, acting as a barrier to the ingress of microorganisms and particulate debris, and preventing the evaporation of the aqueous phase. The liquid-air interface comprises a layer of lipids variously estimated between 10 - 100 nm in thickness. The layer contains amphiphilic components, phospholipids, ceramides and cerebrosides, as well as non-polar molecules such as cholesterol esters, fatty acid esters and triglycerides. There are stringent physiological demands on the dynamics of the tear film, not least because it must regenerate quickly with each blink. Abnormalities in the tear film are related to a wide-spread disorder called dry eye syndrome.

A path of biophysical research was followed by our laboratories in conjunction with academic institutions, profiting of the availability of sophisticated and effective experimental techniques at the interface, with the aim of investigating the non-biological effects of the presence of the ocular lipid layer upon the characteristics of the tear film. Emphasis was given to evaporation phenomena and lipid – proteins interactions.
Experimental data for the retardation of evaporation in a pendant drop model have shown that the meibomian lipid mixture is more effective than the sum of its individual components in retarding the evaporative flux of the underneath aqueous layer and that the effect of protein presence on the evaporative process results in a moderate increment of the evaporation rate. Starting from this evidence it was studied the adsorption of tear protein at the air-liquid interface either in the presence of a spread lipid layer and at a bare saline solution. Comparing the structural conformations of human lactoferrin adsorbed at the air/water interface measured by neutron reflectivity and its solution structure measured by small angle neutron scattering, it was found a strong structural unfolding of the molecule when adsorbed at the nude air saline interface where the extent of lactoferrin adsorption at the interface was found to decrease with increasing surface pressure of a spread lipid monolayer. It was thought that the effectiveness of a meibomian lipid film in preventing protein adsorption and consequent denaturation relies in its peculiar spatial organization and to that end a Grazing Incidence X-Ray Diffraction investigation of the surface lipid layer of the pre-ocular tear film was performed on natural meibomian lipid mixtures and several phospholipids and non polar oil laboratory mixtures. The experiments revealed that despite the complex chemical composition the meibomian lipids show the co-existence of a dilute and a much more condensed phase in the amphiphilic lipid matrix spanning the pressure range 15 – 45 mN/m plus an additional structure due to the much more hydrophobic part of the mixture. This evidence supports the previously hypothesised layered structure of the tear film. Surprisingly, only a restricted number of laboratory compositions reproduced the meibomian structure at the interface and their ability to mimic the natural lipid tear film was reflected in very similar anti-evaporative and protein protecting abilities.

Functional Role Of The Tear Film Lipid Layer. Thomas J Millar. School of Natural Sciences, University of Western Sydney, Australia

Besides lubrication, the foremost function assigned to the Meibomian lipid layer is an outer blanket to reduce evaporation from the tear film, as portrayed in models of the tear film. Like the first models of the cell membrane, substantial experimental and clinical data suggest that this is too simplistic. Dry eye has been associated with Meibomian gland disease or a decrease in the stability of the lipid layer, and it has been tantalizing to equate a thickened lipid layer (>100nm) with reduced evaporation and a stable tear film, and a thin lipid layer (<60nm) with dry eye. On average, this thesis is supported, but there are enough exceptions to negate lipid layer thickness as a reliable indicator of tear film stability, or its thinness as a predictor or dry eye: the lipid layer can be thicker in moderate dry eye than normal, or when the aqueous is inadequate; in contrast, the aqueous levels in people with Meibomain gland deficiency are not necessarily reduced; and evaporation has been reported to be both increased and decreased in dry eye.

These variances indicate that either Meibomian lipid composition differs, or that the model of an outer lipid blanket is too simplistic. With advances in mass spectroscopy, surprising results are emerging from analysis of Meibomian lipids. Common findings are that sterol and wax esters form the bulk of the lipids, and that relatively high levels of cholesterol esters are more likely to have or lead to dry eye. Controversial are the finding of fatty acid amines by one group, and no phospholipids in Meibomian lipids by another. If true, the function of the lipid amines in normal and dry-eye states needs to be determined. Lack of phospholipids provides a conundrum because these were believed to the main surfactants acting between the wax and sterol esters and the aqueous layer of the tear film. Our physical chemistry data show that proteins found in the aqueous phase of the tear film could subserve this surfactant role. Moreover, proteins and mucins are not just interacting with the lipids at the lipid aqueous interface as suggested by others, but form part of the surface layer. Indeed, our data on evaporation under controlled conditions *in vitro* strongly indicate that it is these interactions that prevent evaporation rather than a simple blanket of Meibomian lipids. These concepts form a radical departure from the traditional idea of a three layered tear film and provide a platform for further investigation.

Acinar Cell Paracrine Mediators Enforce Self-Tolerance In Rat Lacrimal Gland. A.K. Mircheff, T. Nakamura, M. de Saint Jean, Y. Wang, University of Southern California, Los Angeles, CA, USA.

Purpose. Lacrimal acinar cells (AC) express a transcytotic apparatus that inserts polymeric immunoglobulin receptors into the basal-lateral membrane to endocytose dimeric IgA. This also may be a constitutive paracrine apparatus that releases both autoantigens and soluble mediators to the underlying tissue space (TOS 4: 182-193, 2005). AC isolated from rabbit lacrimal gland express MHC Class II and stimulate proliferation of autologous lymphocytes in co-culture. In contrast, AC isolated from rat lacrimal gland also express MHC Class II, but they

suppress proliferation of autologous lymphocytes (TOS 3:S56, 2005). Soluble mediators from rat AC suppress proliferation of ConA-stimulated lymphocytes (Regional Immunol. 3:198-203, 1990-91). However, the immunophysiological significance of these observations has not been established. **Methods.** Dendritic cells (DC) were matured ex vivo by culturing adherent rat bone marrow cells in the presence of rat IL-4 and GMCSF. AC were isolated from rat lacrimal glands, and lymphocytes from lymph nodes. Proliferation was measured with [3H]-thymidine. **Results.** Pre-treatment of AC with LPS abolished their inhibitory activity and caused them to stimulate proliferation of lymphocytes in co-culture. The presence of microporous inserts with additional, non-treated AC suppressed lymphocyte proliferation stimulated by LPS-pretreated AC. Ex vivo-matured DC stimulated lymphocyte proliferation. Microporous inserts containing non-treated AC inhibited DC's ability to stimulate lymphocyte proliferation, even when DC were pretreated with LPS. Flow cytometry indicated that inserts with non-treated AC suppressed expression of CD86 and MHC Class II by maturing DC. **Conclusions.** AC from rat lacrimal gland have an unusually robust ability to secrete paracrine mediators that enforce tolerance to autoantigens, in part by modulating phenotypic expression of DC maturing in the local milieu. TLR agonists suppress AC secretion of the immunomodulatory mediators. A similar ability may exist in rabbit lacrimal gland, but may be compartmentalized to the ductal epithelium.
Commercial Relationships: None. Support: EY005801.

Prolactin-Induced Dacryoadenitis In Rabbit. A.K. Mircheff, Y. Wang, P.B. Thomas, S. Song, M.D. Trousdale, J.E. Schechter, University of Southern California, Los Angeles, CA, USA.

Purpose. Prolactin (PRL) and steroid hormones transform the mammary gland during pregnancy. PRL also accumulates within lacrimal epithelial cells and appears to contribute to a mammary-like immunophysiological transformation (Am. J. Physiol. 292: E1122–E1134, 2007). Since elevated PRL is associated with autoimmunity, we hypothesized that locally increasing PRL without also increasing systemic steroid hormones would induce a Sjögren's-like dacryoadenitis. **Methods.** Adenovirus vector for rabbit PRL (AdPRL) was injected into OS lacrimal glands of mature female NZ white rabbits. Controls were not injected or were injected with vector for green fluorescent protein. Three animals each from the injected groups were euthanized on days 4, 12, and 29. Lacrimal glands were removed for histopathology and for real time rt-PCR using rabbit-specific primers and probes designed with vendors' software. **Results.** Lymphocytes extensively infiltrated AdPRL-injected glands, maximally on d4 and d12, less on d29. AdGFP-injected glands were affected much less severely. PRL mRNA increased 130-fold on d4 and declined to near control by d12. IFN-gamma increased 60-fold and TNF-alpha 6-fold on d4; both remained elevated on d12, then declined. CD28 and CTLA-4 increased 2- to 3-fold; CD80 5-fold; CD86 2-fold. CD4 increased 4-fold on d4 and remained elevated on d12. CD8 and CD25 were unchanged on d4, but CD8 increased 5-fold and CD25 2.5-fold on d12. CD4, CD8 and CD25 all declined by d29. **Conclusions.** We conjecture that elevated PRL induces expression of IFN-gamma. PRL and IFN-gamma then support activation of APCs and autoreactive CD4+ cells. Over time, CD4+ and CD8+ regulatory T cells arise and restore immunohomeostasis. Progesterone, as it antagonizes milk production, may prevent autoimmune activation during normal pregnancy. We suspect that the immediate post-partum, with elevated PRL and diminished progesterone, may be a period of increased risk for generation of autoimmune CD4+ T cells.
Commercial Relationships. None.
Support: EY005801 (AKM), EY010550 (JES), EY012689 (MDT).

Influence Of Aspirin Treatment On Lacrimal Gland And Ocular Surface Of Diabetic Rats. Carolina Maria Módulo, Ana Carolina Dias, Angélica Gobbi Jorge, Alexandre Martins Braz, Rubens Bertazolli Filho, Jayter Silva de Paula, Alceu A. Jordão Jr, Eduardo M. Rocha. Departamento de Oftalmologia, Otorrinolaringologia e Cirurgia de Cabeça e Pescoço e Departamento de Clínica Médica da Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo

Purpose. Previous works have observed structural, functional and molecular alterations in lacrimal gland (LG) and ocular surface (OS) after chronic diabetes mellitus in rodents and humans. Those events have been correlated with hyperglycemia and inflammatory signaling mechanisms. The aim of this study is a) to compare the expression of markers of oxidative stress and histological markers of disease and b) to evaluate whether aspirin treatment inhibit LG and OS alterations. **Methods**. Diabetes was induced in male Wistar rats with a single intravenous streptozotocin or vehicle and a sub group was treated with insulin. After 5 and 10 weeks, LG and OS of the three groups (n=5/group) had the structure and morphology compared, and analyzed for expression of peroxidase. Impression cytology (IC) graded and compared the ocular surface epithelia. **Results.** After 5 and 10 weeks, no significant difference was found in ocular surface epithelia morphology (IC), however DM affect tears secretion, which was reverted by aspirin ($P<0.01$) . LG morphology alterations and increased number of lipofucsin-like inclusions was observed in diabetic after 10 weeks, however similar to controls in aspirin treated diabetic rats. Peroxidase levels were significantly higher in DM, but similar to controls in aspirin treated diabetic rats ($P= 0.0025$). **Conclusions.** Diabetes induces significant alterations in rat LG suggesting that hyperglycemia-related oxidative stress may take a part in diabetic dry eye syndrome, as indicated by differences in peroxidase levels. Those events were reverted by aspirin, which may be an effect of anti-inflammatory treatment on oxidative stress pathways in LG.
Support: CNPq, FAPESP, CAPES, FAEPA

Correlation Between Diagnostic Tests Guides The Development And Use Of A Dry Eye Diagnostic Algorithm. Jonathan E Moore,[1,2] Joanna E. Graham,[1] Edward Goodall,[1] Darlene A. Dartt,[3] Antonio Leccisotti,[1,4] Stephen C. Downes,[1] Tara CB. Moore[1]. Centre for Molecular Biosciences, University of Ulster, Northern Ireland,[1] Royal Group Hospitals, Belfast, Northern Ireland,[2] Schepens Eye Research Institute, Boston, USA,[3] Générale-de-Santè Toscana, Siena, Italy[1,4].

Purpose. The existence of large variations in results of diagnostic tests for cases of mild to moderate dry eye is widely recognised. The purpose of this study was to assess if there was correlation between existing dry eye diagnostic tests and attempt to develop a diagnostic algorithm. **Methods.** A total of 91 subjects were recruited to the study. The tear film and ocular surface were evaluated by measuring tear volume using the phenol red thread test, tear break up time using fluorescein, biomicroscopic examination of meibomian glands and impression cytological assessment of conjunctival goblet cells. Dry eye symptoms were assessed using McMonnies dry eye questionnaire and statistical correlations were assessed between all dry eye tests used. **Results.** In this study cohort there were no severe aqueous deficient dry eye patients. Meibomian gland pathology, questionnaire (with a cut off score of 14), impression cytology and an altered tear break up time (with a reference point of 7 seconds) demonstrated correlation, while no other tests demonstrated evidence of correlation. A diagnostic algorithm was devised using these tests alone, with maximum weighting placed on questionnaire with decreasing weighting assigned to impression cytology goblet cell grade, meibomian gland pathology and TBUT. **Conclusions.** We propose this development of a flexible

probabilistic algorithm as a rational approach when diagnosing mild/moderate dry eye in large study groups. Until further studies have demonstrated the true sensitivities and specificities of dry eye tests within the context of test sequences and particular patient cohorts are known, comparison of results between dry eye studies will remain questionable.
[This research was supported by the Department for Employment and Learning in Northern Ireland].

Comparison Of Cytokine Levels In The Tears And Saliva. Carol Morris,[1] Paul Connellan,[1] Linda Banbury,[1] Shelly Ames,[2] Carol Lakkis.[2] Centre for Phytochemistry and Pharmacology, Southern Cross University, Lismore, Australia,[1] Clinical Vision Research Australia, The University of Melbourne, Melbourne, Australia.[2]
Purpose. Despite the fact that the tear film is more stable than saliva, the potential for the tear film to be used as a source of diagnostic information has received limited attention. This study aimed to assess the concentration of cytokines in the tear film and saliva and to determine whether a correlation between the cytokine levels in the two physiological fluids exists. **Methods.** Reflex tears and saliva were collected from n=51 subjects within a 1 hour period. Subjects with ocular or systemic disease and/or using topical or systemic medications were excluded. Cytokines were measured by flow cytometry using a Cytometric Bead Array (Becton Dickinson) with a human inflammation panel to detect IL-12p70, TNF-α, IL-10, IL-6, IL-1_ and IL-8. Duplicate testing of each sample was conducted and concentrations are presented as median (interquartile range) pg/ml. **Results.** IL-12p70, IL-6 and IL-8 were present in both the tears and saliva, with IL-12p70 (saliva 4.2 (1.9-6.6), tears 6.5 (0-13.1)) and IL-6 (saliva 6.8 (5.0-11.6), tears 11.7 (8.0-17.7)) being significantly higher in tears compared to saliva ($p<0.05$). IL-8 levels did not vary significantly between the two fluids (saliva 413.5 (262.4-642.8), tears 356.9 (218.9-587.0), $p>0.05$). IL-1_ was below the detection limit in tears but was significantly higher in saliva (113.3 (50.5-175.2), $p<0.05$). TNF-α and IL-10 were below the detection limit in both tears and saliva. When cytokines were detected in the tears, the levels did not correlate significantly with the concentrations observed in the saliva ($p>0.05$). **Conclusions.** Whilst IL-12p70, IL-6 and IL-8 were reliably detected in the tears, their concentrations were not correlated with levels observed in the saliva. The differences in cytokine levels between the tears and saliva suggest that the tears can be used as a source of diagnostic information; however, are more likely to provide localized rather than systemic information regarding inflammation.
Supported by CIBA Vision GmbH and Southern Cross University.

The Formulation Approach In Ocular Therapy. Valeria Moschetti, Letizia Lo Grasso, Elena Solfato, Anna Claudia Scuderi, Pharma Business Unit, S.I.F.I. S.p.A., Catania, Italy

Purpose. Aim of this study is to provide an innovative approach for the selection of the right dosage form for the treatment of ocular surface diseases. We distinguished the performances of commonly used ophthalmic dosage forms (eye drops, ointment, eye gel), based on their physico-chemical properties. **Methods**. Commercially available ophthalmic dosage forms, such as viscosized eye drops, ointment and ophthalmic gel, with particular emphasis for a new gel formulation, were analyzed in terms of their physico-chemical properties: rheological profile, mucoadhesion performance, refraction index. The rheological profiles were acquired by a rotational cone-plate rheometer; the mucoadhesion properties were evaluated by a tensile strength method; the refraction index values were obtained by means of a refractometer and compared with the normal tears value. **Results**. The rheological profiles at the temperature of the ocular surface (35°C) showed a similar, high value of viscosity for the ointment and the ophthalmic gel, and a low viscosity value for the eye drops. The mucoadhesion test showed a strong interaction between the new gel formulation and the mucin whereas the ointment displayed neither affinity with the mucin nor muchoadesion. The test method was not able to evaluate the eye drops mucoadhesion due to the low viscosity of the formulation. The refraction index values of both gel and eye drops formulations were similar to that one of tears, but the ointment was not evaluated because of its opalescent aspect. **Conclusions**. The physico-chemical properties of each dosage form may affect its *in vivo* performance after ocular administration (i.e. the ocular surface residence time, the patient's comfort). The appropriate selection of a dosage form may have a particular relevance when the ocular surface is compromised such as in dry eye conditions or during wound healing.

REM Sleep And Tear Secretion. A Hypothesis. Juan Murube[1], Joaquin Carbonell[2]. University of Alcalá-Madrid[1], San Francisco Hospital, Madrid[2].

Purpose. When sleeping there is a high brain activity running in different phases. These phases have been included in two primary alternating periods known as REM (Rapid Eye Movements) and non-REM sleep, taking as the main parameter the presence or not of rapid eye movements. At present, there are no scientific theories to justify these eye movements, nor their relation with other concomitant phenomena during the phases of REM sleep.

The ocular surface may play an important role in these eye movements. It is well known that tear secretion is very scarce during sleep, and in persons with dry eye this sometimes results in ocular itching, awaking or keratopathia punctata. The object of this study was to attempt to determine whether or not during the REM periods there is an increase of the tear secretion that protects the ocular surface from the nocturnal sleeping dryness. **Methods**. We have examined many cases of patients by polysomnography observing their eyes during REM and non-REM phases. A simultaneous study of the phases of REM/non-REM and of the tear production was performed in 8 patients, when sleeping. For the polysomnography an EEG-PSG System NIHON KOHDEN 36 channels was used, AND FOR TEAR STIMULATION production along the time of sleep, the relative humidity under goggles spectacles was measured with a humidimeter TestoStor-171-1. **Results**. In all cases, when polysomnography showed a REM phase, it was associated to values of increasing humidimetry. The values of relative humidity under the goggles went up from an average of 68% before the REM sleep, to an average of 81% 5 minutes after beginning the REM sleep phase. No other external circumstances were appreciated that could interfere with the sleep and the measurement process. **Conclusions**. The increase of tear production during the phase of REM sleep was evident. As a result of this finding, we hypothesize that the rapid eye movements may serve to stimulate the ocular surface friction, the eye activity, and consequently the tear production. Other phenomena of the REM sleep phase may be independent of this fact. In the literature review we have found no other explanations for the rapid eye movements phenomenon.

Effect of D- ß-Hydroxybutyrate on Ocular Surface Disorders in a Rat Dry Eye Model. S. Nakamura[1], H. Nakashima[1], R.Hisamura[1], N.Masuda[1] Y.Saito[1] Y.Yabuno[1], and K. Tsubota[2]. 1 Research center, OPHTECS, Toyooka, Japan; [2] Ophtalmology, Keio University, School of Medicine, Tokyo, Japan

Purpose. D-ß-Hydroxybutyrate (HBA) is a ketone body produced by hepatocytes and astrocytes through the degradation of long-chain fatty acids. Recent findings suggest that HBA plays a role in maintaining the viability of neuronal tissues under conditions of hypoxic or ischemic insult. In this study, we investigated the effect of topically applied HBA on chronic SPK and tear film stability. **Methods.** A series of treatments were performed under continuous exposure to low–humidity airflow (25 ± 5%, 2–4 m/s). Female SD rats aged 7 weeks were used for this study. Rats were placed on a Jogging board(JB) made of a plastic pipe for 7.5 h/d, and, for 16.5 hours, they were placed in individual cages without JB treatment.Ten days after rat eyes were applied phosphate–buffered saline (PBS) every 2 hours during JB treatment, eyes that showed SPK were examined. Eyes were randomly selected for 0.04, 0.2, 1% HBA eye drops, or PBS as a control (n=13–14). Five microlitters of eye drops were then given every 2 hours for 8 hours during JB treatment. After the onset of 14 and 21 days application, SPK was recorded according to the protocol described by Shimmura and associates (Shimmura et. al. Br J Ophthalmol 1995). Tear film stability was evaluated using a noninvasive specular reflection video recording system (DR–1, Kowa Company Ltd., Tokyo, Japan) during sustained eye opening following forced blinking. **Results.** For the PBS and 0.04% HBA group, the SPK score were not changed compared to the initial values during experiment. Topically applied 0.2, 1% HBA dramatically recovered the SPK score after 14 and 21 days of treatment and significant decreases were observed compared to the initial ($P < 0.01$) and PBS treatment values ($P < 0.05$) .
For the 1%HBA group, the tear film stability was dramatically recovered after 14 days of treatment. **Conclusions:** This study suggests the potential usefulness of HBA in the clinical treatment of ocular surface epithelial disorders in patients with chronic symptom of dry eye.
Commercial Relationships: S. Nakamura, OPHTECS, E; H. Nakashima1, R.Hisamura, OPHTECS, E; N.Masuda, OPHTECS, E; Y.Saito, OPHTECS, E; Y.Yabuno; OPHTECS, E; K. Tsubota, None.
Support: None

Psychiatric Diagnosis In Dry Eyes. Johannes Nepp. Department of Ophthalmology, Medical. University Vienna, Austria.

Purpose: Patients with dry eyes are known as difficult with noticable manners.Maybe that is the background of less effects of treatments. In this study we wanted to find out, if we find psychiatric diagnosis in combination with dry eyes. **Methods:** All patients with dry eyes more than 1 year resistant on any artificial tears were observed. Records were used with diagnosis by ICD 10, especially Mental and behavioural disorders F00-F99 For dryness slit lamp observation was done including the lipid layer thickness, the break up time of the tear film and the tear meniscus, the Schirmer Test, fluorescein and lissamin green staining. Severeness was determinated by the sicca- score, the mean of measurements of dryness, all equated. With Pearson correlation statistical analysis was performed. **Results**: In 27 of 34 patients (79%) we could find mental and behaverioral disorders. Anxiety was found in 26/34 (76,5%) patients, most of them with generalized ynxiety (F41.1) in 22/34; and social phobia F40.1/ in 10/34 and specific phobie F40.2 in 10/34. Depression was found in 18/34 (52,9%) especially Dysthymia, F34.1 in 6/34 patients. Generally patients reported from vegetative symptoms like nervousity (24/34). There was no patient with severe psychiatric diagnosis.The correlation of dryness to anxiety and depression was not significant, but there was a trend for more mental disorders according to the severness of dry eyes. **Conclusion**: Severness of dry eyes is combined with mental disorders, Therefore effects of relaxation methods like acupuncture or hypnosis calming the nervousness on improving eye conditions should should be considered in patient without effect on conventional treatments.
The author discloses any commercial relationships.

Hyaluronic Acid Versus Hyaluronic Acid Associated With Echinacea Purpurea Extract (Iridium^{tm}) For The Control Of Ocular Surface Disturbances In Patients Treated With Antiglaucoma Polytherapy: A Pilot Study. Piergiorgio Neri, MD, Cesare Mariotti, MD, Manuela Zucchi, Lucia Mercanti, MD, Alfonso Giovannini, MD. The Neurosciences Department, The Eye Clinic, Polytechnic University of Marche, Ancona-Italy

Purpose: To compare the safety and efficacy of hyaluronic acid eye drops versus hyaluronic acid associated with echinacea purpurea extract eye drops (Iridium^{TM}, SOOFT Italia srl) for the control of ocular surface disturbances in patients treated with polytherapy for primary open angle glaucoma. **Methods:** Study design: prospective, consecutive, comparative, randomized, open label trial. Patients have been divided into 2 groups: "Group 1" treated with hyaluronic acid eye drops, "Group 2" with hyaluronic acid associated with echinacea purpurea extract eye drops. The following tests have been performed: slit lamp examination, break up time (BUT) test (seconds), corneal fluorescein staining test, conjunctival rose Bengal staining test, Schirmer test I (mm/5 minutes).
Patients have been checked at: 15, 30, 60, 90 days.
The right eye only has been considered for the statistical analysis. A $p<0,05$ level has been considered statistically significant. **Results:** Group 2 had less redness and photophobia at 15 and 30 days ($p<0,05$) than Group 1. The rose Bengal conjunctival staining and fluorescein corneal staining were persistent in Group 1 at 15 and 30 days ($p<0,05$); no abnormalities were noted in Group 2 at those times. At 60 and 90 days, no differences were observed. Schirmer e BUT test did not show differences at any time ($p>0.05$). **Conclusions:** The combination of hyaluronic acid with echinacea purpurea extract appears to be more effective and faster in resolving symptoms secondary to ocular surface disturbances in patients with antiglaucoma polytherapy. In addiction, it seems to induce a significantly sooner healing of both corneal and conjunctival micro-lesions.
Financial disclosures
Authors disclose financial involvement with companies that directly compete with products named in this manuscript.

T_H17 Cells in Exocine Gland Tissues of an Animal Model for Sjögren's Syndrome (SjS). Cuong Nguyen[1], Minnie Hu[1], Carol Stewart[2] & Ammon Peck[1] Departments of Oral Biology[1] & Oral Medicine[2], College of Dentistry, University of Florida, Gainesville, FL USA

Purpose: In recent years, the T_H1/ T_H2 paradigm has been challenged by the discovery of T_H17 cells, a subset of CD4+ T memory cells characterized by the unique ability to secrete IL17. Differentiation of T_H17 cells is initially mediated by TGF_/IL6, then subsequently by IL23. IL17 cytokines are potent pro-inflammatory factors active in tissue inflammation, inducing expression of other pro-inflammatory cytokines and chemokines (e.g., IL6, TNF, and MIP2) and mediating tissue destruction by upregulation of MMPs. The purpose of this study

was to examine the possible presence of T_H17 cells during development of exocrine dysfunction in C57BL/6.NOD-*Aec1Aec2* mice, a model for SjS. **Methods:** Tears, saliva, sera, and exocrine glands were freshly isolated from 4, 8, 12, 16, and 20 wk old C57BL/6.NOD-*Aec1Aec2* mice. Tears, saliva and sera were analyzed for secreted IL17 by Luminex bead assays, while lacrimal and submandibular glands were stained for IL17 and IL23 using immunohistochemistry. tRNA was extracted from the exocrine glands and quantified by real-time PCR to determine the expression profiles of these cytokines, their receptors, and their transcription factor regulators. **Results:** C57BL/6.NOD-*Aec1Aec2* mice exhibited differential expression of IL17, IL23 and IL17R in the exocrine glands during disease development. IL17 was also present in fluid samples. Expression profiles of IL17 and IL23 correlated with expression of ROR_t, the T_H17 cell master control gene. Immunohistochemical staining revealed diffuse, sparsely staining patterns on epithelial tissues and within lymphocytic foci. **Conclusions:** Results suggest that IL17-T_H17 system may be important in the clinical manifestations of SjS-like disease in C57BL/6.NOD-*Aec1Aec2* mice. Further study is needed to determine its precise role in interacting with other infiltrating lymphocytes in exocrine glands.
Funded by UF Center for Orphaned Autoimmune Diseases and PHS grant T32 DE07200

Contributions Of Evaporation And Dry Eye Status To Pre-Lens Tear Film Thinning. Jason J. Nichols, Elisa Skadahl, Ewen King-Smith. College of Optometry, Ohio State University, Columbus, Ohio

Purpose: We recently reported that the average rate of pre-lens tear film thinning (PLTF) was 6.97 _m/min, although the distribution of thinning rates was bimodal suggesting that there may be more than one mechanism of thinning (Nichols et al, IOVS, 2005). The purpose of this work was to determine the contribution of evaporation and dry eye status to pre-lens tear film thinning. **Methods:** Pre-lens tear film thinning rates and thickness were measured in 40 contact lens wearers (33 female, average age = 26 years) via wavelength-dependent interferometry. Four measurements, separated by 2 mins, were recorded under normal 'evaporative' conditions. Subjects also completed the Contact Lens Dry Eye Questionnaire (Nichols et al, Cornea, 2002). During a second visit, subjects wore goggles for four minutes to allow the ocular surface environment to approach 100% humidity ('non-evaporative' condition) and two measurements were recorded (again separated by two minutes). Repeated measures ANOVA was used to examine the effects of evaporative and dry eye status on PLTF thinning rate. **Results:** There were 30 non-dry eye and 10 dry eye subjects included in the study. Repeated measures ANOVA showed that both evaporative status ($F = 66.4$, $p < 0.0001$) and dry eye status ($F = 5.35$, $p = 0.03$) were related to PLTF thinning rate. In this regard, the average (± SD) PLTF thinning rates under the evaporative condition (open air) were 11.69 ± 5.13 _m/min and 6.49 ± 5.69 _m/min for the dry eye and non-dry eye groups, respectively. The average (±SD) PLTF thinning rates under non-evaporative conditions (goggles) were 1.47 ± 1.55 _m/min and 0.88 ± 2.80 _m/min for the dry eye and non-dry eye groups, respectively. **Conclusions:** Non-evaporative conditions lead to a substantial reduction in pre-lens tear film thinning rate suggesting evaporation is a primary mechanism of PLTF thinning. PLTF thinning also appears to be strongly related to dry eye status, with contact lens-dry eye subjects showing a significantly faster rate of tear film thinning under both evaporative and non-evaporative conditions.
Commercial Relationships. None Support. None

Quality Of Life In Postmenopausal Women With Dry Eye. Kelly K. Nichols and Lisa A. Jones. The Ohio State University College of Optometry, Columbus, OH, USA.

Purpose. Recently there has been an increased interest in the quality of life in dry eye patients with respect to symptoms of visual disturbance and ocular irritation as well as the impact on quality of life (QoL). The aim of this analysis is to explore the association between QoL and dry eye test results in postmenopausal women.
Methods. The NEI-VFQ (short form 25) was completed and a dry eye evaluation performed in 500 postmenopausal women. Overall and sub-scale NEI-VFQ scores were calculated, and patients were queried as to previous dry eye diagnosis, if patients "think" they have dry eye, and if significant symptoms were present. Dry eye examination tests included osmolarity, fluorescein corneal staining, lissamine green conjunctival staining, tear break-up time, and the Schirmer test. **Results.** Of the sample, 46.3% reported dry eye symptoms and 31.1% believed they had dry eye, while 32.1% had been previously diagnosed with dry eye. Statistically significant differences were demonstrated for many of the NEI-VFQ subscales based on the three symptomatic reports (presence of symptoms, "think" dry eye, and previous diagnosis). As expected, the ocular pain subscale demonstrated the largest differences for all three symptom classifications. For the question "Do you think you have dry eye" a 17.7 ± 13.8 point difference ($p < .0001$) was seen between those responding "yes" (Pain score 72.1 ± 15.3) and "no" (Pain score 90.6 ± 12.6). There is some suggestion that the Near Vision and Far Vision subscales are impacted as demonstrated by approximately a 7 unit difference, which is statistically significant. It has been suggested in the literature that a 10 to 12 unit difference is clinically meaningful. Clinical test data used to define dry eye, such as central and total corneal staining, are also used as diagnostic criteria and compared to NEI-VFQ results. **Conclusions.** These data confirm that symptoms are critical in patients' perceptions of dry eye disease, whether they believe they have dry eye or if they have a previous diagnosis. In this sample of postmenopausal women, other vision and health-related subscales may be impacted by these symptoms and warrant further consideration by researchers and clinicians.
Commercial interests: None. Support: This research was supported by NIH NEI K23EY00393 and NIH NEI R24014792.

A Mouse Model Of Lacrimal Keratoconjunctivitis: Evidence That Some Forms Of Dry Eye Disease Are Immune-Mediated. Jerry Y. Niederkorn[1], Michael Stern[2], Stephen C. Pflugfelder[3], Karyn F. Siemasko[2], Jianping Gao[2], Virginia L. Calder[4], and Margarita Calonge[5]. U.T. Southwestern Medical Center, Dallas, Texas[1], Allergan, Irvine, California[2], Baylor College of Medicine, Houston, Texas[3], Univ. College London, London, UK[4], and IOBA, Univ. Valladolid, Spain[5].

The presence of lacrimal keratoconjunctivitis (LKC) during dry eye disease has led many to suspect that dry eye is an immune-mediated disease. We used C57BL/6 mice to determine if desiccating stress (DS) to the ocular surface provokes immune responses to the cornea, conjunctiva, and lacrimal gland (lacrimal functional unit; LFU) and culminates in LKC. There are four hallmarks to an immune-mediated disease: a) it is inducible; b) it is transferable; c) it has memory; and d) it is specific. With this in mind, we explored the immunologic features of LKC induced by DS. The results show that DS induced inflammation of the LFU that is comprised almost entirely of $CD4^+$ T cells, confirming that LKC is inducible and T cell-mediated. Adoptive cell transfer experiments demonstrated that infusing $CD4^+$ T cells from euthymic mice subjected to DS into athymic nude mice produced LKC, yet no inflammation was found in any other major organs examined. Thus, LKC is transferable and antigen-specific. Athymic nude mice

reconstituted with $CD4^+CD25^+$ $FoxP3^+$ regulatory T cells (Tregs) developed milder LKC that was similar to that in euthymic mice that had a normal Treg repertoire. Thus, the severity of LKC is modulated by Tregs, which suggests that dry eye disease may be a consequence of Treg dysfunction at the ocular surface. *In vitro* studies demonstrated that full-thickness corneas from mice subjected to DS stimulated the proliferation of $CD4^+$ T cells from mice subjected to DS, but did not stimulate $CD4^+$ T cells from normal mice. Thus, LKC induced by DS expresses immune memory. The results suggest that some forms of dry eye disease are immune-mediated. Modulation of LKC by $CD4^+CD25^+$ $FoxP3^+$ Tregs raises the possibility that therapeutic maneuvers that reestablish immunological homeostasis at the ocular surface may prove useful in the management of dry eye disease.
All of the authors have a commercial relationship with Allergan.
Supported by unrestricted grants from Research to Prevent Blindness.

Collagen Secretion From Hsp 47-Expressing Lacrimal Gland Myoepithelia In Dry Eye Associated With Chronic Graft Versus Host Disease. Yoko Ogawa [1, 2], Mohammed S. Razzaque[5], Kaori Kameyama[3], Go Hasegawa[2] Shigeto Shimmura[1], Masataka Kawai[1], Kazuto Yamazaki[3], Shinichiro Okamoto[4], Yasuo Ikeda[4], Yutaka Kawakamai[2], Masataka Kuwana,[4] and Kazuo Tsubota[-1] From the [1]Department of Ophthalmology, the [2]Divesion of Cellular Signaling, Institute for Advanced Medical Research, the [3] Department of Diagnostic Pathology, the [4]Department of Internal Medicine, Keio University, School of Medicine, Tokyo, Japan; the [5]Department of Developmental Biology, Harvard School of Dental Medicine, Boston, MA, USA

Purpose. Lacrimal gland fibrosis is a prominent feature of dry eye associated with chronic graft versus host disease (cGVHD). The purpose of this study is to elucidate whether the lacrimal gland myoepithelium and mesenchymal interaction contribute to lacrimal gland fibrosis in the pathogenesis of cGVHD. **Methods.** Lacrimal gland specimens from 8 patients who had presented with dry eye as part of their symptoms of cGVHD were examined and compared with 7 Sjögrern's syndrome (SS). Antibodies to CD4, CD8, HLA-DR, heat shock protein 47 (HSP47), and Ki67 were used for immunohistochemical analysis of lacrimal gland biopsies.The portion of interest in lacrimal gland were further assessed by transmission electron microscopy. **Results.** $CD8^+$ T cells accumulated around lacrimal gland myoepithelium through the disrupted basal lamina. The elevated expression of HSP47 on subepithelial fibroblasts in cGVHD patients was mostly detected in $Ki67^+$ cells. Moreover, HSP47, a marker of collagen synthesis, was detected mainly in the lacrimal gland myoepithelium, but not in the other epithelia. Under electron microscopic observation, collagen bundles were secreted from lacrimal gland myoepithelia, in which collagen bundle possesses abnormal diameter and periodicity. However, these observations were infrequently observed in the specimens from Sjögren's syndrome myoepithelia. **Conclusions.** $CD8^+$ T cells accumulating around the myoepithelia play a critical role in the destructive potential during GVHD pathogenesis. Lacrimal gland myoepithelia may be transformed to a HSP47-expressing mesenchymal phenotype facilitating the production of collagen bundles in lacrimal gland fibrosis. *[This study was supported by grants #18591932 from the Japanese Ministry of Education, Culture, Sports, Science, and Technology]*

Detection Of Objective Ocular Discomfort In Dry Eye-Related Diseases By fNIRS. Masafumi Ono, Hiroshi Takahashi. Nippon Medical School, Tokyo, Japan.

Purpose. Recently, evaluating the symptoms of dry eye has increased in importance. However, the methodologies to evaluate the patient's discomfort have not been established without first learning the subjective symptoms directly from the patient. Previously, we reported that ocular discomfort could be quantified non-invasively and objectively by activating the prefrontal cortex using functional near-infrared ray spectroscopy (fNIRS) in normal controls (NC) after Schirmer 1 test (2004, TFOS), and dry eye patients (DE) (2006, ARVO). This study was designed to determine whether similar detection was evident in dry eye-related diseases. **Methods.** The following subjects were enrolled: 6 NC, 4 DE, 3 blepharospasm (BS), 2 BS with DE, 1 soft CL intolerant and 1 conjunctival condretion. The prefrontal activation was detected by the fNIRS system model OMM-3000 (45 channels)(Shimazu Medical Co., Tokyo, Japan), using semiconductor laser emitting near-infrared rays (780, 810 and 830 nm) in the dark. The number of signal-positive channels in the frontal lobe (FSPC) using the fNIRS system was used to evaluate the extent of prefrontal activity when the eyes were open. Changes in the FSPC count when the eyes were open with spontaneous blinking as a task were analyzed. The required measurement was 5 min. To induce ocular discomfort in NC, wind at 1.8 m/sec was blown. This measurement was repeated, pre and post 0.4% oxybuprocaine hydrochloride instillation as an anesthetic. **Results.** In NC with wind loading, DE, and all other DE-related diseases, prefrontal activity was observed with the eyes opening, and the prefrontal activity was significantly reduced with the instillation of anesthetic. **Conclusions.** These results suggest that ocular discomfort in dry eye-related diseases might be objectified by activating the prefrontal cortex during the task of eye opening using the fNIRS system.

Pathogenesis Of Allergic Conjunctivitis. Santa Jeremy Ono. Emory Eye Center, Emory University School of Medicine, Woodruff Health Sciences Center, Emory University, Atlanta, GA, USA.

Purpose. Allergic conjunctivitis – in its various forms – represents a significant ophthalmic disease for which current treatments are not wholly effective. This laboratory has endeavoured to elucidate cell and molecular pathways contributing to disease pathogenesis and to target specific molecules for rational drug design or high throughput screening with the aim of developing new therapeutics for this and other allergic diseases. In this presentation we will discuss new cell and molecular pathways in disease pathogenesis and progress in the development of new therapeutic compounds. **Methods.** We analyse both samples from patients with allergic conjunctivitis as well as a well characterized animal model of allergic conjunctivitis to determine: cells participating in or regulating the disease process, genes predisposing individuals to develop disease, and molecules contributing to disease pathogenesis. We crosscheck findings in the murine model with our findings in humans. New targets are then prioritised and therapeutic compounds developed either via rational drug design or high throughput screening. **Results.** We will discuss results indicating that regulatory B cells and the cytokine interleukin-6 are very important in regulating the onset of allergic conjunctivitis. New targets defined by gene profiling and proteomic analysis will also be discussed. Finally, results showing the efficacy of new candidate drugs will also be discussed. **Conclusions.** The systematic analysis of pathways underpinning allergic disease in the ocular surface is providing new opportunities for the design of new therapeutics for this disease.

Anatomical And Immunological Changes Of The Cornea In Patients With Pterygium. Marina Papadia[1], Stefano Barabino[1], Cristiana Valente[1], Sebastiano Giuffrida[2], Maurizio Rolando[1]. [1] Ocular Surface Research Center, Department of Neurosciences, Ophthalmology, and Genetics, University of Genoa, Genoa, Italy, [2] Bausch & Lomb IOM, Catania, Italy, [3] IS.PRE Oftalmica, Genoa, Italy.

Purpose. Pterygium is a frequent ocular surface disorder of unknown origin characterized by chronic conjunctival inflammation with a clear central cornea at the slit lamp examination in most patients. The purpose of the present study was to test the hypothesis that anatomical and immunological changes are present in the cornea. **Methods**. The central cornea of twenty eyes of 18 patients (14 males, average age 45 ± 17 yrs.) with primary pterygium was examined by *in vivo* confocal microscopy using a 40x lens and an axial resolution of 5 µm. The size of pterygia was measured by analyzing photographic images. Data from 20 age-matched normal subjects were used as control for analysis. **Results.** The images obtained showed a significant lower number of epithelial cells in patients with pterygium (1043 ± 214 cc/mm^2) compared to controls (1575 ± 238 cc/mm^2), superficial epithelial cell area considerably higher than normal, reduced nucleus/cytoplasm ratio, halos around the nuclei, and sharp borders. Highly reflective dendritic-like cells were present in the epithelial cell basal layer, and their density correlated with the size of pterygium. The stroma changes included loss of keratocytes and presence of lacunae. In particular, the anterior stroma showed a higher reflectivity of the extracellular matrix, and in the middle stroma some fiber-like structures were found. The subbasal nerve plexus showed a higher tortuosity than in controls. The endothelial cell count showed a normal density of cells of this layer. **Conclusions.** *In vivo* confocal microscopy may be helpful in evaluating the immunological and structural changes of the cornea in patients with pterygium, and in understanding its pathophysiology and the possible role of an anti-inflammatory therapy.

The Effect Of An Omega -3 Supplement On Xeropthalmia And Xerostomia In Sjögrens Patients. Athena Papas, Medha Singh and Mabi Singh Tufts University School of Dental Medicine.

Purpose. A previous study found that Sjogrens patients were omega -3 deficient. A prospective, double-blind, randomized, placebo-controlled, clinical trial was conducted to determine if the use of an omega-3 supplement (TheraTears Nutrition ®), could increase unstimulated and stimulated salivary secretion, and improve symptoms of dry eye and dry mouth in Sjögren's patients. **Methods.** The study enrolled 65 Sjögren's syndrome with 61 patients completing, as defined by the European Criteria and a positive blood test or lip biopsy, 61 subjects completed the study. One-third of subjects were randomized to receive a placebo pill (wheat germ oil), and two-thirds were randomized to receive the omega-3 supplement. Concurrent eye or oral therapies were continued unchanged through the study. No patients were excluded. At each visit whole unstimulated saliva was collected by drooling into a pre-weighed container and stimulated saliva was collected by chewing paraffin wax. Both groups were not significantly different for salivary flow at baseline (US was 0.077± 0.091 for active and 0.063±0.081 for placebo SS was 0.784±0.751 for active and 0.919±0.691. A visual analog scale was used to measure the severity of dry eye and dry mouth symptoms at the completion visit. The subject's data from the primary subjective endpoints of improved dry eye and dry mouth symptoms and the objective endpoints of significantly increased unstimulated and stimulated salivary flow were analyzed at 3 months for active and placebo. **Results.** The omega-3 supplement significantly improved dry eye symptoms vs. placebo (45% vs.27% p=0.008, Odds Ratio 2.2). The omega-3 supplement significantly improved dry mouth symptoms vs. placebo (61% vs.35 p=0.0002, 95% Odds Ratio 2.9. Treatment with the omega-3 significantly improved unstimulated ((0.117±0.122, p<0.01) and stimulated salivary flow (0.976±1.068 p<0.048) whereas there was no significant change in placebo flow rate (paired analysis). **Conclusion.** This data demonstrates that treatment with this flaxseed/fish oil omega-3 supplement significantly increases unstimulated and stimulated salivary flow, and significantly improves dry eye and dry mouth symptoms versus placebo control. *(Funded in part by Advanced Vision Research, Woburn, MA)*

Store-Operated Calcium Influx In Epithelial Cells: Recent Advances. Anant B. Parekh. Department of Physiology, University of Oxford, Parks Road, Oxford. OX1 3PT. UK

A rise in cytoplasmic Ca2+ concentration is a key trigger for activating a range of cellular responses. Cytoplasmic Ca2+ can be increased either by releasing Ca2+ from intracellular stores or by opening Ca2+ channels in the plasma membrane. Plasmalemmal store-operated CRAC channels, which are activated by the emptying of the endoplasmic reticulum (ER) Ca2+ stores, are an important and widespread route for Ca2+ influx. In epithelial cells, store-operated calcium influx drives ion secretion, stimulates mitochondrial ATP production and regulates gene expression. Recently, the molecular basis of store-operated entry has been identified with the discoveries of the proteins STIM1 and Orai 1-3. STIM1, which is an ER-resident protein, migrates to discrete puncta just below the plasma membrane upon store depletion where it somehow activates the plasma membrane Orai proteins. Although these recent advances constitute a major step forward, several key questions remain: which Orai proteins contribute to epithelial store-operated calcium entry, how is epithelial cell function affected by suppressing Orai expression; what is the role of other epithelial calcium channels like TRPV6 (CaT1) in cellular function and do Orais represent a valid therapeutic target for treating epithelial cell dysfunction.

Long-Term Outcome Of Limbal Epithelial Cells *In Vivo* Cultivated On Amniotic Membrane (Livam) Transplantation. Woo Chan Park, Dong Jun Lee, Ji Hyun Rho, Hyun Chul Cheon. Dept. of Ophthalmology, Dong-A University, Busan, Korea.

Purpose. The purpose of this study was to investigate the characteristics of limbal epithelial cells cultivated *in vivo* on amniotic membrane (LIVAM) in limbal deficiency and the results of long-term follow-up of transplanted LIVAM. **Methods.** 22 limbal deficiency eyes were removed whole corneal epithelium and grafted amniotic membrane (AM) on epithelial defect area. After confirm the limbal epithelium covered on the AM for a week, the amniotic membrane that was cultivated with limbal epithelium (LIVAM) was detached and then transplanted to limbal deficiency area. Biopsy and immunohistochemical staining (AE5, MUC5AC) of the amniotic membrane cultivated for a week were performed to verify that the cultivated epithelial cells on amniotic membrane are corneal epithelial cells. Impression cytology and immunohistochemical staining (AE5, MUC5AC) were performed to evaluate the characteristics of the transplanted LIVAM at postoperative 1 week, 3 months, 6 months and 1 year. **Results.** Successful epithelial growth was observed on amniotic membrane in a week. The epithelial cells were confirmed to be corneal epithelial cells on immunohistochemical staining. Transplanted LIVAM were confirmed with corneal specificity by impression cytology and immunohistochemical staining at postoperative 1 week, 3 months, 6 months and 1 year. Only one eye showed limbal deficiency at postoperative 6 months. **Conclusions.** *In vivo* cultured limbal epithelial cells showed morphological and immunohistochemical findings similar

to normal corneal epithelial cells. Transplanted *in vivo* cultivated limbal epithelial cells were maintained with characteristics of corneal epithelium. Transplantation of in vivo cultivated corneal limbal epithelial cells will be performed to reconstruct corneal limbus in treating limbal deficiency without limbal damage and immunosuppressive therapy.

A Comparison Of The Numerical Rating Scale (NRS) And The Visual Analog Scale (VAS) In The Assessment Of Ocular Irritation. Jerry Paugh,[1] Robin Sinn,[1] Jennie Fan,[1] Andrew Loc Nguyen,[2]. Southern California College of Optometry,[1] California State University, Fullerton[2].

Purpose. The purpose of this investigation was to compare the NRS and VAS scales directly to determine whether the numerical rating scale might be an acceptable method to gauge ocular irritation. **Methods.** This was a randomized (for test eye and formulation), double-masked study. Saline solutions in a range of osmolalities were instilled (0.9% (control), 4.0, 6.5, and 8.0% NaCl), using a positive displacement pipette (25 microliters) to induce irritation. Ten young, healthy subjects were enrolled and evaluated only during the afternoon. Subjects were seen for 8 sessions, to record their sensation ratings with both the NRS (select an integer from 0 – 100) and the VAS (mark an 11.7 cm horizontal scale). The endphrases for both scales were identical, with "0" as "cannot be felt" and "100" as "worst pain imaginable". Ratings were undertaken immediately, and at 3, 7 and 10 minutes post-instillation. **Results.** The full-scale response for the most irritating solutions was approximately 50%, indicating reasonable scale severity range. Over time, both scales demonstrated overall differences (ANOVA; $p < 0.001$) and highly significant differences at all timepoints compared to the immediate post-instillation value ($p < 0.001$ for 3, 7 and 10 minutes timepoints, 2-tailed, adjusted for repeated measures). **Conclusions.** The NRS appears to be valid against the VAS in assessing ocular irritation with a range of hyperosmolar NaCl solutions. The NRS may provide a rapid and efficient method of gauging irritation concurrently with other measures of topical solution behavior, such as residence time or measurement of non-invasive breakup time.
Commercial Relationships: None

The Effect Of Varying Volumes Of Flourescein On Tear Breakup Time In Dry Eye Subjects. Jerry Paugh,[1] Kashif Qadeer,[1] Hans Steimann,[1] Andrew Loc Nguyen.[2] [1]Southern California College of Optometry, [2]California State University, Fullerton.

Purpose: We examined the effect of varying sodium fluorescein (NaFl) volumes on TBUT in dry eye subjects with a goal of furthering the development of a standardized method for measuring TBUT. **Methods:** A convenience sample of 12 atrophic meibomian gland dysfunction (MGD) subjects was recruited from an existing dry eye database at SCCO. Following qualification for the study via an eligibility visit, they received one NaFl instillation method (of three total) on three separate visits. TBUT was measured in the worse eye of each subject following the instillation of 1.0 and 5.0 µl of 2% non-preserved NaFl using a micropipette, and using a strip with a standard wetting protocol. The order of instillation type was randomized, and the TBUT examiner was masked to the method. Three separate readings were taken, and the times were averaged for each method. **Results:** The TBUT averages were 4.90 (± 3.3) seconds for the eligibility visit (using 5µl of NaFl) and 4.51 (± 2.0) seconds for the 5.0 µl method, which were not statistically different (Pearson's correlation = -0.185; p = 0.565). The TBUTs for the remaining methods averaged 6.92 (± 2.7) seconds for the 1.0 µl volume and 6.62 (± 3.9) seconds for the NaFl strip. There was no statistically meaningful difference in TBUT measurements among the various volumes/methods of NaFl instillation (p = 0.112; ANOVA corrected for repeated measures). This may have been due to Type II error since post-hoc power analysis suggested that a sample of 25 subjects would be required to detect a clinically meaningful difference of 2.0 seconds in TBUT at 80% power. **Conclusions:** This preliminary study of TBUT in dry eyes subjects suggested 1) that the 5.0 µl method gave repeatable results, but 2) that the three methods may provide varying tear stability values. Future work should examine a larger sample size to demonstrate conclusively whether a true difference exists.
Commercial Relationships: None

An Investigtion Of The Direct Retention And Retention Of Effect Of An Artificial Tear In Dry Eye. Jerry Paugh,[1] Julie S. Hwang,[1] Pochi Huang,[1] Andrew Loc Nguyen,[2]. Southern California College of Optometry,[1] California State University, Fullerton[2].

Purpose. The purpose of this investigation was to examine both direct residence time and retention of effect in the same dry eye subjects. **Methods.** This was a randomized, subject-masked crossover study. Formulations were buffered saline (active control) and a marketed artificial tear formulation (test formulation) containing hp-guar. Pre-corneal residence time (RT) was measured directly by monitoring the decay of an FITC-dextran tracer with a scanning fluorometer and estimated as the gross residence time in minutes. Retention of effect was measured with a xeroscope as non-invasive breakup time (NIBUT) and by use of a numerical rating scale (NRS) for comfort. **Results.** Eleven subjects completed all arms of the study. The RT for the saline control and test formulations averaged 17.7 minutes (± 10.0) and 26.8 minutes (± 16.5), respectively. The return to baseline averages for NIBUT were 8.73 (± 6.1) and 19.5 (± 4.9) minutes, for saline and the test formulations, respectively. Similarly, the return to baseline for the NRS comfort scores were 9.91 (± 4.1) and 20.21 (± 6.4) minutes for the saline and test formulations, respectively. When residence time was subtracted from the retention of effect measures, no statistical significance was found (vs. comfort, p = 0.153; vs. NIBUT, p = 0.109). However, *clinical significance* relative to the time in minutes is obvious from the mean values, and demonstrate 1) that the test formulation times were always longer than control, and 2) that the NIBUT and comfort measures were all shorter than the direct residence time measure. **Conclusions.** From these preliminary data it appears that residence time may be much longer than the timeframe of beneficial formulation effects on comfort and tear stability. Future work should aim to confirm these preliminary findings using an adequately powered sample.
Commercial Relationships: None

The Pre-Corneal Residence Time Of Artificial Tears Measured In Dry Eye Subjects. Jerry Paugh,[1], Andrew Loc Nguyen[1,2], David Meadows[3], Mike Christensen[3]. Southern California College of Optometry,[1] California State University, Fullerton[2], Alcon Laboratories, Ft. Worth, TX USA[3].

Purpose. The purpose of this investigation was to measure the pre-corneal residence time of saline and four marketed artificial tears in dry eye subjects using fluorometry. **Methods.** FITC-dextran, 70kD molecular weight, was admixed under sterile conditions (0.1% wt/vol) into buffered saline and four marketed artificial tear formulations of varying viscosity. Pre-corneal residence time (RT) was measured directly in 16 mild to moderate dry eye subjects, classified by sub-type,

in a six-way crossover, masked and randomized study. FITC-dextran tracer decay with a scanning fluorometer was used to estimate the gross residence time (i.e., the time in minutes for the signal to return to baseline). **Results.** All subjects were classified as having non-inflammatory meibomian gland dysfunction (MGD) except one, who had a mixture of aqueous deficiency and MGD. In two separate determinations, the saline RTs were 19.41 ± 7.7 and 17.25 ± 8.3 minutes, demonstrating good repeatability ($p = 0.457$, 1-sample paired t-test). The RTs for the formulations varied somewhat by viscosity, with two higher viscosity formulations demonstrating the longest RTs of 36-41 minutes. An oil emulsion, low viscosity CMC and moderate viscosity HPMC-containing formulation were not statistically different from saline (RTs of 18, 22 and 24 minutes, respectively). The two higher viscosity products demonstrated bi-exponential behavior which may relate to the rheological nature and possibly the relative muco-adhesiveness of the polymeric systems in the formulations. **Conclusions.** More than 2-fold RT differences were found for the higher viscosity, more muco-adhesive formulations compared to saline. However, other formulations provided RTs close to saline, suggesting that residence time is influenced by factors other than simple viscosity. *Commercial Relationships. Funding provided by Alcon Laboratories; Drs. Meadows and Christensen are employed by Alcon Laboratories.*

Trefoil Factor Family Peptide 3 Promotes Re-Epithelialization Of Corneal Wounds. Friedrich Paulsen,[1,] Anne Jansen,[2] Chee-Wai Woon,[3] Fabian Garreis,[1] Kristin Jäger,[1] Deike Varoga,[2] Daniel Podolsky,[4] Nicolas Barker,[3] Saadettin Sel[5], [1]Department of Anatomy and Cell Biology and [5]Department of Ophthalmology, Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany; [2]Institute of Anatomy, Christian-Albrecht-University of Kiel, Kiel, Germany; [3]The GI Company, Framingham, MA, USA; [4]Gastrointestinal Unit, Massachusetts General Hospital, Boston, MA, USA.

Purpose. The trefoil factor family peptide 3 (*TFF3*, also known as intestinal trefoil factor (*ITF*)) has been implicated in epithelial cell restitution. We have recently reported on the expression of TFF3 in both healthy and pathological human corneas. The expression of TFF3 seems to be induced in diseased corneas. The present study examined the biological role of Tff3 in maintaining corneal integrity and investigated the effects of Tff3 on corneal epithelial wound healing. **Methods.** In two different models of corneal injury, alkali- and laser-induced corneal wounding, the wound healing process was evaluated *in vivo* and in a combined *in vivo/in vitro* system in mice with a wild-type ($Tff3^{+/+}$) and *Tff3*-deficient ($Tff3^{-/-}$) genetic background. We extended the study to assess the effects of topically applied recombinant human TFF3 (rTFF3) peptide on the rate of corneal wound healing. **Results.** We found that Tff3 peptide is not normally expressed in intact corneal epithelium but its expression is induced following epithelial injury. Re-epithelialization of corneal wounds is impaired in $Tff3^{-/-}$ mice in comparison to $Tff3^{+/+}$ mice. In addition, exogenous application of rTFF3 to the alkali-induced corneal wounds significantly accelerated healing in the *in vivo* and combined *in vivo/in vitro* systems for both $Tff3^{+/+}$ and $Tff3^{-/-}$ mice. **Conclusions.** These findings confirm a pivotal role for Tff3 in corneal epithelial restitution, opening new prospects to develop treatments that could enhance corneal wound healing following trauma, surgery or disease.

Changes In Visual Acuity Following Meibomian Gland Heat Therapy. E.Ian Pearce[1], M. Anne Pentland,[1] Samina Shabbir,[1] Erin S. McDonald,[1] Khameran Ahmed,[1] Glyn Walsh, [1] Niall C. Strang[1] & Rob J. Fuller[2] Vision Sciences, Glasgow Caledonian University,[1] Glasgow, Royal Eye Infirmary,[2] Plymouth, UK

Purpose. Our previous studies have shown an apparent improvement in low contrast visual acuity (VA) following the use of the EyeCalm heated meibomian goggles. This study was designed to establish the mechanism causing this increase in VA. Tear film and corneal parameters were assessed before and after treatment. Aberrometry was also carried out to establish objectively any improvement in image quality. **Methods.** 31 symptomatic dry eye subjects (2+ symptoms by McMonnies questionnaire) 23 female, 8 male, age 25 ± 7.7 years were recruited. Each subject was treated with the EyeCalm goggles (50˚C for 10 min). Parameters assessed were: VA using a low contrast Bailey-Lovie logMAR chart, non-invasive tear breakup (NITBUT) using a HIR-CAL grid, lipid layer thickness using a Doane interferometer, corneal topography using an Orbscan topographer and aberrations using a Zywave aberrometer. Each was measured before and immediately after treatment. **Results.** A significant improvement in VA of 0.1 log units was observed following treatment ($p=0.029$). NITBUT improved significantly from 11.3 to 17.2 sec ($p=0.000$). Lipid layer appearance was not found to change ($p=0.496$). The corneal topographic data showed that central corneal thickness increased significantly from 586 to 590mm ($p=0.015$). Aberrometry showed a significant reduction in aberrations ($p=0.020$). Although unplanned, the corneal topographer also measured pupil diameter. This was found to be significantly reduced from 4.42 to 4.14mm ($p=0.000$). **Conclusions.** Treatment with the EyeCalm improved VA. This was objectively confirmed by a significant reduction in the total eye aberrations. No significant correlation between the improvement in VA and any single parameter was found. The effect is a combination of improved tear film, increased central corneal thickness and a reduced pupil size, reducing peripheral aberrations. As well as effectively treating MGD this study shows the EyeCalm goggles can cause improved VA through a number of additive mechanisms.

How To Win A Staring Competition. E. Ian Pearce,[1] Alan E.C. Bartholomew,[1] Owen McCann,[1] Sara E. Pearce[2] & Glyn Walsh[1] Department of Vision Sciences, Glasgow Caledonian University[1] Kelvindale Primary School,[2] Glasgow, United Kingdom

Purpose. To investigate if a behavioral strategy could allow a competitor to win a staring competition. This may seem a trivial pursuit, but the winning strategy could prove useful to reduce VDU induced dry eye where blink rate is also substantially reduced. Strategies tested included altered gaze position, increased periocular humidity and the use of topical artificial tears. **Methods.** In all the test conditions the subject was instructed to stare at a target fixed 60cm away. Five test strategies were adopted: normal gaze position, 20° up gaze, 20° down gaze, wearing tight fitting swim goggles and following the use of artificial tears (Hypromellose, 0.32%w/v). The time to the blink was recorded and repeated three times for each condition. The order of the test conditions was randomized using a Latin square. 40 healthy subjects were recruited (22.9 years, range 18-41, 23 males 17 females, refractive correction <3.5±D). **Results.** Median time to blink in seconds were as follows: normal gaze=15.5, 20° up=14.0, 20° down=18.5, goggles 46.5 and artificial tears 13.5. Data did not follow a normal distribution so non-parametric tests were used. A statistically significant difference between conditions was found (Friedman's test $p=0.000$). A *post-hoc* Wlicoxon test was used to find which conditions differed. Performance with the goggles was significantly better than all the other conditions ($p<0.05$). Although indicative trends were seen between the other conditions they did not reach statistical significance. **Conclusions.** Wearing goggles significantly increased the time subjects could hold their eyes open. It is likely that the increased periocular humidity is responsible for this improvement. VDU users could adopt this strategy to alleviate dry eye symptoms. As it may not

be cosmetically acceptable to do this, we suggest that increasing room humidity or wearing close fitting spectacles may provide some of the same benefits. Indicative trends were also seen for the other conditions suggesting that a down gaze, with reduced palpebral aperture may also benefit VDU users.

Building Better Mouse Models To Study Sjögren's Syndrome. Ammon Peck, Department of Oral Biology, College of Dentistry, University of Florida, Gainesville, FL USA.

Over the past two decades, multiple mouse models exhibiting aspects of Sjögren's Syndrome (SjS) have been studied to identify the nature of the underlying autoimmune process. Typically, mouse models show lymphocyte infiltration of exocrine glands, increased expression of inflammatory cytokines, generation of autoantibodies, and eventually decreased tear/saliva flow rates. While no one strain recapitulates completely the pathological characteristics of SjS, it is important to note that, from a genetics perspective, any one inbred mouse model represents a single individual with a specific genetic pre-disposition and immune system shaped by environmental influences. Recent construction of a SjS-susceptible C57BL/6-derived strain, designated C57BL/6.NOD-*Aec1Aec2*, offers many genetic advantages over other models. First, these two genetic regions confer SjS-susceptibility on the C57BL/6 background in the absence of other autoimmune diseases, like diabetes. Second, introduction of genes into C57BL/6.NOD-*Aec1Aec2* mice to study effects of individual genes requires minimal effort and time. Third, the SjS-non-susceptible parent C57BL/6 mouse represents an ideal comparative for studies using C57BL/6.NOD-*Aec1Aec2* mice. Fourth, construction of new recombinant inbred lines defining more precisely the genetic regions conferring SjS-susceptibility and eventually candidate genes within those regions is facilitated through inter-breeding with C57BL/6 mice, concomitantly preserving the genetic background. In this presentation, two questions will be advanced and discussed: (1) "can our nearly 50 newly developed C57BL/6.NOD-*Aec1Aec2* RI lines better delineate the genetic basis for SjS-susceptibility?", and (2) "can the genomic analysis of over 1 million genes define the underlying immune-pathophysiological factors temporally regulating molecular mechanisms during development and onset of SjS-like disease in the lacrimal glands?" Concepts derived from these studies should unravel the nuances of biological processes that regulate SjS, an autoimmunity most likely provoked by pathological events within exocrine glands *per se*.

Self-Renewal Or Aging In Ocular Epithelial Cells. Graziella Pellegrini[1,2], Vanessa Barbaro[1], Anna Testa[2], Enzo Di Iorio[1], Fulvio Mavilio[2], and Michele De Luca[1,2]. [1]Epithelial Stem Cell Research Center, The Veneto Eye Bank Foundation, H. SS Giovanni and Paolo, 30100 Venice, Italy, [2]Department of Biomedical Sciences, University of Modena and Reggio Emilia, 41100 Modena, Italy

Purpose. Stem cells have the unique capacity to self-renew and generate committed, transit amplifying (TA) progenitors that differentiate into the cell lineages of the tissue of origin. We have recently shown that, in the human corneal epithelium, high levels of DNp63a identify limbal stem cells both in vivo and in vitro, whilst DNp63b and DNp63g correlate with corneal regeneration and differentiation In mammary gland epithelial cells, the CCAAT enhancer binding d (C/EBPd)) transcription factor regulates cell cycle by inducing a G0/G1arrest. The purpose of this study is to establish the molecular signatures of the self-renewing/differentiating epithelial cells. **Method.** Experiments were performed on 4 uninjured and 5 wounded ocular surfaces, referred to as resting and activated, respectively. Immunofluorescence analyses were performed on ocular sections and on cultured stem cells versus differentiated cells in vitro. Forced expression of a hormone-inducible ER-C/EBPd chimera in human primary ocular keratinocytes was obtained. Forced expression of C/EBPd and DNp63a by lentiviral vector was perfomed on epithelial clones. **Results.** C/EBPd and DNp63a are co-expressed by human epithelial stem cells in vivo and in vitro, and the expression of C/EBPd is restricted to a subset of mitotically quiescent, DNp63a+/Bmi1+ cells. Forced expression of a hormone-inducible ER-C/EBPd chimera shows that C/EBPd is instrumental in regulating self-renewal and cell cycle length of epithelial stem cells. Upon injury, a fraction of these cells switches off C/EBPd and Bmi1, proliferates and differentiates into mature epithelial cells. Expression of a conditional C/EBPd mutant inhibits the growth of epithelial colonies and increases the cell cycle length of primary epithelial cells, through the activation of p27Kip1 and p57Kip2. **Conclusion.** These effects are reversible, do not alter the epithelial cell proliferative capacity, and are not due to apoptosis, senescence or differentiation. Instead, ectopic C/EBPd, but not DNp63a, promotes holoclone self-renewal, as it prevents clonal evolution, suggesting that self-renewal and proliferation are distinct albeit related processes in epithelial stem cells.

3-D Visualization Of Mucin Release By Laser Scanning Microscopy. Assumpta Peral[1,3], Jesús Pintor[2,3]. Department of Optics II (Optometry and Vision)[1], Department of Bioquemistry and Molecular Biology IV[2], School of Optics, University Complutense of Madrid[3].

Purpose. We have described a method of visualizing the human conjunctiva goblet cell mucin secretion using a combination of the impression cytology and laser scanning microscopy. **Methods**. By assembling a Z-stack of confocal microscopy images taken from human impression cytology samples, 3-D information was obtained about the release and spread of goblet cell secretions above the conjunctival surface. After reconstruction and rendering of these images, analysis of the shape and spreading characteristics of the mucins permitted definition of the following parameters related to goblet cell secretion: Mucin Cloud Height (MCH) as the height of the top of the cloud-like mucin structure visible above the goblet cell opening, while Spread Mucin Thickness (SMT) indicates the thickness of the mucin layer distributed over the surface of the conjunctiva. Immunocytochemical analysis with the antibody against MUC5AC was performed to verify if the staining obtained by PAS corresponds to the secreted mucins. **Results**. Several impression cytology samples of control and muco-deficient patients have been analyzed through the confocal laser scanning technique and significant differences between these groups were found. MCH and SMT values for controls were 8.81±4.00 µm and 2.77±1.00 µm respectively (n=25). These values decreased by about 70% and 40% respectively for moderately muco-deficient subjects and by 84% and 48% for those with severe muco-deficiency. Classifying those individuals having mucin related pathology may thus be possible based on application of these techniques. **Conclusions**. In summary, we present a method of objectively identifying those individuals with problems associated either with a lack of mucins or a reduction in the distribution of these proteins over the ocular surface.

Organized Conjunctival Associated Lymphoid Tissue In The Rabbit. Thomas E. Phillips, Charlette Cain and Carisa Petris. University of Missouri, Columbia, MO, USA

Purpose. Organized conjunctival associated lymphoid tissue (o-CALT) consists of lymphoid follicles covered by an epithelial layer that contains the functionally and morphologically distinct M cell. M cells

are responsible for transcytosis of environmental antigens to follicular cells to initiate a mucosal immune response and can serve as portals of entry for viral and bacterial pathogens. This study investigates whether o-CALT in rabbits, like other mammalian lymphoid tissue, changes with age. Morphological and functional markers of M cells are also reported. **Methods**. Fluorescence stereomicroscopy was used to measure the number and size of conjunctival follicles stained with propidium iodide in rabbits ranging in age from 2 days to 57 months. Confocal microscopy and electron microscopy was used to evaluate transcytosis of latex beads and preferential labeling of M cells by lectins and antisera. **Results**. O-CALT was not present in rabbits younger than 9 days, but was consistently present less than 24 hours after eyes opened around day 11. Follicle numbers increased until adolescence (2-4 months) then stabilized through early adulthood (17-20 months) before declining dramatically in aged rabbits (47-57 months). Follicle diameter increased with age except in the superior conjunctiva of aged rabbits. Functional M cells, capable of transcytosis of latex beads, were present above lymphoid follicles at all ages. Secretory IgA (sIgA) was found to be selectively associated with M cell apical membranes in adult animals and latex beads targeted to sIgA were preferentially transcytosed by M cells. Studies characterizing specific labeling of receptors on the M cell apical surface will be reported. **Conclusions**. Rabbit o-CALT undergoes age-related changes similar to those previously reported for the human conjunctiva. O-CALT at all ages includes functional M cells. The ease and reproducibility in counting follicle numbers and evaluating M cell function make the rabbit an ideal model for studying o-CALT and its role and response in ocular disease. Identification of M cell specific receptors opens the opportunity for targeted immunization studies.
(Support: NEI EY13779)i

What's Wrong With Immunization As An Approach To Preventing Infection? Gerald B. Pier, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115

At the start of the 21st century the United States CDC listed vaccination as the number 1 public health achievement of the 20th century. When immunization against an infectious pathogen works, it generally prevents, and can even eliminate, a disease (e.g., smallpox). With the significant consequences associated with ocular surface infections, developing vaccines would seem like a high priority. But good intentions are not enough, as the challenges to effectively develop and test vaccines for eye diseases may be insurmountable. Probably the biggest challenge is designing and implementing a clinical trial wherein vaccine efficacy can be demonstrated. Almost as big a challenge is the identification of protective antigens and appropriate immune effector mechanisms that prevent infections by the variety of pathogens that can cause serious eye infections. A more rational approach might be the development of specific therapeutic interventions that can be used once a pathogen has been tentatively identified or deemed likely to be an etiologic agent of eye infection, and used to augment standard therapy, thus leading to better outcomes. Using *Pseudomonas aeruginosa* as an example, passive therapy with IgG antibodies to either the cell surface lipopolysaccharide (LPS) or alginate capsular polysaccharide has both prophylactic and therapeutic efficacy in a murine model of ulcerative keratitis. Along similar lines, interfering with pathogenic mechanisms has potential to enhance treatment efficacy. *P. aeruginosa* enters corneal epithelial cells by binding to the cystic fibrosis transmembrane conductance regulator following recruitment to cholesterol-dependent lipid rafts. Such rafts can be disrupted by use of cholesterol-extracting agents, which have also shown efficacy in ameliorating the consequences of *P. aeruginosa* infection during murine ulcerative keratitis. Overall, the most likely immunologic interventions for serious eye disease will be passive therapy and/or inhibitors of essential virulence factors or processes that can be used at diagnosis and in conjunction with standard therapies to obviate the consequences of microbial keratitis.

Transduced dendritic cells: A tool to modulate the immune response? Pleyer U, Lie X*** , Schlieckeiser S, Sawitzki B**, Ritter T**** Department of Ophthalmology, and Institute of Immunology*, Charité – University Medicine Berlin, Germany, Department of Ophthalmology, Union Hospital, Tongji Medical College***, Huazhong University of Science and Technology, China, Regenerative Medicine Institute****, National University of Ireland, Galway, Ireland

Background. Recent strategies to induce donor-specific tolerance focus on the application of regulatory dendritic cells (DC). Adenoviral expression of immunosuppressive molecules is an attractive approach to enhance their tolerogenic potential. **Methods.** In this study, we compared the immunoregulatory properties of CTLA4-Ig and vIL-10 secreting DC in vitro. **Results.** Transduction of DC with AdCTLA4-Ig prior to LPS stimulation dramatically diminished their allostimulatory capacity in vitro, as demonstrated by an inhibition of proliferation of cocultured allogeneic T cells. Compared to controls, AdvIL-10 transduced DC also showed a significant decrease in their ability to activate T cells. In vivo, both CTLA4-Ig and vIL-10 secreting DC lowered serum alloantibody levels and induced anti-inflammatory cytokine mRNA expression in the spleen. **Conclusions.** Genetic modification of dendritic-cell function could to be an attractive approach to modulate the immune response.
(Supported in part by DFG Pl 150-14/2)

An Investigation Of Limbal And Bulbar Hyperaemia In Normal Eyes. Heiko Pult[1], Paul J Murphy[1], Christine Purslow[1], Jeff Nyman[2], Russell L Woods[3]. [1]School of Optometry and Vision Sciences, Cardiff, UK; [2]Pennsylvania College of Optometry, Philadelphia, USA; [3]Schepens Eye Research Institute, Harvard Medical School, Boston, USA.

Purpose To investigate the appearance of limbal and bulbar hyperaemia in normal eyes, their relationship, and the inter-observer agreement of clinical grading. **Methods** Limbal and bulbar hyperaemia were assessed in four quadrants by two trained observers, using the CCLRU grading scale interpolated into 0.1 increments, on the right eyes of 120 healthy, non-contact lens-wearing subjects (m=57, f=63, median age=45 years, range 18-77). In addition, limbal and bulbar overall hyperaemia were assessed and quadrant-average hyperaemia calculated. Inter-observer agreement was assessed at the start and end of the study (20 subjects each). **Results** For limbal hyperaemia, the overall grading (1.62 ± 0.46) (mean units ± sd) was not significantly different from the quadrant-average (1.61 ± 0.40). For bulbar hyperaemia, the overall grading (2.02 ± 0.49) was higher than the quadrant-average (1.82 ± 0.39; $p<0.0001$). Significant correlations were found between bulbar and limbal quadrants (Pearson: $r \geq 0.43$ $p<0.0001$). Significant differences in hyperaemia were found between quadrants (repeated measures, $p<0.0001$), with nasal and temporal redder than superior and inferior quadrants. Small effects of age and gender were found for limbal hyperaemia. The inter-observer 95% limits of agreement were similar at the start and end of the study, and were larger for overall (0.57) compared to quadrant-average (0.28) hyperaemia. **Conclusions** 1) A limbal hyperaemia above 2.5 may be considered abnormal. 2) A bulbar hyperaemia above 2.6 units (quadrant-average) or 3.0 (overall) may be considered abnormal. 3) Limbal and bulbar hyperaemia were moderately correlated. 4) Grading

of overall hyperaemia was less repeatable than using a quadrant average.

The Relationship Between Lid Wiper Epitheliopathy, Lid Parallel Conjunctival Folds And Ocular Surface In Symptomatic And Asymptomatic Contact Lens Wearers. Heiko Pult[1], Christine Purslow[1], Monica Berry[2], Jeff Nyman[3], Paul Murphy[1]. [1]School of Optometry and Vision Sciences, Cardiff, UK. [2]Academic Unit of Ophthalmology, Bristol, UK. [3]Pennsylvania College of Optometry, Philadelphia, USA.

Purpose. Lid wiper epithiolopathy (LWE) and lid parallel conjunctival folds (LIPCOF) are valuable tests in dry eye patients. This study investigates the relationship between lid wiper epithiolopathy (LWE) and lid parallel conjunctival folds (LIPCOF), and their relation to the ocular surface, in soft contact lens wearers. **Methods.** Subjects were divided into two groups (asymptomatic or symptomatic) according to their responses to the Contact Lens Dry Eye Questionnaire (CLDEQ). Pre-lens break-up time (PLBUT), limbal and bulbar hyperaemia, corneal staining, LWE, and temporal and nasal LIPCOF were assessed in the right eyes of 61 (23M, 38F; age 32,1 range= 18-55) experienced contact lens wearers. LWE and LIPCOF were classified using a four grade scale, the further objective signs were classified into four grades, interpolated in 0.1 increments. Differences between groups and relationship between LWE, LIPCOF and objective signs were examined using non-parametric analysis. The predictive values (both positive and negative predictive values; PPV and NPV) of each objective measure for symptoms were calculated. **Results.** 38 subjects were classified as asymptomatic, 23 symptomatic. LWE and LIPCOF severity scores were significantly increased in symptomatic patients (U-test, $p<0.03$), whilst no significant differences were found between groups for PLBUT, corneal staining or hyperaemia ($0.29<p<0.88$). The predictive value of LIPCOF (temporal) was 56.9%/77.1% (PPV/NPV), of LIPCOF (nasal) 70.7%/75.0%, and of LWE 53.1%/81.1%. Significant positive correlations were found between LWE and LIPCOF scores (temporal $r=0.67$, $p<0.001$; nasal $r=0.39$, $p<0.001$), and between LWE and hyperaemia (bulbar, $r=0.28$, $p<0.001$; nasal $r=0.36$, $p<0.001$). **Conclusions.** Contact lens wearers with dryness symptoms exhibit significantly more LWE and LIPCOF, but not corneal staining, bulbar and hyperaemia or decreased PLBUT. LWE and nasal LIPCOF appear to be valuable tests to predict dry eye in hydrogel contact lens wearers. LWE and LICPOF are significantly correlated.

Calcium Entry Mechanisms In Epithelial Cells. James W. Putney, Jr. National Institute of Environmental Health Sciences-NIH, Research Triangle Park, NC 27709 USA.

Purpose. In lacrimal acinar cells and other non-excitable cell types, agonist regulation of calcium signaling often involves a mechanism initiated by release of intracellular calcium and a subsequent entry of calcium across the plasma membrane. This entry of calcium is believed to be signaled by the depletion of calcium stores, and has been termed *capacitative calcium entry*. Recent work from a number of laboratories has highlighted the roles of a Ca^{2+} sensor protein, Stim 1, and a channel subunit, Orai1. The purpose of this work was to understand the mechanisms by which these two key proteins act to produce calcium entry. **Methods.** Calcium entry was monitored either by use of intracellular fluorescent Ca^{2+} indicators, or by measuring membrane currents with the patch-clamp technique. Fluorescently tagged Stim1 and Orai1 were expressed in a human kidney cell line. Intracellular localization of Stim1 and Orai1 was assessed by confocal microscopy and total internal reflection fluorescence microscopy. **Results.** Stim1 was localized to intracellular fibrillar structures. When intracellular stores were depleted, Stim1 redistributed into discrete punctae and neared the plasma membrane to sites where Orai also localized. The Stim1 fibrillar structures coincided with the cellular microtubule network. Disruption of the microtubule network caused Stim1 redistribution within the endoplasmic reticulum (ER) and inhibited capacitative calcium entry and its associated current. This inhibitory effect could be at least partially overcome by overexpressing Stim1. **Conclusions.** Stim1 acts as a sensor for Ca^{2+} within the ER. Depletion of ER Ca^{2+} causes Stim1 to move toward the plasma membrane and collect at sites adjacent to plasma membrane Orai channels. Knowledge of the actions of these mediators in lacrimal and other cell types may form the basis for the design of novel pharmacological strategies for managing a myriad of diseases associated with the store-operated Ca^{2+} entry pathway.
Supported by the Intramural Research Program, NIEHS, National Institutes of Health.
There are no relevant commercial relationships.

Evaluation Of Optive In Patients Previously Using Systane For The Treatment Of Dry Eye Signs And Symptoms. Rajesh K. Rajpal, M.D.; Lorie A. Logan, O.D. Cornea Consultants, 8180 Greensboro Drive, Suite 140, Mclean, VA.

Purpose. The purpose of this study was to evaluate the effectiveness of Optive vs. Systane Tears for patients with moderate to severe dry eye disease. **Methods.** Patients (n=50) using Systane for at least 1 month were switched to Optive for 1 month. **Results.** One month after switching from Systane, patients had a significant improvement in staining (mean score was 2.2 ± 1.1 at baseline and $1.5 \pm .87$ at month 1, $P<.001$), Schirmer's (mean score was 7.2 ± 3.8 mm at baseline and 10.1 ± 3.4 mm at month 1, $P<.001$), and OSDI (mean score was 44.8 ± 15.7 at baseline and 30.5 ± 13.7 at month 1, $P<.001$). Patients used Systane an average of 3.6 times/day but only used Optive 2.6 times/day ($P=.005$). Patients preferred Optive to Systane and noted that Optive did not blur their vision. **Conclusion.** Optive improved dry eye signs and symptoms in patients previously using Systane.
This research was funded by an unrestricted educational grant from Allergan, Inc.

Long-Term Follow-Up Of Autologous Cultured Limbal Stem Cell Transplantation. Paolo Rama, [1] Stanislav Matuska[1], Giorgio Paganoni[1], Alessandra Spinelli[1], Maurizia Viganò[1], Chiara Insacco[1], Graziella Pellegrini[2], Michele De Luca[2]. [1]Ophthalmology, San Raffaele Hospital, Milano, Italy, [2] Epithelial Stem Cell Lab, Veneto Eye Bank Foundation, Venice, Italy.

Purpose. To report on long-term survival of autologous cultured limbal stem cells after transplantation in limbal stem cell deficiency. **Methods.** One hundred-four procedures in 95 patients have been done between July 1998 and April 2007. Eighty-one patients (88 transplants) with a follow-up longer than one year were included. Six patients were reoperated, one patients underwent bilateral transplantation. Stem cells were obtained from a 2X2 mm limbal biopsy and cultivated on a fibrin substrate. All patients had moderate or severe limbal stem cell deficiency due to chemical burns in most of the cases (87%). Diagnosis of limbal stem cell deficiency was based on clinical signs and expression of CK 3/12 and 19 on impression cytology. **Results.** Two patients were lost to follow-up. Success was reported in 55 eyes (68,75%) after a single procedure and in 60 eyes (75%) after a second procedure. Twelve cases (15%) had partial success with improvement of signs and symptoms but mixed phenotype population (K3/K19) after

impression cytology with mild signs of conjunctival migration. Thirteen procedures (16,25%) were considered unsuccessful, with five early failures (within three months) and eight late failures (within one year). We did not report any failure after one year. **Conclusions.** Cultured limbal stem cell transplantation is a new procedure that holds out fresh possibilities for the treatment of limbal stem cell deficiency. This procedure has a very low risk for the donor eye and allows repeating the biopsy in case of need. The cells can be stored frozen thus permitting to schedule the surgery and banking. It allows also treating bilateral diseases when there is a spared limbal area, even small. The results are stable after one year and in case of failure the procedure can be successfully repeated, even more than once. The handiness and ease of long-distance transportation of the fibrin-cultured epithelial sheets suggest that this method can now be widely applied.

Transplantation Of Labial Salivary Glands To Conjunctiva In Cases Of Severe Dry Eyes. Peter Raus, Miró, Center for Eyelid Surgery and Aesthetic Medicine of the Face, Mol, Belgium.

Purpose. To demonstrate that Radiosurgically assisted transplantation of labial salivary glands to conjunctiva can be an excellent solution in cases of severe dry eyes when other therapies fail. **Methods**. I use Radiosurgery to take a free graft of mucosa and labial salivary glands from the lower lip and transplant it to the conjunctival side of upper ande lower eyelid. The graft is sutured with a submucosal running 4/0 Prolene suture that can be taken out after 2 weeks. The original technique was first published by Prof. Juan Murube; the actual version using Radiosurgery will be published in a book by Prof. Gerd Geerling, Prof. Juan Murube and myself in 2007. **Results.** Thanks to Radiosurgery the results of the first 17 transplantations are excellent with fast recovery and only minimal discomfort for the patients. Biopsies of the transplanted glands after 18 and 36 months have proven the survival of functioning glands that continue to produce a secretion product that is very similar to natural tears. **Conclusions.** Radiosurgically assisted transplantation of labial salivary glands promises to be a good alternative for patients with severe dry eyes when other therapies fail. Also patients acceptance is excellent thanks to the fast recovery with only minimal discomfort.

Toll-Like Receptor Expression And Dry Eye. R.L. Redfern, J.A. Baxter, R.Y. Reins, A.M. McDermott. College of Optometry, University of Houston, Houston, TX.

Purpose. Dry eye is a multifactorial inflammatory condition that affects millions of individuals yet the pathogenesis is poorly understood. The ocular surface expresses several toll-like receptors (TLRs) that stimulate the production of proinflammatory cytokines upon activation. Here we investigated the potential role of TLRs in dry eye by determining if their expression is modulated by dry eye simulated culture conditions. **Methods.** Total RNA was extracted from a normal human conjunctival epithelial cell line (IOBA-NHC), SV40 human corneal epithelial cells (HCEC), or primary HCEC that were treated with either 10ng/ml of cytokines (n=2-4, IL-1_, IL-1_, TNF_, or TGF_), hyperosmolar media (n=2-3, 400, 450, 500mOsm) or serum-free media alone for 24 hours. TLR4, 5, and TLR9 mRNA expression was determined by real-time RT-PCR. Additionally, human corneas were incubated at 37oC for 24 hours with the epithelial surface exposed or completely submerged in culture medium. The epithelium was then collected and TLR4, 5, and TLR9 (n=3) mRNA expression was determined by real-time RT-PCR. **Results.** Cytokines did not significantly modulate the expression of TLR4, 5, and TLR9 in HCEC and IOBA-NHC cells. TLR5 expression was not significantly modulated in response to hyperosmolar stress in SV40 HCEC; however TLR4 was upregulated by 1.53, 1.83, and 1.75 log2 fold in IOBA NHC and 0.481, 1.44, and 3.14 log2 fold in SV40 HCEC in response to 400, 450, and 500mOsm stress. TLR9 was downregulated by 0.762 and 1.18 log2 fold in HCEC in response to 400 and 450mOsm stress respectively. TLR4 and 5 were upregulated in the desiccation culture model by 2.26 and 1.27 log2 fold respectively, whereas TLR9 was downregulated by 3.11 log2 fold compared to the submerged control. **Conclusions**. These data indicate that TLR expression is modulated during dry eye conditions in vitro and suggests that TLR4 and 5 may stimulate ocular surface inflammation in severe dry eye.
This research was supported by EY13175 NIH Grant to AMM and NIH-NEI T32 EY07024 to RLR.

Do Genetic Alterations In Sex Steroid Receptors Contribute To Lacrimal Gland Disease In Sjögren's Syndrome? Stephen M. Richards, David A. Sullivan. Schepens Eye Research Institute and Harvard Medical School, Boston, MA, USA

Purpose. Defects in sex steroid receptors have been linked to the onset, progression and severity, as well as the sex-related prevalence, of a variety of autoimmune disorders, including lupus, rheumatoid arthritis, multiple sclerosis and diabetes. These defects, which are often due to gene polymorphisms or alternative splicing, may lead to significant changes in the affinity or specificity of ligand binding, nuclear translocation, receptor dimerization, DNA association and transcriptional activation. We hypothesize that defects in estrogen receptor a (ER1), estrogen receptor b (ER2) and/or the androgen receptor (AR) may also contribute to the development of lacrimal gland autoimmune sequelae in Sjögren's syndrome. To test this hypothesis, we examined whether mutations in ER1, ER2 and AR transcripts exist in lacrimal tissues of mouse models of Sjögren's syndrome. **Methods.** Lacrimal and submandibular glands were collected from adult age-matched male and female MRL/Mp-lpr/lpr, non-obese diabetic and/or BALB/c mice (n = 5-10 mice/sex/experiment; n = 3 experiments). Glands were pooled according to sex and experiment and processed for cDNA generation. PCR primers were designed to amplify 566-875 base pair segments of the entire open reading frame of each receptor. Segments were amplified from reverse transcribed cDNA, purified and then sequenced with an automated fluorescent sequencer. Receptor sequences were assembled and compared to each other and to known sequences from NCBI with CLC Gene WorkBench. **Results**. Our results show that almost all ER1, ER2 and AR sequences in exocrine tissues of male and female autoimmune and non-autoimmune mice were identical to those of NCBI standards. There was a G→A shift at position 998 of the ER2 complete coding sequence when compared to NCBI reference sequence U81451.1, but this polymorphism was not found in other ER2 reference sequences. **Conclusions.** Our findings do not support our hypothesis that sex steroid receptor defects contribute to the pathogenesis of lacrimal gland disease in Sjögren's syndrome.
(Supported by NIH grant EY05612)

Surfactant Protein Gene Expression In Ocular Surface Tissues: Sex And Hormonal Influence. Stephen M. Richards, David A. Sullivan, Schepens Eye Research Institute & Harvard Medical School, Boston, MA, USA

Purpose. Surfactant proteins (SPs) play a critical role in reducing surface tension at the lung air-liquid interface, as well as in modulating pulmonary innate and adaptive immunity. These proteins include hydrophobic species that interact with lipids to decrease surface tension, and host defense-related SPs that are members of the collectin

family. We hypothesize that SPs serve an analogous function on the ocular surface and promote both tear film stability and immune defense. To begin to test this hypothesis, we evaluated whether the genes for SPs and their receptors, other collectins, and SP-associated proteins (e.g. transporters, enzymes) are expressed in mouse lacrimal and meibomian glands and human corneal epithelial cells. We also analyzed whether gene expression: [a] is influenced by sex and sex steroid hormones, given that these factors modulate lung surfactant production; and [b] occurs in mouse submandibular, parotid and sublingual tissues. **Methods.** Lacrimal, meibomian and salivary glands were obtained from adult, male and female BALB/c, C57BL/6, MRL//lpr, non-obese diabetic and/or aromatase knockout mice that were either intact or castrated, and/or treated with testosterone, estradiol-17b, progesterone or vehicle for 2 weeks. Human corneal epithelial cells were isolated from the corneoscleral rims of male and female donors. Samples were processed for the analysis of differentially expressed mRNAs by using GE CodeLink Bioarrays and Affymetrix GeneChips, and data were evaluated with statistical software. **Results.** Our data demonstrate that the mRNAs for SPs (A, B, C & D) and their receptors (e.g. GP340, SIRPa, CD93, TLR2, TLR4, calreticulin, MD2, CD14, SPR-210, CD91), other collectins (e.g. MBL, sub-family members 10, 11 & 12) and SP-associated proteins (e.g. Es1, Masp1, Masp2, ficolin A) are expressed in ocular and salivary tissues. In addition, our results show that the levels of many of these mRNAs are significantly influenced by sex and sex steroids. **Conclusions.** Our findings support our hypothesis that SPs may play multifaceted roles on the ocular surface.
(Supported by NIH grant EY05612)

Does Artificial Tear Use Alter The Tear Layer? William H. Ridder, III, James LaMotte, Robin Sinn and Jonathan Q. Hall, Jr. Southern California College of Optometry, Fullerton, CA, USA.

Purpose. Dry eye is frequently encountered and often treated with artificial tears (AT). The administration of an AT can disrupt the tear layer resulting in an immediate decrease in contrast sensitivity (CS) and visual acuity. While this immediate effect was shown to be dependent on viscosity of the AT, the longer-term effect of continuous use of ATs has not been investigated. The purpose of this study was to determine if long-term use of ATs altered the immediate effect of AT administration on CS. **Methods.** Thirty-two subjects (10 normal, 13 mild and 9 moderate/severe dry eye) used either Refresh Plus (Allergan) or Optive (Allergan) on a daily basis for two weeks. The subjects were examined before and at 1 and 2 weeks after daily AT use. At each visit, the subjects answered a Schein dry eye questionnaire, underwent a slit lamp evaluation, and had CS measured. CS to a 14 cpd sine wave grating was continually tracked (using a 2 AFC technique) before and after (minimum of 35 minutes) a drop of the AT was instilled in the test eye. **Results.** The Schein questionnaire indicated subjective improvement in dry eye symptoms with both ATs (all p values < 0.05), whereas, the slip lamp findings did not change ($p > 0.05$). There was no significant change in CS with Refresh Plus for the normal or dry eye subjects across visits (ANOVA, all p values > 0.05). Optive did not produce a change in CS over time for the normal ($p = 0.38$) but it did for the dry eye subjects ($p = 0.03$). Dry eye subjects using Optive showed a significant decrease in CS at pretreatment and week one ($p < 0.05$) not seen after two weeks of treatment.
Conclusions. These results confirm that the viscosity of an AT has a significant effect on CS. Refresh Plus (3 cps, no effect on CS) and Optive (15 cps) have low viscosity and Optive may marginally alter CS. The long term use of Optive by dry eye subjects altered the immediate effect of AT administration on CS. The administration of a drop of Optive had less of an effect on CS after 2 weeks of treatment. This suggests that continued use of Optive may normalize the tear layer.
[This research was supported by a grant from Allergan]

The Influence Of Aging On The Tear Film And Ocular Surface. Eduardo M. Rocha. Department of Ophthalmology, Otorrinolaringology and Head & Neck Surgery, Faculty of Medicine of Ribeirão Preto, São Paulo University, Brazil.

Aging is a major cause of lacrimal gland dysfunction and dry eye syndrome. Clinical and pathological observations are now reinforced by several epidemiological surveys and the impact of dry eye in life quality is also well documented.

The increase in life expectancy and behavior changes of this population are challenging but also expanding the opportunity for research in age related tear film, lacrimal gland and ocular surface disorders in order to improve healthy ocular aging.

The experimental studies to improve mechanistic and therapeutic knowledge are driven by theories related to metabolic disturbance, oxidative stress, inflammation, DNA damage and neuroendocrine impairment. In the clinic, besides pharmacological approaches, it has been investigated correlation with systemic diseases, nutritional habits and environmental conditions.
Supported by CNPq, FAPESP, Capes, FAEPA.

A New Test To Quantify Lipid Layer Behavior In Normal And Keratoconjunctivitis Sicca Patients. Maurizio Rolando, Cristiana Valente, Stefano Barabino. Ocular Surface Research Center, Department of Neurosciences, Ophthalmology, and Genetics, University of Genoa, Genoa, Italy.

Purpose. Tear film lipid layer alterations have important consequences on visual functions and ocular surface homeostasis. Diagnostic procedures to evaluate the lipid layer are of limited application and do not provide quantitative results. The purpose of the present study was to develop a non-invasive quantitative test to measure tear lipid interference patterns in dry eye patients. **Methods**. The dynamic lipid layer interference patterns (DLIP) test was performed on 21 patients with dry eye, and 21 age-matched controls. In the same examination room with controlled environment, subjects in the study and control group were asked to perform 5 forced blinks and 10 consecutive non-forced blinks every 2 seconds. After recording the shape of the lipid layer interference patterns obtained with the Tearscope, we counted the number of blinks to observe significant changes of shape, position, and number of waves of the interference patterns. The results are expressed as the number of blinks preceding the inability to further recognise the identified interference pattern. Patients with dry eye were identified on the basis of the typical symptoms measured by a validated questionnaire (OSDI Questionnaire Score >10), Schirmer I test scores <10 mm/5 minutes, tear break-up time <7 seconds, and lissamine green conjunctival staining >4. **Results.** Significant differences in Schirmer test, BUT and lissamine green were recorded between groups. The DLIP test in the dry eye group (2.4 ± 3.1 blinks) was statistically decreased compared to the control group (18.1 ± 5.9 blinks, $p< 0.0001$, *t*-test). A significant Pearson's correlation ($r= 0.788$) was found between DLIP test and BUT. Receiver operating characteristic (ROC) curve defined a cutoff value of 6.5 blinks to separate normal from dry eyes (sensitivity of 100%, specificity of 95%). **Conclusions.** The DLIP test may be helpful in quantifying the behavior of the tear lipid layer in the clinical practice and may be used to evaluate the efficacy of new treatments for dry eyes.

Mechanism Of Secretion In Lacrimal Gland Of Diabetic Rats. Leticia P. Roma[1], Daniel A. Cunha[1], Ana Carolina Dias[2], Carolina Maria Módulo[2], Angélica Gobbi Jorge[2], Alexandre Martins Braz[2], Eduardo Melani Rocha[2].[1]Department of Physiology, Institute of Biology, Unicamp, Campinas, SP, [2]Department of Ophthalmology, Faculty of Medicine of Ribeirão Preto, USP, Ribeirão Preto, SP Brazil.

Purpose. In lacrimal glands (LG), cholinergic agonists transmit signals that regulate release of secretory products and those events are governed in cytoplasm by groups of proteins known as rab and SNARE. The aim of the present work was to compare the expression of the secretory apparatus in lacrimal glands of diabetic and control rats. **Methods.** Diabetes was induced in male Wistar rats with a single intravenous streptozotocin or vehicle and a sub group was than treated every other day with insulin. After 10 weeks, LG of the three groups (n=5/group) had the structure compared, and analyzed for expression of acetylcholine (Ach). Western blot and RT-PCR was used to compare the expression of rab and SNARE secretory factors. **Results.** After 10 weeks of diabetes, it was not observed significantly differences in the Ach levels among the three groups. The mRNA expression of Rab3D, Rab 27b and VAMP2 were not changed by diabetes or insulin treatment. On the other hand, western blot analysis had shown that Rab 27b, syntaxin and VAMP2 proteins were significantly reduced in LG of DM compared to controls ($P<0.05$). Insulin treatment increases the expression of Rab 27b and syntaxin to the control levels while Vamp 2 reduction was not reverted by insulin. **Conclusions.** The significant alterations in LG structure and function in DM also includes reduction in proteins of the exocytosis machinery revealing a pos-transcriptional regulation in diabetic LG. Those events were, in part, reverted by insulin replacement, which may be directly driven to acinar cells or secondary to glycemia control.
Financial Support: CAPES, CNPq, FAPESP, FAEPA.

Expression of Semaphorin and VEGF Ligands and Receptors following Corneal Injury. M.I Rosenblatt, C. Yu, D. Eliason, E. Graue, M. Zhang, Department of Ophthalmology and Vision Science, University of California, Davis, California, 95616

Purpose. To investigate the expression of semaphorin and VEGF ligands and receptors in the corneal epithelium and trigeminal ganglia in a murine model of superficial corneal injury. **Methods.** A 2 mm circular corneal epithelial defect was made in thy1-YFP mice using a blade. Regeneration of corneal nerves in the sub-basal plexus was imaged at time points after the initial injury via fluorescence microscopy. Epithelium from the injured ocular surface as well as the contralateral trigeminal ganglion was analyzed by non quantitative PCR for the expression of semaphorins (Sema) 3A, 3B, 3C, 3D, 3E, 3F, plexin A3, neuropilin-1 (NP-1),VEGF164 and VEGFR2. Quantitative PCR (qPCR) was subsequently performed for Sema 3A and 3F, plexin A3, NP-1,VEGF-A and VEGFR2 (Taqman). **Results.** Superficial corneal scraping results in complete loss of the sub-basal neuronal plexus. Regeneration was seen to occur from existing sub-basal nerves peripheral to the injury, or nerves "blooming" from deeper stromal nerves underlying the injury. At 28 days, the density of the regenerated plexus was still less than that seen pre-injury. By non-quantitative PCR, Sema 3A, 3B, 3C, 3D, 3E, 3F, plexin A3, NP-1, VEGF164 and VEGFR2 were expressed both in corneal epithelium and trigeminal ganglia. By qPCR, VEGF164 expression was increased after injury in both epithelium (day 1) and trigeminal ganglia (days 1, 3, 5), while VEGFR2 expression was increased only in the trigeminal ganglia (days 1, 3, 5). In the epithelium, Sema 3A was increased (day 1), while semaphorin 3F, plexin A3, and NP-1 were decreased (days 1, 3, 5). Trigeminal expression of Sema 3F and plexin A3 was decreased (days 1, 3, 5). **Conclusions.** After superficial corneal injury, the corneal epithelium and trigeminal ganglion display altered expression of semaphorin and VEGF ligands and receptors. The regulation of these axonal guidance genes after injury suggests a role for these molecules in the regeneration of corneal nerves.
Supported by Research to Prevent Blindness and NIH K08EY015829.

Dry Eye Syndrome-Related Quality Of Life In Glaucoma Patients. Gemma CM Rossi[1,2,] Carmine Tinelli[3]. [1]UO Oculistica, AO Bolognini, Seriate Bg; [2]University Eye Clinic of Pavia, Pavia; [3]Lab. Epidemiologia e Statistica, IRCCS Policlinico S. Matteo Pavia

Purpose. To verify the presence of dry eye syndrome (DES) in treated glaucoma patients and to analyze DES impact on quality of life of such patients versus a control group. **Methods.** 61 consecutive primary open-angle glaucoma or ocular hypertension patients were enrolled in this cross-sectional study. Patients were divided into three groups on the basis of the number of glaucoma drops instillation per die (G1=1 drop/die, G2=2drops/die, G3=3 drops/die). A control group of 20 subjects was selected too (G0). All subjects were submitted to a complete ocular examination including some tests of tear function and ocular surface status (fluorescein break-up time, presence of punctate keratitis, ocular hyperaemia). All subjects completed the self administered versions of the 25-item NEI-VFQ, the GSS questionnaire, and the OSDI. The Kruskal-Wallis ANOVA and Mann-Whitney U Test were used to compare median values between groups. The $\chi 2$ test and Fisher's exact test were used to verify statistically significant differences between groups. **Results.** 40% of G3 and 39% of G1 patients presented a DES versus 11% of G2 and 5% of G0 ($p=0.01$). Quality of life (QL) was significantly influenced and altered when evaluated both by NEI-VFQ 25 total mean and by GSS total mean and symptoms average ($p=0.0085$, $p=0.006$ and $p=0.03$, respectively). OSDI pointed out differences by group: 26% of G1 and 15% of G3 presented moderate OSDI; and 15% of G3 and 8.7% of G1 presented severe OSDI ($p>0.05$). **Conclusion.** Topically treated glaucoma patients present a DES more often than a similar control group ($p=0.01$). The presence of DES negatively influences the patient's QL. Patients care about worsening symptoms as about worsening disability. The ocular surface status should be regularly evaluated before starting and during the assumption of topical chronic glaucoma therapy to early recognize and cure the ocular surface pathological signs.

Loss Of BMP Signaling Results In Meibomian Gland Dysplasia And An Altered Palpebral Conunctival Epithelium. David G. Ryan and Robert M. Lavker; Dept. of Dermatology, Northwestern University Medical School, Chicago, IL

Purpose. Interference in BMP signaling causes generalized abnormalities in cell proliferation, differentiation, and apoptosis. BMP signaling also plays a crucial role in the development of appendages (e.g., hair follicles, sebaceous glands). To analyze the in vivo effects of BMP signaling on meibomian gland development, we characterized transgenic mice that ectopically express Noggin, a potent BMP inhibitor. **Methods.** We generated transgenic mice that ectopically express Noggin under the control of the neuron specific enolase (NSE) promoter. Upper and lower eyelids were dissected from transgenic and wild-type mice at various stages of development. Whole mount specimens containing meibomian glands, mucocutaneous junctional (MCJ) epithelium and palpebral conjunctiva as well as frozen and paraffin embedded tissues were examined histologically. **Results.** Individual meibomian glands were missing or markedly disorganized in whole mounts prepared from Noggin transgenic mice. This phenotype was more severe in the lower lids. Eyelashes were missing from the upper lids, whereas hairs resembling eyelashes were observed on the

lower lids, apparently replacing meibomian glands. The overall organization of the conjunctival tissue was also altered; increased goblet cells were observed on the conjunctival epithelium of upper lids while a decrease in goblet cells was seen in the conjunctival epithelium of lower lids. **Conclusions.** Loss of BMP signaling affects the normal development of the meibomian gland and conjunctival epithelium. We suggest that BMP signaling is inhibited in the meibomian gland and MCJ epithelium by misexpression of Noggin. We propose that a "dorso-ventral midline shift" might account for the observed phenotypic changes. Noggin specifies the dorsal field, whereas high levels BMP maintain the ventral nature of the opposing field. Overexpression of noggin thus shifts the position of the midline across the ventral surface. This dorsalization could explain the replacement of meibomian glands by eyelashes.

Protein Array Characterization Of The Secretion Of Inflammatory, Immune And Angiogenic Modulators By Immortalized Human Cornea Epithelium In Response To Bacterial Stimulation. Robert Sack[1], Sonal Sathe[1], Ann Beaton[1], Nancy McNamara[2], Minjian Ni[3], Suzanne Fleiszig[3]. [1]SUNY Opt, [2]UCSFMS, Proctor Foundation, [3]UC Berkeley Opt

Purpose. The corneal epithelium actively contributes to the innate host defense system by sensing potential pathogens through interaction with pattern recognition receptors and secreting proteins including defensins, IL-8 and IL-6. This study was designed to address whether other bioactive proteins are similarly up regulated. **Methods.** Immortalized human corneal epithelium cells were grown in culture, starved and exposed to heat-killed *Pseudomonas aeruginosa* in a dose dependent manner. Culture medium of exposed cells was screened for > 50 growth factors, inflammatory and immune modulators and angiogenic proteins by sequential development using a series of membrane protein arrays and micro-well plate arrays with identification based upon sandwich dot ELISA assays carried out using biotin-streptavidin amplification and a femtogram-sensitive substrate. **Results.** Immortalized corneal epithelial cells grown in culture secrete detectable levels of only three of the > 50 screened proteins. These consist of IL-6, IL-8 and GRO (generic). Exposure of the cells to killed *P. aeruginosa* caused a marked increase in the level of secretion of all three proteins with the intensity of the signal for GRO particularly striking. Also evident in tissue culture was the emergence of new sets of proteins including an exceptionally strong signal for GM-CSF and moderate/weak signals for MCP-1, MMP-9 as well as leptin. **Conclusions.** Protein array analysis allows the identification of several bioactive proteins that are up regulated and secreted by immortalized corneal epithelium in response to the presence of killed bacteria. The data supports a role for GM-CSF (as suggested by others) in PMN cell sIgA-receptor activation and reveals other possible contributors to the ocular host defense system, including GRO, which may play a significant role in PMN cell recruitment.

Micro-Well Plate Array Characterization Of Inflammatory Mediators In Normal And Dry Eye Tears. Robert Sack[1], Sonal Sathe[1], Ann Beaton[1], Trinka Vijmesi[2] and Nancy McNamara.[2] [1]SUNY Opt, [2]UCSFMS.

Purpose. To adapt a micro-well protein array to characterize cytokines in clinically obtainable dry eye and normal tear samples. **Methods.** Micro-well plate kits from 2 manufacturers specific for IL-1a, 1b, 2, 4, 5, 6, 8, 10, 12, 13, IFNg, TNFa and TNFb were evaluated in tears using a laboratory protocol designed to minimize confounding tear matrix effects. Microliter size tears samples were self-collected during the day (open tear fraction, OTF) and immediately upon eye opening after overnight sleep (closed tear fraction, CTF) using filter strips from normals (N) and labial gland biopsy-positive individuals with Sjörgen's syndrome (SS). Preliminary analysis revealed that the signal for IL-8 in N OTF greatly exceeded that for any other cytokines. To avoid a blooming artifact, arrays were sequentially developed first with a cocktail of biotinylated secondary Abs excluding the Ab for IL-8 followed by amplification using a biotin-Streptavidin- HRP reporter enzyme and imaging using a long decay time femtogram sensitive chemoluminiscent substrate. After imaging, the plates were redeveloped by exposure to the biotinylated Ab to IL-8, with the HRP detection process repeated. **Results.** Tear samples were assayed for the presence of 14 inflammatory mediators. Mediators were detected even in trace tear samples collected from individuals with severe dry eye deficiency without a confounding matrix effect. Data shows that the N OTF almost invariably contains high levels of IL-8 and trace to negligible levels of all other cytokines. In some samples, a small percentage of the signal for IL-8 was shown by further analysis to be due to an artifact, a possible putative IL-8 complex. Comparative assays revealed intense signals for several cytokines common to both the OTF and CTF samples in the majority of the SS positive individuals. **Conclusions.** Protein arrays can be successfully adapted to assay cytokines in N and SS samples with marked differences evident in most SS patients. Advances in protein array methodology will make it possible to monitor the effectiveness of therapeutic intervention on a molecular level.

Antimicrobial Peptides Are Lytic To *Acanthamoeba Castellanii.*
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Acanthamoeba species are an important cause of keratitis, mainly in contact lens wearers. Because of its poor response to conventional antimicrobial agents at concentrations tolerated by the eye the outcome is generally severe visual impairment. We evaluated the *in vitro* efficacy of two classes of antimicrobial peptides against *Acanthamoeba castellani* trophozoites compared to rabbit corneal epithelial (SIRC) cells. We used Gomesin, a b-hairpin peptide, and peptides derived from the N-terminus of trialysin (P5 and P6), which form amphipathic a-helix structures. Gomesin was more effective in promoting amoeba (LC_{50} = 15 _M) than SIRC cells permeabilization (LC_{50} = 25 _M), resisting proteolytic degradation. It was less effective in preventing growth because its action decreased in amoeba growth medium. P5 and P6 peptides promoted amoeba permeabilization at higher concentrations (LC_{50} = 36 _M and 40 _M, respectively) and were very sensitive to proteases secreted by amoeba. Nevertheless, peptide P5 prevented amoeba growth at concentrations as low as 5 _M. Addition of PMSF increased P5 and P6 lytic efficiency. We concluded that although _-hairpin peptides are effective to kill amoeba at safe concentrations, their effect depends on the culture medium, which increases parasite resistance to lyses. In contrast, amphiphathic a-helix peptides are effective in preventing growth but their action would depend on the susceptibility to amoeba proteases.

Omega-3 Fatty Acids and Dry Eye: Clinical, Interferometric, and Proteomic Considerations. Satiani NG,[1] Green-Church KB,[2] Nichols JJ,[1] King-Smith PE,[1] Nichols KK.[1] The Ohio State University, College of Optometry,[1] Mass Spectrometry and Proteomics Facility[2]

Purpose. To examine the effect of omega-3 fatty acids in the form of fish oil supplements on the tear film. **Methods.** Twenty-six participants with either a previous diagnosis of Dry Eye Syndrome (DES) or significant symptoms of dryness consumed six capsules of omega-3 fatty acids in the form of fish oil for a period of eight weeks. Each capsule contained 102.74 mg EPA and 197.37 mg DHA. Assessments of symptoms and signs were performed at the baseline visit, the intermediate visit (week four), and the final visit. Symptoms were assessed by the Ocular Surface Disease Index (OSDI, Allergan Inc.) and by querying symptoms of dryness and irritation. Dietary intake of omega-3 fatty acids was assessed using a food frequency questionnaire. Corneal staining with fluorescein, conjunctival staining with lissamine green, wavelength-dependent interferometry, Schirmer testing without anesthesia, and tear break-up time were also assessed. Nonparametic statistical analyses were used to compare the baseline and final visit. **Results.** Statistically significant improvement in symptoms was noted by both methods of symptom assessment – OSDI ($p = 0.001$) and the two symptom questions ($p < 0.001$). Twenty participants stated feeling relief from their dryness symptoms (76.9%). Corneal staining with fluorescein improved significantly ($p = 0.003$) and clearance of central staining was observed in both of the participants who had central staining at baseline. A trend in the reduction in conjunctival staining was also noted ($p = 0.06$). Interferometry results showed a trend toward an increased rate of tear film thinning ($p = 0.09$) although there was no difference in overall tear film thickness ($p = 0.78$). Schirmer testing and tear break-up time did not change ($p = 0.60$ and 0.44, respectively). **Conclusions.** A larger, randomized, double-masked, placebo-controlled clinical trial is warranted to determine if omega-3 fatty acid supplementation reduces the signs and symptoms of DES. Initial results are promising.
Commercial Relationships. None. Support. Pharmavite, LLC provided the supplements.

Epidemiology Of Dry Eye Disease. Debra A. Schaumberg, ScD, OD, MPH, Brigham and Women's Hospital, Harvard Medical School, Boston MA USA.

An eruption of knowledge about dry eye disease (DED) over the past decade has resulted in a broad based recognition of its importance. Rather than an annoyance inherent in normal aging, DED is now viewed as a common multifactorial disease of the tears and ocular surface that results in debilitating irritative symptoms, tear instability, and fluctuating visual disturbances. Study of the epidemiology of DED continues to be challenged by a number of factors but some measure of progress has been made through adopting the view that since 1) clinically important degrees of ocular surface damage rarely occur without symptoms, 2) symptoms contribute to care-seeking behavior, and 3) a major goal of therapy for DED is relief of symptoms, assessment of symptoms could be used as a common basis to study DED. Epidemiological studies have provided useful data on the prevalence of DED in various populations, identified important risk factors, and begun to address its impact on quality of life. In the largest studies, the age-adjusted prevalence of DED in the US was 7.8%, or 3.23 million women, and 4.3%, or 1.68 million men aged ≥50 y. There appear to be no substantial differences in prevalence between blacks and whites, but there is a question of higher prevalence in Hispanic and Asian populations. In the Women's Health Study (WHS), use of estrogen replacement therapy increased the risk of DES by about 70% if used alone, and by 30% if used in combination with progesterone/progestins, regardless of the dose of estrogen. There is also emerging evidence that androgen deficiency, e.g. among men taking anti-androgen medication, is associated with DED. Essential fatty acids appear important in DED, with a 20% reduction in DED among those in the highest versus lowest fifth of dietary intake of omega-3 fatty acids in the WHS. Consumption of five to six four-ounce servings of tuna fish was related to a nearly 70% reduction in risk of DED. In addition to experiencing irritative symptoms, patients with DED often complain of problems with their vision in spite of normal visual acuity. Overall, patients with DED are about 3 times more likely to report problems with common activities such as reading or driving. Through such work, epidemiological studies have helped place DED in its proper context as a significant public health problem.

Androgens Regulate Autophagy In The Mouse Meibomian Gland. Frank Schirra, Berthold Seitz. Eye Infirmary, Saarland University Hospital, Homburg/Saar, Germany

Purpose. To demonstrate the relevance of androgen-regulation of autophagy related genes in the mouse meibomian gland. **Methods**. Orchiectomized mice were systemically treated with either testosterone or placebo for two weeks. The mRNA was then extracted from the meibomian glands and differential gene expression was investigated by microarray hybridisation and evaluation with GeneSifter software and pathway information of the Kyoto Encyclopedia of Genes and Genomes (KEGG). **Results**. One of the most significantly testosterone-controlled pathways is the regulation of autophagy. Interferon alpha 7, GABA receptor-associated protein-like 1, beclin 1 and autophagy-related types 3, 5, 7, and 12 were up-regulated. In contrast, phosphatidylinositol 3 kinase, regulator subunit, polypeptide 4, p150 was down-regulated in the mouse meibomian gland. **Conclusions**. Autophagy may play an important role in the supply of lipids in the meibomian gland. The control of genes involved in the regulation of autophagy in the meibomian glands by testosterone provides further insight into the nature of androgen regulation of this tissue.
[This research was supported by NIH grant EY05612, as well as the German Research Society DFG SCHI 562/1-1 and 1-2]

Cultivation Of Lacrimal Gland Acinar Cells In A Microgravity Environment. S. Schrader*, C. Kremling*, M. Klinger**, H. Laqua*, G. Geerling***, *Department of Ophthalmology, University of Luebeck, Germany, **Department of Anatomy, University of Luebeck, Germany, ***Department of Ophthalmology, Julius-Maximilian-University Wuerzburg, Germany

Purpose. A Rotary Cell Culture System (RCCS) allows the creation of a microgravity environment of low shear force, high-mass transfer, and 3-dimensional cell culture of various cell types. Aim of this study was to evaluate the growth pattern and the secretory function of rabbit lacrimal gland acinar cells in a microgravity environment using a RCCS. **Methods.** Lacrimal gland acinar cells from New Zealand White rabbits of both sexes were isolated and cultured in a RCCS up to 28 days. Cells were analysed by light and electron microscopy at day 7, 14, 21 and 28. Secretory function was tested by measuring the ß-hexosaminidase activity. **Results.** After seeding to the RCCS, the lacrimal gland cells formed spheroidal aggregates. The acinar cells inside the spheroids retained their histotypic features, but in the center of the spheroids groups of necrotic cells became more abundant during the culture period. The evaluation of the secretory function showed a response to stimulation with carbachol until day 7. **Conclusions.** Acinar lacrimal gland cells can be successfully cultured in a RCCS up to 28

days, with a secretory response to carbachol up to 7 days. A simulated microgravity environment allows to maintain long-term cultures of lacrimal gland acinar cells and promises opportunities for further applications in basic and applied cell research on lacrimal gland cells.
[This work was in part supported by a research grant of the University of Luebeck, Germany (A03-2007)]

Lowering The Graft's Age Turns The Heavy Rejection After Keratoplasty In Baby Rats To A Low Risk Situation. Johannes Schwartzkopff, Florian Birnbaum, Thomas Reinhard. Eye Hospital, University of Freiburg, Germany.

Purpose. It has been shown that 3 week old rats reject corneal grafts statistically significantly faster than 8 week old rats. In this study we wanted to analyze the impact of the donor's age during this process. **Methods.** Penetrating keratoplasty was performed between Lewis recipient and Fisher donor rats. The donor's and the recipient's age were varied between 8 and 3 weeks, respectively. This let to the following groups: a) Donor and recipient: 8 weeks, b) donor 8 weeks and recipient 3 weeks, c) donor 3 weeks and recipient 8 weeks and d) donor and recipient 3 weeks. All groups a)-d) were controlled in a syngenic setting. Postoperatively, the transplants were monitored by two independent investigators for their grade in opacity, oedema and vascularisation. Finally, histological evaluations for mononuclear infiltrates were performed on the day of rejection. **Results.** Animals of group a) rejected allografts with a mean survival time of 15 days. Contrary to that, the mean survival time was 9 days in group b). This time was prolonged to 18 days in group c). As in the syngenic controls, no rejection at all was observed in group d). A dense infiltrate of mononuclear cells could be stained in groups a) and c). By contrast, only few infiltrating cells were found in group b). Along with the clinical findings, very few cells invaded in group d) as in the syngenic control groups. **Conclusions.** After 9 days already few infiltrating cells lead to rejection of corneal grafts in 3 week old rats compared to a dense infiltrate leading to graft failure after 15 days in 8 week old rats. By lowering the graft's age to 3 weeks this process was delayed to 18 days in 8 week old recipients. Surprisingly, reducing the donor's age turned the heavy rejection process in 3 week old recipients to a "low risk" situation, i.e. no signs of rejection at all could be observed anymore. These results propose that an infant's corneal allograft primes the recipient's immune system weaker and thus might antagonize the otherwise vigorous immune reaction in an infant's eye after a penetrating keratoplasty.
Commercial relationship: JS: none FB: none TR: none.
This work was supported by the Ernst-und-Berta Grimmke Stiftung, Duesseldorf, Germany.

A New Oil-In-Water Emulsion For The Treatment Of Dry Eye. C. Scifo[1], G. De Pasquale[1], M. Pistone[1], S. Barabino[2], A.R. Blanco[1], M.Rolando[2]. [1]Pharma Business Unit S.I.F.I. Spa Lavinaio (Catania), [2]Department of Neurosciences, Ophthalmology, and Genetics University of Genoa[2], ITALY

Purpose. The aim of this study was to test the efficacy of a new oil-in-water emulsion, containing natural apolar triglycerides and phospholipids, in the *in vivo* experimental model of dry eye described by Barabino et al (IOVS, 2005). **Methods.** C57BL/6 female mice (8-12 weeks old) were kept for 3 to 7 days in a controlled environment chamber (CEC) under both low humidity (RH< 20%, 21°C) and constant air flow. Scopolamine transdermal patch (0.75 mg) was also administered to animals in order to make dry eye symptoms more severe. A control group of mice were kept under standard temperature and humidity to confirm the occurrence of dry eye in the CEC conditions. Animals in CEC were divided into 3 groups: 1 group remained without treatment, the other 2 groups received 10µl of emulsion or sodium hyaluronate (NaHa) eye drops *quid* (*quater in die*). Tear production and corneal damage were assessed after 3 and 7 days of CEC by cotton thread test and fluorescein staining, respectively. **Results.** Tear production and corneal staining were significantly ($p<0.001$) worsened in the group of mice maintained under CEC with respect to the control group. On the other hand, in animals treated with emulsion for 7 days tears were less reduced than the in the untreated group (40% vs. 70%) with $p<0.05$ (ANOVA test). No differences were observed in the NaHa treated group with respect to untreated group. Corneal damage was improved in both treated group; this effect was statistically significant after 3 days in the emulsion group only ($p<0.05$, Kruskal-Wallis test) and after 7 days in both groups ($p< 0.01$ and $p< 0.05$, emulsion and NaHa, respectively). **Conclusions**. Our results indicate that the new oil-in-water emulsion is able to reduce the tear evaporation and to improve the corneal damage caused by this experimentally-induced dry eye model. This product may be an useful tool for the treatment of dry eye syndrome.

Application Of Bone Marrow Cells And CD117+ Stem Cells Promotes Corneal Ulcer Healing. Saadettin Sel,[1] Martin Schilling,1 Kathrin Friebel,[1] Elke Vetter,[1] Andreas Simm,[3] Norbert Nass,[3] Hassan Nakhai,[4] Thomas Kalinski,[5] Gernot Duncker,[1] Friedrich Paulsen[6] [1]Department of Ophthalmology, Martin Luther University Halle-Wittenberg, [2]Department of Ophthalmology Vogtland-Klinikum Plauen, [3]Department of Cardio-thoracic Surgery, Martin Luther University Halle-Wittenberg, [4]Department of Internal Medicine II, Klinikum Rechts der Isar, Technical University of Munich, [5]Department of Pathology, Otto-von-Guericke-University, Magdeburg, [6]Department of Anatomy and Cell Biology, Martin Luther University Halle-Wittenberg.

Purpose. Bone marrow cells have the ability to differentiate into different cell types and produce a variety of growth factors and cytokines. In this study, we verify the hypothesis whether topically applied bone marrow cells and CD117+ stem cells play a role in healing of corneal ulcers. **Methods.** Bone marrow cells from syngenic Balb/c mice were isolated. Half of the volume of the bone marrow cells were transferred into cell culture medium and the other half of the volume were used to isolate CD117+ cells by means of the MACS method. The quality of CD117+ stem cells were verified by FACS analysis. Corneal ulcers were created on mice eyes by application of alkali-soaked filter paper disk. 3 mice treatment groups were build: Group 1 (control): cell culture medium. Group 2: cell culture medium plus bone marrow cells. Group 3: cell culture medium plus CD117+ cells. All treatments were applied as eye drops 3 times per day. The corneal ulcers were visualized by fluorescein eye drops and the healing process was photo-documented for 7 days. The defect area was measured with Sigma Pro 5.0 and statistical analysis was performed using SPSS for Windows 12.0. **Results.** Kaplan-Meier analysis revealed that the topical application of bone marrow cells (log rank test $p<0.0001$) and CD117+ stem cells (log rank test $p<0.0001$) as ophthalmic eye drops accelerates the healing of corneal ulcers in comparison to the control group. There was no statistically significant difference between the application of bone marrow cells or CD117+ stem cells ($p=0.91$). **Conclusions.** Topically application of bone marrow cells and CD117+ stem cells promote the healing of corneal ulcers. This could be another more effective option for the treatment of patients with therapeutically resistant corneal ulcers.

Ion Fluxes Across Rabbit Acinar Cell Monolayers On Polyester Membrane Scaffolds. Shivaram Selvam[1,2], Padmaja B. Thomas[1], Hovhannes J. Gukasyan[3], Douglas Stevenson[1], Alan S. Yu[4A], Melvin D. Trousdale[1,4B], Joel E. Schechter[4B,4C], Austin K. Mircheff[4B,4D], Ronald E. Smith[1,4B], Samuel C. Yiu[1,4B]. [1]Ocular Surface Center, Doheny Eye Institute, Los Angeles, CA; [2]Mork Family Department of Chemical Engineering and Materials Science, Depts of [A]Medicine, [B]Ophthalmology, [C]Cell and Neurobiology, [D]Physiology and Biophysics, [4]Keck School of Medicine, USC, Los Angeles, CA; [3]La Jolla Laboratories, Pfizer Inc., San Diego, CA, USA.

Purpose. To test the hypothesis that rabbit lacrimal acinar cell monolayers (RLACMs) on polyester membrane scaffolds generate active transepithelial ion fluxes. **Methods.** Purified acinar cells were seeded onto polyester membrane inserts and cultured to apparent confluency. RLACMs were evaluated by transmission electron microscopy (TEM) and immunofluorescence staining for Na,K-ATPase. Active ion fluxes were evaluated in Ussing chambers by measuring short circuit currents (I_{sc}) under various conditions. _-hexosaminidase secretion was measured by determining catalytic activity in aliquots of the apical bath medium. **Results.** TEM revealed well-maintained epithelial characteristics, i.e., apical (AP) secretory granules, microvilli and junctional complexes. Immunofluorecence staining showed Na,K-ATPase expression on both the AP and basolateral (BL) membranes. Stimulation of RLACMs with carbachol (CCh) induced a large I_{sc} (33.93 ± 12.25 μA/cm^2), positive in the AP_BL direction. Addition of the Na,K-ATPase inhibitor, ouabain, to the BL medium completely abolished I_{sc}. Replacing both the AP and BL media with Cl^--free buffer solution also returned I_{sc} to baseline values. Neither the Na/H exchange inhibitor, amiloride, nor the NaK2Cl cotransport inhibitor, bumetanide, caused a significant change in I_{sc} when added singly. However, addition of both inhibitors in combination reduced I_{sc} by 65%. Secretion of b-hexosaminidase to the AP medium was six-fold greater than secretion to the BL medium, and it was increased two-fold ($P<0.05$) by stimulation with CCh. **Conclusions.** RLACMs generate a Cl^--dependent, ouabain-sensitive, AP_BL I_{sc} in response to CCh consistent with current models for Na^+-dependent Cl^- secretion.
[Supported by EY15457, EY03040, EY10550, EY12689, DK062283 and RPB. CR: none].

Myoepithelial Cells Originate From Nestin-Positive Precursors In The Lacrimal Gland. Marie A. Shatos, Linda Jonsson, Robin R. Hodges, Laura M. Tarko and Darlene A. Dartt. Schepens Eye Research Institute and Harvard Medical School, Boston, MA. USA.

Purpose. To investigate the origin of myoepithelial cells *in vitro.* **Methods**: Rat lacrimal glands were dissociated by repetitive cycles of digestion in Type I collagenase. Liberated cells were first grown in serum-supplemented RPMI-1640 medium. To examine plasticity of precursor cells, they were cultured in either serum-free RPMI medium or in X-Vivo medium containing various growth factors. Characterization medium consisted of either RPMI or X-Vivo medium supplemented with 10% FBS. Cells were evaluated for expression of the progenitor cell marker, nestin; the proliferation marker Ki-67; the myoepithelial cell markers smooth muscle actin, α-actinin, vimentin, and adenyl cyclase II; the neuronal markers NF-200 and MAP 5; and the glial cell marker, GFAP. Tissue sections of lacrimal gland were also examined for the presence of nestin. **Results**. Following digestion with collagenase, a variety of cells immediately attached to the culture vessel and became confluent. Gradually, clusters of round immature cells which were nestin and Ki-67 positive began to form and overgrew the underlying monolayer. In FBS-supplemented RPMI medium these cells differentiated into myoepithelial cells that expressed smooth muscle actin, α-actinin, vimentin and adenyl cyclase II. When tissue sections of lacrimal gland were evaluated for nestin expression, nestin was observed in a population of myoepithelial cells surrounding both acini and ducts. In separate experiments to examine plasticity of these immature precursor cells, they were grown in X-Vivo neural cell medium that caused them to differentiate into neuronal-like cells which expressed NF-200 and MAP-5, but not the glial cell marker GFAP. **Conclusion**. Lacrimal gland myoepithelial cells appear to originate from progenitor cells within the gland and may retain some progenitor cell functions.
Support: NIH grant EY06177

Expression Of Tight Junction-Related Proteins In Cultivated Oral Mucosal And Limbal Epithelial Sheets. Jun Shimazaki, Kazunari Higa, Fumito Morito, Yoshiyuki Satake. Department of Ophthalmology, Tokyo Dental College.

Purpose. Transplantation of cultivated epithelial sheets is a recently developed method for
ocular surface reconstruction. Several clinical studies reported promising outcomes; however, it is unclear whether epithelial function is also recovered or not following surgery. Here we study expression of tight junction-related proteins in the cultivated oral mucosal epithelial (COMES) and limbal epithelial (CLES) sheets. **Methods.** Immunohistochemistry for ZO-1 and occludin was performed in the human oral mucosa and limbal/corneal tissues. The results were compared with those in the COMES and CLES after cultivation on the human amniotic membrane. Histology and transmission electron microscopy were performed in selected samples. **Results.** Histological examinations revealed that the epithelial cells were thick and formed 10-20 multi-layers in the oral mucosal tissue, however, only 6-7 layers of epithelial cells were found in the COMES. ZO-1 and occludin were expressed not only at the surface epithelial cells but also at the cell-cell junctions throughout the epithelial layer in the living oral mucosal tissue. However, the expression patterns of ZO-1 and occludin were considerably altered in the COMES; they were predominantly expressed in the uppermost cell layer. Electron microscopy demonstrated the tight-junction like structure in the superficial epithelial cells of COMES. The ZO-1 and occludin were exclusively distributed in the uppermost layer in both living limbal/corneal epithelium and CLES. **Conclusions.** Distribution of tight junction-related proteins were considerably different between living oral mucosal tissues and COMES. [The authors do not have any proprietary interest in the products mentioned used in this study.
The research is supported in part by a Grant of the Ministry of Health and Welfare, Japan (H15-Saisei-013).]

Physiological Mechanisms And Clinical Impact Of Aging. Andreas Simm, Department of Cardiothoracic Surgery, Martin-Luther University Halle-Wittenberg, Halle, Germany

The changes in the age structure of many human populations in the last 200 years lead to the fact that part of the old people from the entire population will increase drastically. In order to understand the notion of aging better, the antagonistic pleiotropy, an evolution biology based theory, may give some explanations how a beneficial mechanism which effectively protects the body during youth, contrary transform itself by aging. Additionally, some mechanisms of aging have become clearer during the last decades. Genes and pathways were elucidated that are involved in the regulation of longevity. The limitation of human cells to proliferate (replicative senescence) is caused by the erosion of telomers and stress induced expression of cdk inhibitors. There are nutritional

effects on aging as dietary restriction extends lifespan in many species. Oxidative stress is an important contributor to the mechanisms of aging as well as it stimulates the modification of long-lived molecules like DNA or proteins. Indeed, the current understanding of intrinsic aging is that it is due to the progressive accumulation of molecular and cellular defects. However, degenerative diseases like sarcopenia, cataract, ostheoarthitis and even cancer showed an almost equivalent situation. Protein modifications like the products of the sugar based Maillard reaction, the advanced glycation endproducts, accumulate with age and are discussed to be responsible for many degenerative diseases. The damage that leads for example to the accumulation in the aged brain of the amyloid plaques, which are associated with Alzheimer disease, appears to be associated with intrinsic processes of brain aging that we probably cannot understand the one without understanding the other. Therefore, basic and medical scientists should work integratively to solve age associated problems. Unfortunately, geriatrics tend to treat mostly the symptoms of old age whereas biogerontologists on the other hand, study the biological basis of aging. Until now, they have no real tradition to work to a common goal.

Selection Of Compatible Solutes For Inclusion In A Lubricant Eye Drop. Peter A Simmons[1], Joan-En Chang-Lin[1], Joseph G Vehige[1], Quang Chung[2], Devin Welty.[1] Allergan R&D[1], Irvine CA USA; Southern California College of Optometry[2], Fullerton CA USA.

Purpose. Compatible solutes (CS) are a class of small nonionic organic molecules that are synthesized or taken up by cells and tissues in order to build internal osmotic strength without perturbing cell function in the presence of osmotic shifts in the extracellular environment. In dry eye, ocular surface cells are exposed to chronic hyperosmolar stress, and preliminary data suggests they may be able to accumulate CS to protect themselves. Identification of a potential CS to use as a supplement in an artificial tear formula would include demonstration of both uptake by ocular surface cells and benefit to cells and tissues, as well as compatibility with other formula components. **Methods**. Candidate CS identified from the literature were screened for compatibility with standard artificial tear components including lubricant, buffer, and preservative. A primary culture of rabbit corneal cells grown under airlift conditions was used to assess potential benefit of topically applied CS by measuring changes of the transepithelial electrical resistance (TEER) response to hypertonicity in the presence or absence of CS. CS demonstrating formula compatibility and in vitro benefit were obtained in radio-labeled form and tested for uptake by the rabbit corneal cell model. **Results**. Of several candidate amino acid CS, l-carnitine was the most clearly compatible with other artificial tear components, and demonstrated both benefit to the TEER response under hypertonic conditions and specific uptake in radiolabel studies. Of polyol candidates, glycerol and erythritol but not xylitol or inositol demonstrated a benefit in the TEER response. Glycerol entered cells very rapidly, and erythritol appeared to share the glycerol channels in competition experiments. **Conclusions.** L-carnitine, glycerol, and erythritol were identified as CS suitable for inclusion in an artificial tear formula designed to provide osmoprotection for the ocular surface.
Research supported by Allergan LLC.

The Effect Of Hormone Replacement Therapy On Ocular Surface Disease In Women With Premature Ovarian Failure. Janine A. Smith, MD[1], Susan Vitale, PhD, MHS[1], Serena Morrison, MD[1], Linda A. Goodman, COT [1], George F. Reed, PhD,[1] Roula Nashwinter, COA,[1] Vien H. Vanderhoof, RN, CRNP[3], Dessie Koutsandreas, COA[1], Karim A. Calis, [2] Lawrence M. Nelson, MD [3]. [1] Division of Epidemiology and Clinical Research, National Eye Institute, [2] Mark O. Hatfield Clinical Research Center, [3]Developmental Endocrinology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland

Purpose. To examine the effect of estrogen and progestin replacement therapy (HRT) on the ocular surface of women with premature ovarian failure. **Methods**. 45 women with premature ovarian failure (POF) being treated with hormone replacement therapy (HRT) were examined for dry eye. Symptoms of dry eye were assessed with the Ocular Surface Disease Index questionnaire©. Standardized, masked assessments of the ocular surface, vital dye staining (Oxford scale) and tear status (Schirmer test with and without anesthesia, tear break-up time, TBUT) were performed. Serum sex hormone levels (follicle stimulating hormone, luteinizing hormone, estradiol, and testosterone) were measured. The ocular signs and symptoms of these women were compared with a previously reported series of 65 women with POF not receiving HRT. **Results**. There were no differences in age, race, ethnicity or visual acuity between the POF and POF-HRT groups. Women with POF on HRT showed significantly more disease than women with POF not receiving HRT: significantly reduced TBUT (5.0 vs. 6.3 seconds, p=0.01), worse meibomian gland plugging (1.11 vs 0.57, p=.004) , more frequent meibomian gland dysfunction (38% vs. 14%, p=0.007), more frequent abnormally reduced TBUT (62% vs. 35%, p=0.011), and more frequent conjunctival hyperemia (96% vs. 68%, p=0.001) and chemosis (42% vs. 15%, p=0.003). There were no differences in staining, tear production or patient-reported symptoms. TBUT was inversely correlated with dry eye symptoms in all women with POF regardless of HRT status. Testosterone level was significantly negatively associated with symptoms in women with POF and with all measures of ocular surface staining: total Oxford score (r=-.40, p=.009), conjunctival lissamine green (-.31, p=.05), and corneal fluorescein staining (r=-.34, p=.03) in women with POF receiving HRT. **Conclusions.** Hormone replacement may alter the ratio of estrogens/ androgens, exacerbating androgen deficiency and promoting meibomian gland dysfunction and tear film instability. The association of lower serum testosterone levels with worse ocular surface staining provides more evidence of a role for androgen deficiency in the ocular surface disease seen in women with POF.
This research was supported by the Intramural Research Program of the NIH and the National Eye Institute. No commercial relationships to disclose for any authors.

Grading Scale For The Time Course Of Corneal Fluorescein Staining. Chris Snyder[1], Mohinder Merchea[2]. [1] Adjunct Professor, University of Alabama at Birmingham School of Optometry, Birmingham, AL, USA; [2]Director, Scientific & Medical Affairs, Bausch & Lomb.

Purpose. The factor of TIME is used in clinical practice and research for assessment of corneal staining. The dimension of TIME COURSE has received increased attention of late, particularly in regard to low severity staining levels associated with contact lens care product usage. **Methods.** This grading scale incorporates TIME as a factor in corneal staining and is designed to address the clinical dimension of staining over time (TIME COURSE). This scale was applied to various clinical conditions and presentations. **Results.** The grading scale demonstrated distinctions in time course between conditions. Contact lens solution-induced transient staining was not clinically relevant in a sample of normal and dry eye patients. **Conclusions.** The use of this grading scale allows grading of the dimension of staining TIME COURSE to document time-related characteristics known clinically and familiarly as "acute", "chronic", "compounding", "cumulative", "worsening" and

"improving". The presented grading scale is relevant to ocular surface response to gas-permeable and soft contact lens wear and to various conditions of acute or chronic nature such as dry eye. This scale should be particularly useful in defining levels of clinical significance for cases of low-severity (trace and mild) levels of staining.
Disclosure: Dr. Snyder serves as Director of Professional Relations for Bausch & Lomb.

The Improved Surgical Technique For Conjunctivochalasis. Yukiko Sonomura, Norihiko Yokoi, Aoi Komuro, Masakazu Nishii, Kayoko Inagaki, Hidemi Chihara, Shigeru Kinoshita. Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan

Purpose. To report the postoperative results of an improved surgical technique for treating conjunctivochalasis. In a previous report, we reported a newly designed operation for conjunctivochalasis to reconstruct the lower tear meniscus (Yokoi N, et al. Cornea 24, S24-31, 2005). In that report, postoperative wound breaks were noted as a postoperative complication (11/168 eyes). One of the possible important causes for this complication is that all procedures for resecting redundant conjunctiva were performed at the frontal eye position and as a result, excessive tension was exerted on the closed wound by sutures during postoperative lateral eye movement. To avoid this complication, we improved the original operation for conjunctivochalasis. **Methods.** Sixty-eight eyes of 57 patients with conjunctivochalasis (12 males and 45 females; mean age: 70.7 yrs) who received the improved conjunctivochalasis operation were enrolled. In this improved operation, redundant conjunctiva at the temporal and nasal portions was resected with the eye being positioned in a contra-lateral direction. All patients had severe symptomatic conjunctivochalasis and could not be treated successfully with the usual eye drops alone. Postoperative complications and improvement in symptoms of patients were evaluated. **Results.** The average follow-up period was 550.1±161.6 (SD) days (164-775 days). Four eyes had complications including lymphangiectasis (2 eyes), and suture-associated granulation (2 eyes). Symptoms 3 months after the operation were improved in 61 eyes (89.7 %), unchanged in 7 eyes (10.3 %), and there was no deterioration. **Conclusions.** This improved surgical technique might very well be the perfect conjunctivochalasis operation for reconstruction of the lower tear meniscus due to the fact that it successfully eliminates wound breaking.

Assay Of MUC16 In Conjunctiva And Tears Of Postmenopausal Women With And Without Dry Eye. Sandra Spurr-Michaud[1], Michelle Senchyna[2], Sruthi Srinivasan[3], Robert Ritter III[2], Pablo Argueso[1], Elizabeth Joyce[3], Miriam Heynen[3], Lyndon Jones[3], Daniel A. Gamache[2], Ilene K. Gipson[1] Schepens Eye Research Institute and Department of Ophthalmology, Harvard Medical School[1], Boston MA, Alcon Research Ltd[2], Ft. Worth Texas, and Center for Contact Lens Research, School of Optometry, University of Waterloo[3], Ontario, Canada.

Purpose. Membrane-associated mucins are heavily glycosylated, high molecular weight glycoproteins that provide a protective barrier at the ocular surface. MUC16 is a membrane-associated mucin, which carries the H185 carbohydrate epitope, shown to be altered on conjunctival epithelium of dry eye patients. We have examined the expression, protein biosynthesis, and protein glycosylation of MUC16 in the conjunctival epithelium and tears of postmenopausal women with and without mild or moderate dry eye as determined by Allergan Ocular Surface Disease Index© symptoms scores, NITBUT (Alcon Eyemap®) and mean tear volume measured by phenol read thread. **Methods.** 20 controls, 10 mild dry eye, 14 moderate dry eye postmenopausal women (no menses for at least 12 months), aged 49 to 91 (mean=62±8yrs), were included in this study. Conjunctival impression cytology samples were collected for assay of MUC16 mRNA by real time-PCR, MUC16 protein by western blot assay, and MUC16 glycosylation using the H185 antibody in ELISA. Saline tear washes were collected for MUC16 protein assay and H185 ELISA. Statistical significance was determined using Instat3 statistical software. **Results.** Data indicate a significant increase in H185 epitope in conjunctival impression cytology samples in moderate dry eye patients ($p=0.0279$, unpaired t-test). There was a trend towards increased MUC16 mRNA in these patients, although it did not quite reach the level of significance ($p=0.0899$, Mann-Whitney test). No statistical differences were noted between normal and mild dry eye patients. **Conclusion.** Increase in H185 epitope in moderate dry eye suggests either a compensatory increase in glycosylation of cell surface mucins or an increase in H185 epitope in goblet cell mucins (as shown by impression cytology).
Funded by Alcon Research Ltd.

Expression Of Soluble And Membrane Bound MUC16 In Dry Eyed Postmenopausal Women. Sruthi Srinivasan[1], Elizabeth Joyce[1], Miriam L. Heynen[1] Lyndon Jones[1], Trefford Simpson[1], Daniel A Gamache[2], Michelle Senchyna[2]. [1]Center for Contact Lens Research, School of Optometry, University of Waterloo, Ontario, Canada, [2]Alcon Research Ltd, Fort Worth, Texas, USA

Purpose. To quantify the expression of both soluble and membrane bound MUC16 in a group of symptomatic dry eyed post menopausal (PM) women compared to aged matched controls and to investigate the potential relationship between MUC16 expression and tear film break up time. **Methods.** 37 healthy PM females (>50 years of age) were categorized as being symptomatic (dry eye (DE)) or asymptomatic (no symptoms of dry eye (NDE)) based on their responses to the Allergan Ocular Surface Disease Index© (OSDI) questionnaire. Non-invasive tear breakup time (NITBUT) was evaluated using the ALCON Eyemap®. Tears were collected from the inferior tear meniscus using a disposable glass capillary tube. Total protein was isolated from epithelial cells collected via impression cytology. Soluble and membrane bound MUC16 were quantified via Western blotting. A standard curve of MUC16 standard (CA125, Biodesign) was run on each blot to facilitate quantitation. Data was compared using a t-test and linear regression. **Results.** OSDI responses revealed 19 symptomatic (age 60.7±8.9 yrs) and 18 asymptomatic (age 59.4 ± 6.9yrs) participants. The DE group exhibited a significantly shorter NITBUT (DE = 4.12 ± 1.30 sec; NDE = 6.56 ± 3.30 sec; $p=0.005$). No difference in either soluble or membrane bound MUC16 expression was found between DE and NDE. Weak correlations were found between the NITBUT values compared with either soluble or membrane bound MUC16 expression ($r^2 = 0.04$ and $r^2 = 0.05$, respectively). **Conclusions.** No difference was found in the expression of either soluble or membrane bound MUC16 between symptomatic postmenopausal women and aged matched controls. Symptomatic women did differ from controls with respect to significantly reduced NITBUT. NITBUT values do not appear to be associated with MUC16 expression. Further research is required to investigate the potential use of biomarkers such as MUC16 in the characterization of dry eye disease.
Funded by Sponsored Research from Alcon Laboratories.

Effect Of Temporary Collagen Inserts On Ocular Comfort And Osmolality During Contact Lens Wear. U.Stahl,[1,3] M. Willcox,[1,2,3] F. Stapleton[1,2,3]. The VisionCRC,[1] Institute for Eye Research,[2] School of Optometry and Vision Science, University of NSW,[3] Sydney, Australia

Purpose. A reduced tear volume is frequently seen in contact lens wearers and dry eye patients and has been suggested as a cause of hyperosmolality leading to discomfort. This pilot study aimed to evaluate the role of tear volume on tear film and lens osmolality and discomfort and dryness sensations during contact lens wear. **Methods.** 10 symptomatic subjects wore Lotrafilcon A lenses bilaterally for 6 hours on 3 different days each; directly following insertion of temporary collagen plugs in the upper and lower puncta of both eyes, 60 hours after plug insertion and without plugs. Comfort, dryness symptoms, tear volume, tear break up time and lipid layer were evaluated. Tear and contact lens osmolality was measured with a Wescor Vapor Pressure Osmometer. Mechanical sensitivity to warmed air (34ºC) was measured for the central corneal and inferior conjunctiva with the CRCERT-Belmonte aesthesiometer. Punctal occlusion was confirmed using Jones testing after each day of lens wear. **Results.** Tear volume, comfort and dryness significantly improved when lenses were worn directly after plug insertion (all $p<0.05$), but not when lenses were worn with plugs in place for 60hours (all $p>0.05$). Lens osmolality significantly decreased when lenses where worn directly after plug insertion (364.8±88.0 mmol/kg, 283.8±30.1 mmol/kg, respectively, $p=0.022$), but not when lenses were worn 60 hours after plug insertion (342.6±80.4, $p=0.156$). Lipid layer, tear break-up time, tear osmolality and ocular sensitivity were not affected by plugs (all $p>0.05$). Jones testing was negative at all visits. **Discussion.** Whilst punctal plugs gave an initial positive effect, the effect was lost after 60 hours, despite confirming punctual occlusion. This finding may support the existence of a rapid feedback mechanism between the lacrimal drainage system and lacrimal gland in individuals with mild symptomatology only in association with contact lens wear.
Commercial Relationships: None; Support: Australian Federal Government through the CRC Programme, Contact Lens Society Research Award.

Effects Of Contact Lens Wear On The Tear Film And Ocular Surface: Future Directions. Fiona Stapleton, Institute for Eye Research, School of Optometry and Vision Science and Vision Cooperative Research Centre, University of New South Wales, Sydney, Australia.

All types of contact lens wear impact upon the ocular surface and tear film; corneal homeostasis is slowed, conjunctival integrity is altered, the structure and physiology of the tear film is disrupted and the lid/ocular surface/tear resurfacing mechanism is altered. The magnitude or impact of these effects varies with lens material type, wear modality and characteristics of the individual wearer. New generation silicone hydrogel lenses have clearly reduced corneal signs associated with hypoxia, however, the risk of corneal infection and inflammation appear to not be reduced, with overnight wear of any modality persisting as the main risk factor. In daily wear, corneal infiltrates appear to be more common in wearers who experience corneal staining as a result of particular lens/care solution combinations.

Future research will focus on individual differences that influence wearer success and the development of lens related inflammation and infection; improving lens biocompatibility with the ocular environment, underpinned by better understanding of how the contact lenses interact with the ocular surface, lid margin and tear film at a cellular, biochemical and molecular level and the impact of combinations of lens type and care solution on the development of adverse responses. The recent increase in reports of contact lens-related fungal and Acanthamoeba keratitis, may direct lens care solution research to a focus on products which enhance antimicrobial efficacy and new strategies for limiting microbial contamination of the storage case.

Intravital Real-Time Imaging Of Conjunctiva-Associated Lymphoid Tissue. Philipp Steven[1,2], Gereon Huettmann[3], Norbert Koop[3], Andreas Gebert[2] Eye Hospital, UK-SH, Campus Luebeck[1], Institute of Anatomy[2] and Institute of Biomedical Optics[3], University of Luebeck, Germany

Purpose. Conjunctiva-associated lymphoid tissue (CALT) has been proposed to be involved in numerous diseases of the ocular surface. Functional studies of CALT are rare, due to a lack of an animal model and insufficient imaging techniques. We demonstrated that two-photon microscopy is a suitable optical method to investigate CALT in a newly established mouse model. Here we present data from intravital real-time investigations of murine CALT. **Methods.** Female Balb/c mice were topically challenged with either Chlamydia trachomatis serovar C or ovalbumin/choleratoxin B to induce CALT in the nictitating membrane of the eye two weeks prior to the investigation. A two-photon microscope equipped with a near infrared femtosecond-laser and a fluorescence-lifetime detector was used. Mice were deeply anesthetized for intravital analysis of CALT with additional application of fluorescent microspheres to demonstrate transepithelial particle transport. **Results.** High resolution images of lymphoepithelium, follicle and adjacent vessels are obtained in tissue depths up to 80 µm. Time-lapse image series of CALT within the nictitating membrane demonstrate that macrophages and lymphocytes rapidly migrate within the subepithelial space. Macrophage-like cells move along elastic fibres. Vascular blood flow is detectable. Topically applied microspheres are tracked over hours on their passage through the epithelium. **Conclusions.** For the first time two-photon microscopy enables intravital real-time investigations of immunological mechanisms related to CALT. This new optical method will be a useful tool to evaluate the physiological function of CALT and to elucidate its functional relation to ocular surface diseases such as dry eye or Sjögren syndrom.
(Supported by University of Luebeck Research Grant A02-2007)

Conjunctival Bacterial Flora In Contact Lens Wearers. Jasmina Stojsic[1], Dragan Stojsic[1], Vladislava Masulovic[2], Milos Vejnovic[1], Novkovic Mile[3]; Dept.of Ophthalmology, [1]General Hospital, Sombor, Serbia; Dept.of Microbiology [2]Sombor, Serbia; General Hospital, [3]Vrbas, Serbia.

Purpose. Contact lens wearers are at high risk to have keratoconjunctivitis. Poor handling and inadequate cleaning compliance can occur, and allergic and toxic reactions can arise in predisposed patients, with resultant consequences. The aim of our study was to investigate the conjunctival bacterial flora in contact lens wearers and to determine the most frequent bacterial agents. **Methods.** This study includes nonrandomised group of contact lens wearers (n - 54; aged 33,7±7,2); soft contact lens 34 (62.9%); RGP 17 (31.5%) and PMMN 3 (5.6%) , examined in Cabinet for Contact lens, General Hospital, Sombor, during Year 2005. Material for bacteriological study was obtained by conjunctival swabs. To analyse these specimens we used: standard microbiological evaluation (Giemsa staining, agar plating and conventional identification tests for bacteria). **Results.** In 42 (77,8%) contact lens wearers, we found sterile bacteriological culture. The most frequent isolated agent was Staphylococcus spp. coagulasa negative (isolated in 9 - 16,8% patients). In rest of the

examined patients, we isolated Citrobacter spp in 1 (2.7%) patient and E.coli in 1 (2.7%) patient. **Conclusions.** Our results confirm importance of carefully handling and adequate cleaning compliance by contact lens wearers. Contact lens patients are at high risk for bacterial conjunctivitis, because of hand to eye transmission.

Herpes-Like Keratitis Associated With Acute Febrile Neutrophilic Dermatosis. (Sweet's Syndrome). Min Hee Suh,[1,2] Joon Young Hyon,[1,3] Won Ryang Wee,[1,2] Jin Hak Lee.[1,3] Seoul Artificial Eye Center, Seoul National University Hospital Clinical Research Institute,[1] Seoul National University Bundang Hospital,[2] Department of Ophthalmology, Seoul National University College of Medicine,[3] Seoul, Korea

Purpose. To report a case of Sweet's syndrome with herpes keratitis like corneal involvement. **Methods.** A 43-year-old woman with a history of seropositive rheumatoid arthritis presented with conjunctival injection of her right eye and redness, swelling of her both upper eyelids. Clinical examination showed conjunctival injection and inferior corneal stromal infiltration, which later progressed to the geographic epithelial defect of her right eye and papillary lesion on her both upper eyelids. **Results.** Neutrophilic infiltration in conjunctival stroma was confirmed by conjunctival biopsy based cytopathologic examination. **Conclusions.** Corneal involvement in Sweet's syndrome is rare. We describe the first case of Sweet's syndrome with herpes like keratitis of which the diagnosis was confirmed by conjunctival biopsy.
Commercial Relationships : None

Recent Advances In Point Of Care Nanoliter Tear Collection. Benjamin Sullivan,[1,2] Steve Zmina,[2] Michael Berg,[2] Sasha Miu,[2] Graeme Bullock,[2] Eric Donsky.[2] University of California, San Diego, La Jolla CA,[1] OcuSense Inc., San Diego, CA,[2] USA.

Purpose. OcuSense is launching a microfluidic, electrochemical platform intended to diagnose and manage Dry Eye Disease (DED), a disease that affects roughly 40 million Americans and 100 million people worldwide. Current DED tests are qualitative and highly subjective, making it difficult for eye care practitioners to definitively diagnose the majority of DED patients, most of whom suffer from mild to moderate forms of the disease. **Methods & Results.** Conventional tear testing systems are not suitable for clinical use due to their high cost, complexity, the need for large sample volumes, and the lengthy time required for testing. OcuSense's TearLab™ requires nanoliters of tears for testing, significantly reducing collection time and the risk of diagnostic variability caused by reflex tearing. At the core of the TearLab system is a disposable lab-on-a-chip that functions as both a tear collection device and a measurement system. Tears are collected directly from the eye, eliminating the need for a standard glass capillary tube. Material interactions at the entrance of the lab-on-a-chip facilitate tear collection in less than 0.1 seconds. A fulcrum located below the disposable chip improves tear collection ergonomics, providing a means to translate gross wrist movements into very small displacements at the tear lake. The system also provides audio feedback to the user to indicate when sufficient tears have been collected.

The Effect Of Menstrual Cycle On The Meibomian Gland Physiology. T.Suzuki[1,2], N.Yokoi[2], A.Komuro[2], S.Kinoshita[2]. Department of Ophthalmology, Kyoto City Hospital[1] and Department of Ophthalmology, Kyoto Prefectural University of Medicine,[2] Kyoto, Japan.

Purpose. Since the menstrual cycle is known to have effects on pilosebaceous gland physiology, we hypothesize that meibomian gland, which is a large sebaceous gland as well as a hormone target organ, changes during the menstrual cycle. Thus, the objective of our study is to assess the cyclic change of meibomian gland physiology during the menstrual cycle. **Methods.** Six female in the twenties with regular 28-day menstrual cycle were enrolled in this study, with informed consent. The diameter of meibomian gland orifice (MGO) using slit-lamp photography with high-magnification, the volume of meibum by meibometry, meibomian gland morphology by video-meibography, and fluorescein tear-film breakup time (F-BUT) were evaluated every three days for 5 consecutive weeks. Serum concentration of sex steroid hormones and basal body temperature were evaluated to confirm the menstrual cycle. **Results.** The menstrual cycle was divided into 6 phases- 2 days before menstruation (phase VI) and the first 2 days of menstruation (phase I), and the remaining time was divided into four 6-day periods (phase II-V). The diameter of MGO was the smallest at phase VI (0.157±0.003 mm, p=0.002). The volume of meibum, the width & length of meibomian gland, and F-BUT were also smallest at phase VI. **Conclusions.** The meibomian gland physiology during the menstrual cycle, especially at post-ovulation, may influence on tear film instability, resulting in the evaporative type of dry eye. Serum levels of sex steroid hormones should be considered as causative factors for meibomian gland physiology. The cyclic change of meibomian gland physiology may impact on meibomitis-related keratoconjunctivitis in young females.

Non-Invasive *In Vivo* Investigation Of The Tear Film Stabilization Process On Cornea And Soft Contact Lenses Using Interferometry. Dorota Szczesna[1], Henryk Kasprzak[1], Ulf Stenevi[2,] Institute of Physics, Wroclaw University of Technology, Wroclaw, Poland[1], Department of Ophthalmology Sahlgren's University Hospital, Mölndal, Sweden[2]

Purpose. To investigate the tear film build-up kinetics and its stability on cornea and different soft contact lenses. The aim was to illustrate the changes of smoothness of tear film surface just after a blink and during tear film drying and as well as a better understanding the physical properties of the tear film. **Methods**. The tear film was examined in vivo by used the lateral shearing interferometer. Pattern of interference fringes carries information about the geometry of the tear film surface. Fast Fourier Transform was used to quantitative evaluation of the tear film surface irregularities. **Results**. For evenly spread tear film, regular and smooth fringes are observed on the cornea. During tear film build-up process on the cornea after every blink a bright pattern has been observed in the background of interference fringes. The semi-vertical orientation of this pattern and its symmetrical inclination for the right and left eyes indicate that the pattern is likely to be related to movements of the upper eyelid wiper. After about 1-3 sec the background of the interferogram becomes uniform and the interference fringes are smooth. The build-up phase is not as clearly observed and the tear film layer is never as perfectly smooth on contact lenses as on the cornea. Irregularities on the tear film surface vary faster in time on the contact lenses than on the cornea. The differences in the tear film stability have been observed on different type of contact lenses and on the cornea before the contact lens was applied. **Conclusions**. The interferometric method allows non-invasive, high accurate, dynamic measurements of the tear film kinetics in real time. Our results indicate differences in the tear film stability on cornea and various contact lens materials as well as in different periods of contact lens wearing. The tear film smoothness on contact lenses is correlated with the tear film quality on the cornea.

Immunohistochemical Investigation of the Filament in Filamentary Keratitis. Hidetoshi Tanioka, Norihiko Yokoi, Aoi Komuro, Takasumi Shimamoto, Satoshi Kawasaki, Akira Matsuda, Shigeru Kinoshita. Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan

Purpose. It is reported that the filament of filamentary keratitis is composed of epithelial cells and mucin through histological examination with a light microscope and an electron microscope. However, up until now, no studies have elucidated the composition of the filament in detail. In this experiment, we used an immunohistochemical technique to clarify the exact composition of the filament. **Methods.** The filaments were obtained from filamentary keratitis patients receiving treatment at an outpatient facility, and these tissues were later embedded in optical cutting temperature (OCT) compound and snap-frozen with liquid nitrogen. Frozen tissues were then sectioned to an 8mm thickness and subjected to an indirect fluorescent immunohistochemical analysis with primary antibodies including cytokeratin (CK1, CK4, CK6, CK10, and CK12), mucin (MUC1, MUC4, and MUC5AC), and markers of infiltration cells (CD3, HLA-DR, and Neutrophil Elastase). Fluorescent images of the sections were inspected and photographed with a fluorescence microscope and a chilled CCD camera (Olympus Corporation, Tokyo, Japan). **Results.** Immunohistochemical analysis showed strong positive staining for CK4 and weak positive staining for CK6 and CK12 in the cellular components of the filaments. The mucin was densely stained by MUC5AC and weakly stained by MUC1 and MUC4. Infiltrating cells were positive for Neutrophil Elastase and HLA-DR. **Conclusions.** We found that the filament was composed of CK4 positive epithelial cells, MUC5AC that is a mucin of Goblet cell origin, and inflammatory cells. We believe that the results of this research will open new pathways towards understanding the mechanism that generates the filament in filamentary keratitis, as well as new methods of treatment in the future.

An Essential Role For MYD88 And IL-1R1, But Not TLR2 Or TLR4, In A Murine Model Of Fusarium Solani Keratitis. Bakir Tarabishy, Mahmoud Ghannoum and Eric Pearlman. Case Western Reserve University, Cleveland, Ohio.

Purpose. Toll Like Receptors (TLR) have an important role in host inflammatory responses to microbial pathogens. Functional TLRs and signaling pathways are present in the cornea, and their activation induces corneal inflammation. The current study examined the role of TLRs and MyD88 in a murine model of *Fusarium solani* keratitis. **Methods.** 10,000 *Fusarium solani* conidia in 2 µl were injected into the corneal stroma of C57BL/6 and MyD88$^{-/-}$ mice, and clinical and histopathological outcomes were measured in relation to fungal growth and survival. **Results.** Fusarium Conidia germinated within 6h, and hyphae were detected in the corneal stroma of all mice. C57BL/6 mice developed a pronounced corneal opacification within 24h, consistent with an intense neutrophil infiltration in the corneal stroma and anterior chamber. After 48h, organisms had been eliminated, although the cornea remained opaque for several days. In marked contrast, MyD88$^{-/-}$ corneas infected with Fusarium remained transparent for the first 24h, but became opaque within 48h, often with ulceration. In contrast to C57BL/6 mice, hyphae penetrated to the anterior chamber of MyD88$^{-/-}$ mice, and although neutrophils were detected in contact with hyphae, fungal growth continued unchecked, and corneal rapidly ulcerated. Analysis of TLR2$^{-/-}$, TLR4$^{-/-}$, and TLR2/4$^{-/-}$ mice showed that although there was a delay in fungal killing in TLR4$^{-/-}$ and TLR2/4$^{-/-}$ mice, neutrophil infiltration and clinical response was similar to C57BL/6 mice. In contrast, IL-1R1$^{-/-}$ mice showed a similar phenotype as MyD88$^{-/-}$ corneas, with delayed pathology, and impaired ability to clear the organisms. **Conclusion.** Together, these findings demonstrate an essential role for the MyD88 dependent pathway in regulation of *Fusarium solani* keratitis, and that MyD88 activation is initiated by IL-1R1 rather than TLR2 or TLR4.

This study was supported by a Senior Investigator Award (EP) from the Research to Prevent Blindness Foundation.

Human Tear Fluid Phospholipid Transfer Protein (PLTP) Interacts with Lysozyme. Timo Tervo[1], Niko L. Setälä[1,2], Matti Jauhiainen[2] and Juha M. Holopainen[1]. [1]Department of Ophthalmology, University of Helsinki, Finland, [2]Department of Molecular Medicine, National Public Health Institute, Biomedicum, Helsinki, Finland.

Purpose. In the circulation PLTP facilitates phospholipid transfer between apolipoprotein B-containing lipoproteins and HDL. Surprisingly, we have shown that active PLTP is found in human tear fluid. We have also elucidated the molecular level mechanism how PLTP facilitates the transfer of phospholipid between donor and acceptor particles. Yet, we have no clue what is the function of PLTP in tear fluid. To address this in this study we have approached this question by first identifying the source of PLTP is tear fluid and then seeked to identify the proteins that interact with PLTP in native human tears. **Methods.** Immunohistochemical analysis of human lacrimal glands using monoclonal antibody against PLTP. Immunoprecipitation and co-immunoprecipitation of PLTP and binding proteins using monoclonal antibody against PLTP. The precipitated proteins were first separated by gel electrophoresis and the visible protein bands were excised and analyzed by MALDI-TOF mass spectrometry (ms) and Western blot analysis. **Results**. PLTP is highly expressed in human lacrimal glands as evidenced by immunohistochemistry. The expression pattern resembles that of tear lysozyme and lipocalin. Immunoprecipitation (IP) of PLTP with monoclonal anti-PLTP antibody was then utilized to identify proteins that are interacting with PLTP in native human tears. Following IP and separation of proteins by electrophoresis several visible protein bands were observed, excised, and identified using MALDI-TOF mass spectrometry (ms). Among these putative interacting proteins were lysozyme, lactoperoxidase, and lipocalin. The interaction of these proteins with PLTP was further studied by Western blot analysis of IPs using specific antibodies against proteins that were identified by MALDI-TOF ms. Yet, only the presence of lysozyme in IPs was confirmed. **Conclusions**. Since lysozyme is known to interact with other lipophilic proteins the above results suggest that PLTP is part of a larger protein complex that may be involved in stabilization of the lipid layer of human tear film.

Autoimmune Dacryoadenitis Induced by Auto-Adoptivee Transfer of CD4+ T Cells. Padmaja.B. Thomas[1], D. M Samant[1], S. Selvam[1, 5], D. Stevenson[1], J.D Gray[4], A.K. Mircheff1, [3], J.E. Schechter[1, 2], M.D. Trousdale[1]; [1]Doheny Eye Institute, Depts. of [2]Cell & Neurobiology, [3]Physiology & Biophysics, [4] Division of Rheumatology & Immunology, and [5]Mork Family Dept. of Chemical Engineering and Materials Science, Keck School of Medicine, University of Southern California, Los Angeles, CA

Purpose. Regulatory mechanisms involving CD4$^+$ and CD8$^+$ T cells are important in the maintenance of immune homeostasis. In Sjögren's syndrome the infiltrates in lacrimal glands consist primarily of CD4$^+$ T cells and IgG$^+$ B cells**.** This study evaluates CD4$^+$ T cell proliferation in an ex vivo autologous acinar cell-peripheral blood lymphocyte (PBL) mixed cell reaction and assesses their ability to induce experimental autoimmune dacryoadenitis. **Methods.** One inferior lacrimal gland was excised from each rabbit. Epithelial cells were purified, cultured for 2 d,

gamma irradiated, and then co-cultured for 5 d with autologous PBL. Cells from the mixed cell reactions were sorted by FACS analysis after staining with $CD4^+$ or $CD8^+$ antibodies. Separated $CD4^+$T cells were then injected into the remaining inferior lacrimal gland of the donor rabbit (auto-adoptive transfer). After 4 weeks, ocular surface status was assessed and the rabbits were sacrificed. Inferior and superior lacrimal glands were removed for analysis. **Results.** FACS analysis showed that the number of $CD4^+$ T cells increased significantly in the mixed cell reactions compared to non-stimulated control lymphocytes. Rabbits injected with $CD4^+$ T cells showed clinical signs of ocular surface disease. Inferior lacrimal gland showed increased lymphocytic infiltration, eosinophils within acinar lumens, and morphologically altered acini. **Conclusions.** The majority of lymphocytes proliferating in mixed cell reaction with autologous acinar cells are $CD4^+$ T cells. The auto-adoptive transfer of these $CD4^+$ T cells induces autoimmune dacryoadenitis resulting in dramatic histopathology in the inferior lacrimal gland and severe dry eye symptoms. These results suggest a strong involvement of $CD4^+$ T cells in the auto-adoptive transfer model of dacryoadenitis and dry eye.
Support: EY12689, EY05801, EY10550, EY03040 and grants from RPB and Allergan.

Direct Evaporation Measurements on Cornea and Bulbar Conjunctiva. John Tiffany, Aravinthan Varatharaj. Nuffield Laboratory of Ophthalmology, University of Oxford, Oxford UK

Purpose. Previous studies had suggested that the evaporation rate from the tear film over the conjunctiva was higher than from the cornea. Whole-eye measurements such as goggle methods would require some method of discriminating the relative areas, so a more direct technique was sought. We attempted to take direct readings from defined small areas on the surface of the open eye (cornea or bulbar conjunctiva) following a series of modifications to the ServoMed evaporimeter probe. **Methods.** In vitro tests made on a standard moist surface explored the effects of height of probe above the surface, and contribution from surrounding areas. The standard ServoMed probe was modified by replacement of the central 12mm "chimney" containing the humidity sensors by a longer 8mm chimney with the entry tip extended downwards, and placement of a small drying sachet of silica gel at the top of the chimney. Tests showed that acceptable readings were obtained from moist surfaces at any surface-tip distance in the range 0–20mm. A correction factor of 2.25 was used to give readings equivalent to the unmodified probe. The probe was mounted on a spectacle frame and readings taken on supine subjects over the cornea (direct upward gaze) and conjunctiva (with sideways gaze). Subjects were 12 normal undergraduates (7M, 5F, age range 20-23, mean 20.5). The mean of 2 readings was taken from both surfaces on the right eye only. **Results.** Corneal evaporation rate was 0.75 ± 0.19 _g/cm2/sec for cornea and 0.81 ± 0.18 _g/cm2/sec for conjunctiva (mean ± SD). The difference is significant at the level $p<0.005$ (Wilcoxon Signed-Rank test). Typically, comparing palpebral height of 8mm in downgaze with 15mm in upgaze, the total rate of evaporative loss is 250% greater in upgaze. **Conclusions.** This modification gives a simple direct measurement of evaporation rate from the eye, independent of room humidity, and would be easy to use in the clinical setting as one of several dry-eye tests. Measuring, it can discriminate between corneal and bulbar conjunctival rates from an 8mm spot, and shows the protective effect of downgaze eg. in computer-screen use.
[Support: none]

Evaporation Through Defined Surface Lipid Layers. John Tiffany, Naomi Lizinde. Nuffield Laboratory of Ophthalmology, University of Oxford, Oxford UK

Purpose. The meibomian lipid layer over the tear film helps to reduce evaporative loss from the open eye. Its thickness is inferred from its interference colours. The overall evaporation rate has been estimated by several methods (with considerable errors in estimating areas) but not related to the interference colours. **Methods.** Layers of oils were examined by surface spreading on Langmuir troughs at 34ºC. We mainly used the model substance "indicator oil" (used engine oil, which contains polar components generated by combustion, as well as non-polar oils), but also expressed meibomian oil from horse eyelids. This spreads to give layers of near-uniform thickness. The refractive index (RI) of oils was measured since it determines the relation of interference colours to thickness. Digitised images of spread layers of oil were quantified using a simple colour recognition scale developed using Adobe Photoshop. Evaporation through defined surface lipid layers was measured with the ServoMed evaporimeter probe placed directly over the trough under constant humidity of 35-37% and 50-53% RH. **Results.** RI for both indicator oil and horse meibomian closely resembled that of human meibomian (so the interference colour/thickness relationship is similar). Uniformly-coloured films were achieved with indicator oil on distilled water over the thickness range 50-600nm, in a black trough to eliminate internal reflections. Black (trough) and white (Teflon barriers) were taken as the limits 0 and 255 on a 256-step intensity scale, and RGB values determined as a function of thickness. The variation of evaporation rate with thickness was measured at several RH values for both oils. Curve-fitting gave exponential expressions, and a family of curves was obtained for different RH conditions. **Conclusions**. These experiments establish the colour/thickness and the evaporation/thickness relationships; hence the rate of evaporative loss can be deduced from interference colours in vivo. The RGB technique makes it possible to create defined oil thicknesses on the Langmuir trough from collected lipid samples even when too small to determine by weighing.
[Support: none]

Punctal Plugs For Patients With Post-Lasik Dry Eye. Ikuko Toda[1], Chikako Sakai[1], Takahiro Yamamoto[1], Yoshiko Hori-Komai[1], Kazuo Tsubota[2]. Minamiaoyama Eye Clinic[1] and Keio University, School of Medicine[2], Tokyo, Japan.

Purpose. Post-LASIK dry eye sometimes affects visual performance, such as conventional and/or functional visual acuity. We evaluated the efficacy of punctal plugs on visual performance in patients with severe post-LASIK dry eye. **Methods.** Twelve eyes in 9 patients were inserted puctal plugs in upper and lower puncta at 1 month after LASIK. All eyes had chronic symptoms of dryness and foreign body sensation, unstable vision and refraction, fluorescein score > 2 and tear break-up time (BUT)< 5 sec. Uncorrected (UCVA) and best corrected visual acuity (BCVA), fluorescein score, BUT, and symptoms were compared before and 1 month after the plug insertion. The change of visual acuity (functional visual acuity: FVA), surface regulatory index (SRI) and surface irregularity index (SAI) in topography immediately and after 10 seconds of prolonged eye opening were compared before and after plug insertion. **Results.** UCVA and BCVA were significantly improved after the plug insertion. Fluorescein score, BUT and dry eye symptoms were significantly improved. FVA was increased more than 2 lines in 8 eyes, no change in 2 eyes, and decreased in 2 eyes due to too much lacrimation. The change of SAI and SRI between 0 sec and 10 sec of eye opening was decreased (improved) in 9 and 9 eyes, unchanged in 1 and 0 eyes, and increased (worsen) in 2 and 3 eyes, respectively.

Conclusion. Punctal plugs are effective to improve symptoms and visual performance in some patients with severe post-LASIK dry eye, which lasts long and affects patient satisfaction for the LASIK outcome.

Objective Tests in the Differential Diagnosis of Dry Eye. Alan Tomlinson[1], Santosh Khanal[1], Angus McFadyen[2], Charles Diaper[3]. [1&2]Vision Sciences and Mathematics, Glasgow Caledonian University, Glasgow, [3]The University Hospital Trust, Southern General Hospital, Glasgow, Dept of Vision Sciences_, Cowcaddens Road_, Glasgow_, G4 0BA_

Purpose. Determination of the most effective objective tests, applied singly or in combination in the diagnosis of dry eye disease and blepharitis. **Methods**. 92 subjects, 41 with dry eye and 19 with blepharitis (by ophthalmological diagnosis) and 32 subjects with no ocular surface disease, were assessed for symptoms, tear film quality, evaporation and tear turnover (TTR), volume and osmolarity and meibomian gland dysfunction (MGD). Dry eye subjects had significantly different TTR, tear evaporation and osmolarity than healthy normals. Cut-off values between the groups were determined from distribution curves for each aspect of tear physiology and the effectiveness of the cut-off determined from the receiver operating characteristic ROC curves. The differential diagnoses yielded by the objective tear tests, (singly and in combination) compared to ophthalmological classification, were determined. **Results**. Values of 12%/min for TTR, 33g/m^{-2}/h for evaporation and 317mOsm/l for osmolarity were found to give sensitivities and specificities and overall accuracies of 80%, 72% and 77% (TTR); 51%, 96% and 67% (evaporation); and 78%, 78% and 79% (osmolarity) when applied singly as diagnostic criteria in dry eye. In combination they yielded sensitivities, specificities and overall accuracy of 100%, 66% and 100% (in parallel) and 38%, 100% and 63% (in series). Discriminant function analysis incorporating these three factors allowed diagnosis with a sensitivity of 93%, specificity at 88% and overall accuracy of 89%. The latter result compared favourably with the efficacy of other diagnostic tests reported in the literature. **Conclusions.** A battery of tests employing a weighted comparison of results from tear turnover, evaporation and osmolarity measurements derive from discriminant function analysis was found to be the most effective in differentiating and diagnosing dry eye.
(Alan Tomlinson has commercial relationships with Allergan and Pfizer; there was no grant support for this research study.)

Reactive Oxygen Species Can Be Controlled By The Secretory Glycoprotein, Clusterin, From Side Population Cells In The Lacrimal Gland: A New Intervention For Age-Related Dry Eye Disorders. Kazuo Tsubota,[1] Kenji Mishima,[2] Kumi Obara, [2] Hiroyuki Yamada,[2] Hiroko Inoue, [2] Ichiro Saito.[2] Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan[1] Department of Pathology, Tsurumi University School of Dental Medicine, Yokohama, Japan[2]

Purpose. This study was performed to determine the function of the secretory glycoprotein, clusterin, a side population (SP) cell-specific gene and its role in restoring secretory function of the lacrimal gland (LG). **Methods.** SP cells were purified from the LG of heterozygous enhanced green fluorescent protein (EGFP)-transgenic (GFP-Tg) mice of a C57BL/6 background. Two weeks following irradiation, the SP cells were injected with a microinjector directly into the LG of each experimental mouse. Eight weeks after the injection the mice were sacrificed for analysis and the engrafted cells were identified by the presence of GFP. The engrafted cells were then transplanted into mice with irradiation-induced hypofunction in the LG. Using the fluorescent indicator dichlorofluorescein diacetate, intracellular levels of ROS were assayed. **Results.** Two months after the transplantation, secretions from the LG in the recipient mice were restored; however, since no outgrowths were produced and the transplanted cells were only sparsely distributed, it is likely that soluble factors secreted by the SP cells, and not reconstituted cells, restored the functions of the residual glands. We found that clusterin is the SP cell-specific secretory glycoprotein, and it can rescue cell death through the suppression of ROS accumulation induced by irradiation or oxidative stress. Our results indicate that the advantages of SP cell transplantation are attributable to the functions of the SP cell-derived soluble factor, clusterin, but not to the cell transplantation-mediated reconstitution of the glands. **Conclusions**. We propose that SP cells in each organ might also have specific factors that prevent cell stress, including oxidative stress. Here we provide initial evidence suggesting the possibility of clinical application of the SP cell-related factor, clusterin, to treat oxidative-stress related aging diseases, including age-related dry eye disorders. *[K. Tsubota and I. Saito have applied for a Japanese patent.]*

Dry Eye after Hematopoieic Stem Cell Transplantation. Miki Uchino[1], Yoko Ogawa[1], Yuichi Uchino[1], Takehiko Mori[2], Shinichiro Okamoto[2], Kazuo Tsubota.[1] Department of Ophthalmology[1]Keio Bone Marrow Transplant Program, Division of Hematology, Department of Internal Medicine[2], Keio University, School of Medicine, Tokyo, Japan.

Purpose. To compare the incidence and clinical course of dry eye (DE) after allogeneic hematopoietic stem cell transplantation (HSCT) according to the stem cell sources. **Methods.** At a tertiary care hospital, 99 patients undergoing allogeneic HSCT followed at least 180 days were studied prospectively. Examination include corneal vital staining with rose bengal and fluorescein, tear film break up time, and Schirmer test. We analyzed the incidence of DE and the relationship with systemic graft-versus-host disease (GVHD). **Results.** Of the 99, 42 patients (42.4%) developed DE or their pre-existing DE worsened after HSCT. The incidence of DE between groups was 31 patients (46.3%) in bone marrow transplant (BMT) group, 8 patients (44.0%) in peripheral blood stem cell transplants (PBSCT) group, and 3 patients (21.4%) in umbilical cord blood transplantation (CBSCT) group (p=0.57). The mean onset time of DE was 287.8±369.4 days in BMT group, 474.1±472.0 days in PBSCT group, and 168.3 ± 209.7 days in CBSCT group (p=0.23). Severe dry eye (S-DE) was observed 12 patients (38.7%) in BMT group, 7 patients (87.5%) in PBSCT group, and 1 patient (33.3%) in CBSCT group. **Conclusions.** Depend on the stem cell sources, the severity and the onset time of DE were different. For the future study, more large number of patients and longer follow up are necessary.
This work was supported by a grant from the Japanese Ministry of Education, Science, Sports and Culture #18591932.

Prevalence of Dry Eye Syndrome among Japanese VDT Users. Yuichi Uchino[1,2], Miki Uchino[1,2], Murat Dogru[1], Kazumi Fukagawa[1,2], Shigeto Shimmura[1,2],Toru Takebayashi[3], Debra A. Schaumberg[4], Kazuo Tsubota[1,2] Department of Ophthalmology, Keio University School of Medicine,[1] Ryogoku Eye Clinic,[2] Department of Public Health, Keio University School of Medicine, [3] Tokyo, Japan, Division of Preventive Medicine, Brigham and Women's Hospital, and the Schepens Eye Research Institute, Harvard Medical School[4], Boston, MA, USA

Purpose. To determine the prevalence of dry eye syndrome (DES) and risk factors among Japanese office workers using visual display terminals (VDT). **Methods.** We carried out a cross-sectional survey in 4,393 Japanese office workers using VDT Office workers completed questionnaires designed to ascertain a prior diagnosis of DES, current symptoms of DES, as well as information on possible risk factors such as age, duration of VDT use, type of VDT work, environmental factors, presence of systemic diseases, and contact lens (CL) use. We used logistic regression analysis to examine the associations between DES and other factors. **Results.** Of the 4,393 office workers, 3,549 (80.1%) completed the questionnaire. Clinically diagnosed DES was present in 266 male subjects (10.1%) and 195 female subjects (21.5%). Severe symptoms of DES were observed in 711 male (26.9%) and 436 female participants (48.0%). Combining the men and women, VDT use over 4 hours was associated with an increased risk of DES (OR= 1.50, 95% CI=1.11-2.03). In addition, contact lens use (OR= 3.48, 95% CI= 2.74-4.43) increased the risk of severe dry eye symptomatology, and smoking was associated with a higher risk of diagnosed DES among women (OR= 1.25, 95% CI= 0.83-2.18). **Conclusion.** Dry eye syndrome leading to a clinical diagnosis or severe symptoms is prevalent among Japanese office workers using VDT. The condition is more prevalent among females, CL wearers, prolonged VDT users, and among cigarette smokers. Relevant measures directed against these risks could provide a positive impact on public health and quality of life of office workers.
This work was supported by a grant from the Japanese Ministry of Health, Labour and Welfare # H17-025.

Tear Function And Ocular Surface After Muller Muscle-Conjunctival Resection. Suat Hayri Ugurbas[1,] Atilla Alpay[1], Burak Bahadır[2], Zonguldak Karaelmas University, Faculty of Medicine, Department of Ophthalmology1 and Department of Pathology2, Zonguldak, Turkey.

Purpose. Müller muscle- conjunctival resection is a precise technique to correct mild and moderate ptosis cases. On the other hand, resection of conjunctiva during the surgery may lead some problems in tear function. In this study, tear function tests and ocular surface are evaluated in patients who underwent unilateral surgery. **Methods**. A dry eye assessment questionnaire, Schirmer's test, tear film break-up time, fluorescein stain, rose bengal stain, and conjunctival impression cytology were used to assess the tear film functions and ocular surface changes in the operated and non- operated eyes of 16 patients with normal preoperative tear function tests. **Results.** There was no statistically significant difference in the tear function tests and goblet cell densities between the operated and non operated eye of the patients. **Conclusion.** The results of this study indicate that, the Müller muscle-conjunctival resection procedure has no apparent effect on tear function tests and goblet cell density in patients with normal preoperative tear function.

Dry Eye Disease In The Young: Relevance Of Corneal Dyrstrophies In The Differential Diagnosis. Gysbert van Setten, Sankt Eriks Eye Hospital, Karolinska Insitutet, Stockholm, Sweden.

Purpose. Evaluate the clinical importance of corneal dystrophies in the patients admitted for differential diagnosis of dry eye disease. **Method.** Over a three year period of time patients admitted for the clinical differential evaluation of dry eye disease were screened for the presence of corneal changes possibly mimicking dry eye disease. Patients detected were followed up and evaluated. **Results.** Amongst all corneal dystrophies observed only microcystic changes could constitute a challenge for the differential diagnosis of dry eye disease. Clinically significant complaints and findings were observed two patients, both females, 9 and 25 years of age. In both patients the clinical picture resembled that of severe dry eye disease when low magnification was used in the clinical examination. Higher magnification easily revealed the difference. Initially intensive treatment with SVV (surface viscous vehicles) and steroids was required to alleviate the significant clinical symptoms. It was observed that even with following constant low dose application of SVV, recidives of intense pain and symptoms did occur, although they became less frequent over time of treatment. Corneal neovascularisation is however, a future threat for one of the four eyes. **Conclusion.** Although the clinical symptoms do not differ initially from the classical dry eye disease, chronic epithelial abnormality may cause secondary complications such as neovascularisation and may give a strong rational for a different therapy. Although itself a rare corneal disorder the congenital nature of corneal abnormalities must kept in mind when young patients are admitted for dry eye disease.
[The author is very thankful to the Synfrämjandets Forskningsfond, Stockholm, Sweden för financial support.]

A Four Week Therapy With Systane Improves Ocular Surface Parameters In Dry Eye Patients. Piera Versura, Vincenzo Profazio, Emilio C Campos Dept Ophthalmology Alma Mater Studiorum University of Bologna, Italy. piera.versura@unibo.it

Purpose. Systane® Lubricating Eye Drops (Alcon Forth Worth, Inc.) contains PEG 400/PG and the gelling agent hydroxypropyl-Guar (HP-Guar). Systane is designed to provide dry eye relief by means of longer retention time on the ocular surface that may promote epithelial repair. We evaluated Systane in an open trial in patients complaining of moderate dry eye symptoms. **Methods**. A total of 50 subjects were enrolled (age 57.6 $\pm$ 15.4; 40 women, 10 men) ; inclusion criteria were based on a tear break-up time (TBUT) < 10 sec without corneal epithelial staining. Six symptoms of ocular irritation were rated on a four-point scale. Conjunctival injection was graded. Schirmer test, conjunctival imprint cytology, inflammation score given by scraping cytology, tear protein content (serum albumin exudated in tears, lysozyme, lactoferrin, lipophillin, lipocalins) were also evaluated in both eyes. Patients were instructed to instill Systane four times a day for four weeks. Satisfaction with the product was rated at the end of the study. Media of results in both eyes was considered for statistical evaluation; the Wilcoxon test for paired data was applied, $p< 0,05$ was considered as statistically significant. **Results.** All patients regularly instilled Systane and completed the study; results are expressed baseline vs endpoint. Significant reduction was demonstrated for ocular irritation symptom scores (1.44 vs 0.94, p 0.0001) and exudated serum albumin in tears (0.268 $\pm$ 0.035 vs 0.198 $\pm$ 0.075 mg/ml, p 0,03), while improvement was shown for patient's satisfaction score (3.41 vs 3.94, p 0.0001), TBUT (6.9 $\pm$ 0.9 vs 8.5 $\pm$ 1.5 sec, p 0.0001), tear lipocalins (9.4 $\pm$ 2 vs 11.3 + 2.3, results in %, p 0.03). A significantly increased proportion of

patients with normal conjunctival injection (10 vs 22, results in %, p 0.01) was observed after one month follow up. Tear production, conjunctival cytology dryness, conjunctival inflammation score, tear protein content showed no significant change. **Conclusions.** Systane proved effective in reducing the symptoms of dry eye and it was overall well accepted. This finding is likely to be related with improved stability of tear film associated to tear lipocalin increase. *Research supported in part by Alcon.*

Corneal Involvement In Rheumatoid Arthritis: An *In Vivo* Confocal Study. Edoardo Villani, Daniela Galimberti, Francesco Viola, Chiara Mapelli, Roberto Ratiglia. Eye Clinic University of Milan; Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Fondazione IRCCS, Milan, Italy

Purpose. To analyze the in vivo morphology of corneal cells and nerves in rheumatoid arthritis (RA) patients with or without Sjögren's syndrome (SS), and to investigate the correlations between corneal alterations and RA activity. **Methods.** Fifty patients with RA and 30 age- and gender matched control subjects (C) were studied. SS was diagnosed according to the American-European Consensus Group criteria and RA activity was evaluated by the Lansbury index (LI). Each participant completed a questionnaire for a standardized evaluation of dry eye symptomatology (Ocular Surface Disease Index) and underwent an ophthalmologic examination (including Schirmer test, corneal sensitivity, fluorescein staining and tear break-up time) and a confocal microscopic examination (to investigate corneal and stromal thicknesses, cell density of different corneal layers, number of "activated keratocytes" and sub-basal nerves number, tortuosity, reflectivity and "bead-like formations"). **Results.** 16% of RA patients (8/50) were diagnosed with secondary SS. No statistically significant difference was found in LI and in clinical and confocal parameters between SS and non-SS patients. Significant differences were found between RA patients and C for all the parameters studied, reflectivity excepted. LI showed a significant correlation with the number of "bead-like formations" and the number of "activated keratocytes"; these correlations were found in both the RA with SS ($P<0.01$ and $P<0.05$ respectively) and the RA non-SS groups ($P<0.05$ and $P<0.05$ respectively). **Conclusions.** In RA patients, confocal microscopy showed several changes in corneal cells and nerves. The number of "bead-like formations" and the number of "activated keratocytes" could be interpreted as confocal signs of ocular surface disease activity; their correlation with an index of systemic disease activity (LI), both in RA with SS and in RA without SS patients, could be an interesting element to better understand the pathogenetic mechanisms of dry eye in RA patients. *[Disclosure all authors: none]*

Histopathological Alterations In Senescent Cu, Zn–Superoxide Dismutase-1 (SOD–1)–Knock-Out Mice: A New Model For Dry Eye. Tais Hitomi Wakamatsu[1], M. Dogru[1,2], Y. Sasaki[1], S. Ward[1], Y. Imamura[1], Y. Ogawa[1], A. Igarashi[2], T. Shimizu[3], T. Shirasawa[3], J. Shimazaki[2], K. Tsubota[1]. Ophthalmology Department, Keio University, Tokyo, Japan[1], Ophthalmology Department, Tokyo Dental College, Ichikawa, Japan[2], Gerontology, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan[3].

Purpose.: The purpose of our study was to determine the histopathological alterations in the lacrimal gland of Copper/zinc superoxide dismutase (*Sod*1)–knock-out (–/–) mice as a dry eye model. **Methods.** Tear function tests (BUT and cotton thread), corneal sensitivity and corneal fluorescein staining tests were performed on *Sod*1 –/– mice (n=4) aged 18 ~ 23 weeks (average age= 20 weeks) and wide type (+/+) mice (n=5) aged 18 ~ 22 weeks (average age= 20 weeks). After a follow up for 44 weeks, the mice were sacrificed and the lacrimal glands were collected for histopathology analyses and immunohistochemistry for inflammatory and oxidative stress markers. Haematoxylin and Eosin, Periodic Acid Schiff and Mallory staining were performed. The study was conducted in compliance with the ARVO statement for the use of animals in Ophtahlmic and Visual Research. **Results.** Tear quantity values in *Sod*1 -/- mice were lower compared to the *Sod*1 +/+ mice detected by the cotton thread test throughout the study. The mean BUT values of the *Sod*1 -/- mice were also consistently lower compared with *Sod*1 +/+ mice throughout the follow up. Fluorescein staining scores were higher in the *Sod*1 -/- mice compared to the wild type mice. Histopathological analysis showed the hallmarks of lacrimal gland inflammation: presence of intense lymphocytic infiltration, periductal fibrosis and periacinar CD45 positivity in the lacrimal glands of *Sod*1 -/- mice. Immunohistochemistry using 4-Hydroxy-2-nonenal (4-HNE) monoclonal antibody showed prominent positive staining in the lacrimal glands of *Sod*1 -/- mice. **Conclusion.** Although the S*od*1 -/- mouse is known as a model mouse of aging, abnormal quantitative and qualitative findings observed in relation to the tear functions, ocular surface and lacrimal gland histopathology suggest that it can also serve as a mouse model of dry eyes.

Diurnal Variation Of Visual Function And Corneal Keratitis In Patients With Dry Eye. [1]Pamela M. Walker, [1]George W. Ousler III, [1]Michael Schindelar, [1]Donna Welch, [1,2,3]Mark B. Abelson. ORA Clinical Research and Development, North Andover, MA[1], Schepens Eye Research Institute, Boston, MA[2]; Harvard Medical School, Boston, MA[3].

Purpose. Dry eye patients often complain of disturbances in visual function and worsening of dry eye signs and symptoms in the evening. To better understand these reports of diurnal variations, the present study tested dry eye patients on a series of visual function and ocular physiology measures. **Methods.** Twenty-one dry eye patients underwent ophthalmic examinations including BCVA, visual function decay as measured by the interblink-interval visual acuity decay (IVAD) test without ocular anesthetic, reading rate test, slit lamp biomicroscopy, TFBUT, corneal keratitis, conjunctival redness and corneal sensitivity assessments once during the morning and for a second time in the evening. Patients also completed a modified version of the Ocular Surface Disease Index (OSDI) at both study visits. **Results.** Dry eye patients showed impaired visual function in the evening as compared to the morning condition; they maintained their BCVA for a shorter time between blinks ($p<.01$), and had longer readings rates ($p<.05$) in the evening. Patients also demonstrated an increase in corneal keratitis and conjunctival redness from morning to evening testing. Less ocular discomfort was reported in the evening than in the morning, but greater subjective visual impairment was reported in the evening. **Conclusions.** The present data show robust diurnal variations of visual function and ocular surface physiology in dry eye patients. These findings directly demonstrate the role of time-of-day in fluctuations of dry eye patients' ocular signs, symptoms and visual function abilities.

Tear Distribution On Ocular Surface Imaged With Ultra-High Resolution Optical Coherence Tomography. Jianhua Wang, Shuliang Jiao, Jayachandra Palakuru. Bascom Palmer Eye Institute, University of Miami, Miami, FL, USA

Purpose. To develop an ultra-high resolution optical coherence tomography (OCT) for imaging the tear distribution on ocular surface. **Methods.** A custom ultra-high resolution (3 μm) spectral domain OCT was developed and connected with a telecentric light delivery system for imaging anterior segment of the eye. Very broad bandwidth (100nm) with a center wavelength of 840 nm light source was used. The scan width was up to 15 mm with the scan depth of 3 mm. The speed of the system was set up to 48 frames per second. Eyes were imaged before and after instillation of artificial tears (Optive, Allergan). These eyes were also imaged with different types of contact lenses (PureVision, Bausch & Lomb, Acuvue Oasys, Vistakon). Two dimensional images of the central cornea and contact lens edge were obtained. **Results.** The epithelium (including the basal cell layer) and Bowman's layers were also visualized. Vertically scanned images showed thinner tear film at the upper cornea than the lower cornea after instillation of the tears. From the central cornea with a contact lens, pre- and post- tear films were clearly visualized immediately after lens insertion. The edge configurations of different types of lenses and tear meniscus around the lens edge were shown. Pre- and post-lens tear films at the lens edge area were seen after instillation of artificial tears. **Conclusions.** We have demonstrated the feasibility of using ultra-high resolution spectral domain OCT for imaging the tear distribution on ocular surface and contact lenses. It appears that ultra-high resolution OCT is a powerful tool for studying the tear system. Further studies on tear dynamics and dry eye could be done using this tool.
This study was supported by research grants from NEI (R03 EY016420) and an unrestricted grant from Research to Prevent Blindness (RPB). The authors have no proprietary interest in any materials or methods described in this abstract.

Implementation Of A New Questionnaire Into The Recently Revised Japanese Dry Eye Diagnostic Criteria. Samantha Ward,[1] M. Dogru, [1] T. Wakamatsu, [1,2] O. Ebrahim, [1] M. Kaido,[2] Y. Matsumoto, [2] N. Yokoi, [3] M. Ueda, [4] A. Tsuyama, [4] K. Tsubota[2] Keio Univ, J&J OSVO,[1] Dept of Ophthalmology[2] Kyoto Prefectural Univ of Medicine[3] Japanese Preventive Medicine Society, Tokyo, Japan[4]

Purpose. To evaluate the implementation of a new symptom questionnaire into the recently revised Japanese Dry Eye Diagnostic Criteria and to investigate the changes in dry eye diagnosis using the revised diagnostic criteria. **Methods.** Subjects seen for general and dry eye examination filled out a questionnaire with questions pertaining to alterations in visual function, ocular symptoms, and environmental triggers often associated with dry eye. Answers, with the corresponding point score included: always (4); frequently (3); occasionally (2); rarely (1) and never (0). A Dry Eye Severity Score (DESS) was given based on the score total. From the results of the Schirmer I test (ST), BUT evaluation, and fluorescein (F) and Rose Bengal (RB) staining, patients were diagnosed with Definite Dry Eye (DDE), Probable Dry Eye (PDE), or No Dry Eye, using both the old and revised diagnostic criteria. Under the old criteria, DDE was defined as abnormal tear function (ST value ≤ 5 mm or BUT ≤ 5 s), in addition to corneal staining (F or RB score ≥ 1 pt) and PDE was defined as the presence of either abnormal tear function or corneal staining. Under the new criteria, a diagnosis of DDE required the presence of symptoms and to meet the corneal staining requirement, ≥ 3 pts was needed. **Results.** A higher DESS was seen in subjects with DDE compared to those with PDE. In both the DDE and PDE groups, points obtained from the questions pertaining to ocular symptoms contributed most significantly to the overall DESS. Due to the more stringent corneal staining requirement, many diagnosed with DDE under the old criteria were diagnosed with PDE under the new criteria. **Conclusions.** The DESS obtained from the new symptom questionnaire was found to correspond well to the dry eye diagnosis and was useful in elucidating which symptoms are prominent in dry eye subjects. The number of patients diagnosed with DDE is expected to drop under the new diagnostic criteria.

Epithelial Damage Of The Conjunctiva_In Sjogren Syndrome Is Not Restored By Standalization Of Tear Volume. Hitoshi Watanabe, MD[1,2] Takeshi Soma, MD[1,], Shizuka Koh[1,] MD**,** Koji Nishida, MD[1,] , Naoyuki Maeda, MD[1], Osaka University Medical School[1], Osaka, Japan, and Kansai Rosai Hospital[2,] , Hyogo, Japan

Purpose. The damage of the conjunctival epithelium as well as the corneal epithelium by dry eye is restored by plug treatment. However, we noticed that there was a difference on the impairment of the damage of the conjunctival epithelium after plug treatment between Sjogren and non- Sjogren dry eye. To evaluate the difference, we examined the damage of the corneal and conjunctival epithelium of Sjogren and non-Sjogren dry eye patients before and after plug treatment. **Methods.** Twenty-four eyes of 24 patients with Sjogren dry eye and Thirty-six eyes of 36 patients with non- Sjogren dry eye were enrolled. Schirmer I test was examined in each eye and fluorescein staining score was evaluated in the corneal and conjunctival epithelium before and after punctual plug treatment. **Results.** After treatment, the values on Schirmer I test significantly increased 3.8±1.3 in Sjogren patients and 4.6±1.9 in non-Sjogren dry eye respectively (Wilcoxon test; P<0.001 each)., but there is no significant difference between two groups. (Mann-whitney Rank Sum Test;) . By the increase of the tear volume, the damage of the corneal epithelium was significantly decreased either in Sjogren (from 5.4±1.8 to 1.1±1.7) and non-Sjogren dry eye (from 5.0±2.6 to 1.2±0.9) (Wilcoxon Test, P<0.001, respectively) and there is no significantly difference between two groups. The conjunctival epithelium was significantly decreased in non-Sjogren dry eye (from 5.0 ±1.6 to 1.2±0.9) but not so much in Sjogrendry eye (from 5.6 ±0.3 to 4.8 ±0.9). There existed a significantly difference on FL score after treatment between Sjogren and non- Sjogren dry eye (Mann-Whitney Rank Sum Test; p<0.001) although there was no statistically difference before treatment **Conclusion.** The damage of the conjunctival epithelium is well restored in non- Sjogren dry eye, but not so much improved in Sjogren syndrome. This suggests that the damage of the conjunctival epithelium in Sjogren syndrome may be caused by not only tear abnormality but other reasons such as some inflammation.

Protein And Lipid Deposits On Lenses Are Affected By Lens Material And Solutions, And Associated With Clinical Performance. M. Willcox, T. Naduvilath, N. Carnt, Z. Zhao. Institute for Eye Research, University of New South Wales, Sydney, 2052, Australia.

Purpose. The aim of the current investigation was to compare the amount of protein and cholesterol bound to silicone hydrogel contact lenses and to find correlations between deposition and clinical or subjective variables.
Methods. Data from non-randomized clinical studies were analysed retrospectively. Subjects wore various commercially available lenses bilaterally on a daily wear schedule for 3 months with overnight disinfection. Lenses were analysed for levels of total protein or cholesterol adsorbed to lenses using standard extraction and

biochemical techniques. The amount of protein/lipid was analysed to test for lens-solution differences. The varying lens types, solutions and their interactions were tested for association with log transformed protein and lipid levels using linear mixed model. The association of lens age, clinical grades and subjective ratings were also tested for significance. Level of significance was set at 5%. **Results.** Balafilcon A lenses adsorbed more protein on their surface compared to other lens types,whereas both balafilcon A and galyfilcon A lenses adsorbed the most cholesterol. The effect of solution type was different depending on lens material type. The amount of protein/lipid adsorbed correlated with increasing lens age on eye (p=0.002). Higher concentrations of protein was associated with higher grades of front surface deposits (p=0.001), haziness (p=0.015) and mucin balls (p=0.022). Higher concentration of cholesterol was associated with higher grades of front surface deposits (p=0.006) and corneal staining (p=0.039), but associated with lower grades of bulbar redness (p=0.009). There was no correlation between protein/lipid levels and any subjective symptoms (i.e. comfort, dryness). **Conclusions.** The contact lens material has a major affect on amount of protein and lipid that can be removed from lenses after daily wear. The levels of protein or lipids are correlated with a number of clinical variables.
[Support: the Australian Federal Government through the CRC Scheme, and CIBA Vision]

Increased Expression Of The Autoimmune-Related Genes, FGG And PADI2, In Nod Mouse Lacrimal Glands Relative To Balb/C Mouse Lacrimal Glands Characterized By Cdna Microarray And Real-Time PCR. Kaijin Wu1, Xiaodong Li[1], Michelle MacVeigh[2], Sarah F. Hamm-Alvarez[1]; [1]Department of Pharmacology and Pharmaceutical Sciences, and [2]Center for Liver Disease, University of Southern California, Los Angeles CA, USA

Purpose. To characterize changes in gene expression which may contribute to the development of Sjögren's syndrome (SS)-like disease in lacrimal glands (LG) of NOD mice. **Methods.** cDNA microarray was utilized to identify genes of interest and the resulting data were validated and extended by real-time PCR and confocal and electron microscopy (EM). **Results.** Microarray analysis revealed elevated expressions of numerous genes associated with autoimmunity in LGs from NOD male mice aged 12 weeks compared to gender- and age-matched BALB/c controls. Among these, fibrinogen gamma (FGG) and peptidyl arginine deaminase type II (PADI2) were of special interest as they have been implicated in rheumatoid arthritis and/or multiple sclerosis. Expression levels of these two genes in NOD LGs aged 4 and 12 weeks were analyzed relative to paired samples from BALB/c mice by real-time PCR. The results showed that FGG mRNA levels in NOD LGs of male and female at 12 weeks were 994-fold and 4767-fold respectively as high as in the BALB/c controls. FGG mRNA levels in NOD LGs of male and female at 4 weeks were 80-fold and 59-fold respectively as high as in the BALB/c controls. Real-time PCR also showed consistently higher PADI2 mRNA levels of 8-20 folds in the NOD LGs of both genders compared to the BALB/c controls in the two age groups. Preliminary immunofluorescence microscopy of FGG verified the increased expression of protein in NOD LGs relative to BALB/c LGs, and suggested accumulation in the endoplasmic reticulum (ER) within acinar cells. EM data confirmed a swollen and distended ER in NOD LGs of both genders, suggesting an association between altered FGG expression and inappropriate ER processing and function. **Conclusion.** Our study suggests that FGG and PADI2 participate in the development of SS- like autoimmunity in the NOD mouse model.
Support. NIH EY011386 and EY016293
No commercial relation for the research presented.

The Proinflammatory Response Of Human Corneal Epithelial Cells To Toxigenic *Staphylococcus Aureus*. Ai Yamada, Susan R. Heimer, Michael S. Gilmore. Schepens Eye Research Institute, Harvard Medical School, Boston, MA, USA.

Purpose. *Staphylococcus aureus* is a major cause of bacterial keratitis. The pathology results from a combination of bacterial toxins and the host immune response. Most of these toxins are modulated by global regulators Agr and Sar. Little is known regarding the global effects of *S. aureus* and its toxins on human corneal epithelial cell (hCE) in the early phases of infections. The objectives of our study are (1) To identify changes in gene expression of hCE in response to *S. aureus*; (2) To identify changes in gene expression of hCE that are attributable to *S. aureus* toxin production; and (3) To compare the hCE response to *S. aureus* infections with other types of epithelium. **Methods.** *S. aureus* RN6390 and an isogenic Agr/Sar mutant ALC135 were incubated with confluent monolayers of hCE (primary and Araki-Sasaki) in serum-free defined keratinocyte medium for 6 h. RNA was extracted from Araki-Sasaki cells and cDNA quantification was assessed by Affymetrix Gene Chip Human Genome U133 Plus 2.0 Array. Culture supernatants were analyzed for cytokine production. **Results. (**1) 515 genes were up-regulated, and 78 genes were down-regulated in hCE infected with RN6390. Principle changes were up-regulation of inflammatory molecules such as CCL20, IL8, CSF2, IL6, and TNF_. Secretion of these molecules were increased in the supernatant cytokine assay in both primary and Araki-Sasaki cells. Signaling molecules such as HSP70B, SerpinB2, EGR4, and TNF_AIP3 were up-regulated. (2) 37 genes were significantly differently expressed when hCE were exposed to toxigenic *S. aureus* as opposed to non-toxigenic *S. aureus*. The majority of these genes were heat shock protein-related. (3) The responses of hCEs to *S. aureus* were broadly similar to the responses seen when vaginal epithelial cells were exposed. **Conclusions.** The principle early response of hCEs to *S aureus* is the induction of pro-inflammatory and related-signaling molecules. Another component of the hCEs early response is up-regulation of stress-response, which is effected by *S. aureus* toxins regulated by Agr/Sar.
Acknowledgments: NEI #017381-01A1 and Japanese Eye Bank Society

A Case Of Severe Meibomitis During The Toxic Epidermal Necrolysis, Stevens-Johnson Syndrome. Hiroko Yamagami,[1] Akihiro Kakehashi,[1] Kozue Ishizaki,[1] Fumihiko Toyoda,[1] Chiho Mameuda,[1] Maki Kakurai,[2] Toshio Demitsu,[2] [1]Department of Ophthalmology, Jichi Medical University, Omiya Medical Center, [2]Department of Dermatology, Jichi Medical University, Omiya Medical Center, Omiya, Saitama, Japan

Toxic epidermal necrolysis (TEN) and Stevens-Johnson syndrome (SJS) are drug-induced, severe acute exfoliative skin and mucosal disorders, and many associated ocular disorders have been reported. We report a patient with severe meibomitis after TEN.

A 55-year-old woman received indomethacin to treat a common cold. The next day, she developed conjunctivitis, photosensitivities, and skin eruptions. The conjunctivitis caused severe conjunctival erosion. The eruptions developed into bulla, erosions, and ulcers. She was diagnosed with TEN. Necrotic keratinocytes were found at all epidermal levels in fully evolved lesions by skin biopsy.

Systemic methylprednisolone pulse therapy was ineffective. She received therapeutic plasmapheresis with total plasma exchange using fresh frozen plasma four times. The conjunctivitis was treated with topical steroids and antibiotics. The conjunctival erosions healed with a complication of symblepharons within 1 month.

After recovery of the skin erosions and ulcers (about 1 month after onset), the meibomian glands became whitish and nodular changes

developed in both lower eyelids. Two months later, the upper lid margins also became inflamed. Two and half months after onset, the meibomitis peaked, and the nodular changes were maximal. The lower lid margins appeared scallop-shaped and improved gradually. The lid margins appeared flat after 6 months. The Schirmer test showed decreased lacrimal secretion of 4 mm bilaterally and she had dry eye and dry mouth. Corneal superficial punctate keratopathy and symblepharons were present.

We managed the case of TEN with severe ocular disorders from the acute phase. The meibomitis appeared during the late phase. The skin was treated successfully but the mucosal dysfunctions still exist and need treatments over the long term.

A New Surgical Punctal Occlusion Using Fibrous Tissue Under Lacrimal Caruncle. Norihiko Yokoi, Hidemi Chihara, Masakazu Nishii, Aoi Komuro, Takasumi Shimamoto, Shigeru Kinoshita. Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan.

Purpose. Surgical punctal occlusion is indispensable to the severe aqueous-deficient dry eye cases where there is no indication for punctal plugs. However, there are in fact some cases in which obtaining a complete and permanent punctal occlusion is difficult. We attribute the postoperative recanalization of the closed punctum to the negative pressure exerted on the punctum during postoperative blinking. To overcome this recanalization of the closed punctum, we present a new surgical punctal occlusion technique consisting of new procedures for obtaining complete and permanent punctal occlusion. **Methods.** We performed new surgical procedures which include the use of gentle diathermy and a drill to remove epithelium from the lacrimal canaliculs. Next, fibrous tissue was obtained from under the lacrimal caruncle, placed in the canaliculs just below the punctum, and anchored with nylon sutures positioned to close the punctum tightly. This operation was performed on 18 puncta of 18 eyes of 11 dry eye subjects with repeated recanalization or enlarged puncta due to repeated punctal plug insertion [mean age: 64.7 yrs.; 6 cases of Sjögren syndrome (SS), 1 case of ocular cicatricial pemphigoid, 3 cases of Non-SS, and 1 case of graft-versus-host disease]. **Results.** During the postoperative follow-up periods (mean 4.0 months), complete punctal occlusion with no recanalization was confirmed in all cases, and repeated corneal filaments were resolved in all 3 cases in which they existed. In 8 cases, a significant improvement of superficial punctate keratopathy was obtained according to the A (area) and D (density) classifications (before surgery: A: 1.9±0.6, D: 2.8±0.5; after surgery: A: 0.5±1.1, D: 0.4±0.7;_each $p<0.05$). All cases were maintained postoperatively with eye drops. No postoperative complications were experienced in all cases. **Conclusions.** Early postoperative results of the new punctal occlusion show that it is a satisfactorily safe and effective technique for obtaining complete punctal occlusion for severe dry eye cases with difficult punctal occlusion.

Quantitative Detection Of Cholesterol In Tears By A Simple And Sensitive Mass Sprctrometry Method. Zhenjun Zhao[1,2], John Korth[3], Yulina Aliwarga[1], Todd Mitchell[3], Stephen Blanksby[3] and Mark Willcox[1,2]. [1]The Institute for Eye Research, Sydney, Australia and the Vision Cooperative Research Centre, Sydney, Australia, [2]The School of Optometry and Vision Science, University of New South Wales, Sydney, Australia and [3]The Department of Chemistry, University of Wollongong, Wollongong, Australia.

Purpose. To quantitatively detect the concentration of free cholesterol in tears. **Method.** Human and rabbit basal tear samples were collected and freeze dried in glass vials. The residues were extracted in methanol chloroform (1:2) containing an internal standard D_6-cholesterol (6 hydrogen atoms were substituted with deuterium). The supernatants were then directly analyzed by a mass spectrometry (MS) method recently developed by a team in the University of Wollongong, in which the samples were loaded onto a drawn glass capillary and - using a heated probe - inserted directly into the electron ionisation source of a quadrupole MS. Temperature programmed heating of the probe results in sublimation of the cholesterol and the internal standard (D_6-cholesterol) and the relative abundance of the resulting spectra can be integrated to quantify the cholesterol present. **Results.** Preliminary work has already shown that this method is applicable to the analysis of tear free cholesterol and has identified a possible difference in tear cholesterol concentration between male and female human tears (20.2 ± 2.0 vs. 13.4 ± 1.4 ppm; $P<0.01$). Cholesterol concentration in female New Zealand white rabbit tears was 9.8 ± 4.6 ppm. **Conclusion.** The MS method can easily and accurately detect the amount of free cholesterol in human tears. Only 2-3 µl of tear sample is needed in the assay. The method makes it possible to study the role of cholesterol in dry eye. It is also likely that some 'fine tuning' of the technique will allow the direct quantification of cholesterol esters in tears without the requirement of prior isolation, which is very difficult for tears because of the very small sample volume.

Ocular Cicatrizing Pemphigoid: Is It Still One Of The Worst Disorders Of The Ocular Surface? Manfred Zierhut[1]. Department of Ophthalmology, University of Tuebingen, Germany.

Ocular cicatrizing pemphigoid (OCP) belongs to the group of chronic bullous dermatoses and is (out of this group) the disorder with the highest incidence of induction for ocular inflammation of the conjunctiva. Due to cicatrisation and keratinisation of the conjunctiva with severe dry eye, keratitis, superinfection and finally complete keratinisation of the ocular surface OCP may lead to blindness. In recent years, the pathophysiology of this group of autoimmune disorders has been characterized, the autoantigen structure for most of these different diseases determined, and, at least for OCP, there are multiple immunosuppressive therapy strategies available which can completely stop the disease. This review will summarize our knowledge about this group of disorders, comparing their clinical pictures and their pathophysiological differences, and finally update the therapy strategies.

Sebum, The Lipid Skin Layer: Biogenesis And Functional Properties. Christos C. Zouboulis. Departments of Dermatology, Venereology, Allergology and Immunology, Dessau Medical Center, Dessau, and Laboratory for Biogerontology, Dermato-Pharmacology and Dermato-Endocrinology, Institute of Clinical Pharmacology and Toxicology, Charité Universitaetsmedizin Berlin, Campus Benjamin Franklin, Berlin, Germany.

The sebaceous glands are functional from their formation: sebum is the first demonstrable glandular product of the human body. Their development and function before birth and in the neonatal period appear to be regulated by maternal androgens and by endogeneous steroid synthesis by the fetus, but also by other "morphogens". Hydroxysteroid dehydrogenases, which activate and inactivate androgens, are present after 16 weeks of fetal life. The sebaceous glands reach a peak of activity in the third trimester and their secretion forms a significant portion of vernix caseosa, the variably adherent fetal skin surface film consisting of lipids, epidermal corneocytes, water, and other substances from the amniotic fluid. Vernix caseosa covers the

fetus during the last trimester of pregnancy and acts as a hydroptic and antibacterial barrier. A strong increase in sebum excretion occurs a few hours after birth which peaks during the first week. The excretion slowly subsides with very low sebum levels after 6 months. A new rise takes place with adrenarche and continues up to 17 years when the adult level is reached. The endocrine environment of the neonate correlates and may influence the sebaceous gland development in puberty. Human sebum contains a lipid mixture of squalene and wax esters, as well as cholosterol esters, triglycerides, and possibly some free cholesterol. Although widely suggested that bacterial hydrolases convert triglycerides to free fatty acids on the skin surface, sebaceous glands are able to synthesize considerable amounts of free fatty acids. The fatty acids of the ester lipids include species with chain branching or with unusual double-bond positions. The alcohol moieties of the wax esters contain unusual chain types similar to those of the fatty acids. Genetic and hormonal factors cause individual differences in sebaceous lipid composition. The vernix lipids resemble sebum in their content of fatty acids, squalene and wax esters, but also contain lamellar lipids, such as sterols and sterol esters. Sebaceous lipids are responsible for skin surface lipids at most areas of the human body where casual surface lipids are greater than 100 µg/cm^2; in the forehead 150-300 µg/cm lipids can be recovered. Lower trunk and arm/leg lipids make an exception with 5-10 µg/cm^2 recovered lipids, which is the rate of lipids originating from epidermal keratinocytes. As a consequence, squalene and wax esters cannot be identified at the latter areas. Changes in lipid composition of sebum are associated with age or with sebaceous gland activity. Skin surface lipids maintain their protective mechanisms after birth, where sebum lipids play a major role. Total sebum lipids are a relevant physiologic pathway for the delivery of vitamin E to the skin; they exhibit photoprotective, thermoregulatory and mosquito repellent effects. Moreover, sebaceous lipids cause a reduction in growth of gram-positive and anaerobic bacteria, while they are ineffective against the most gram-negative ones.

Biomarkers For Sjögren's Syndrome Detected In Saliva Using High-Resolution Mass Spectrometry And Bioinformatics. Driss Zoukhri[1], Mabi Singh[1], Claire Kublin[1], Athena Papas[1], Ian Rawe[2], Kevin Dawson[3], William F. Haddon[3], Earl White[3], Kathy Hanley[3], Daniel Tusé[3], and Wasy[l] Malyj[3]. Department of General Dentistry, Tufts University School of Dental Medicine, Boston, MA[1]. Schepens Eye Research Institute and Department of Ophthalmology Harvard Medical School, Boston, MA[2]. Predictive Diagnostics, Inc., Vacaville, CA[3].

Purpose. Sjögren's syndrome (SjS) is considered to be the most common and under-diagnosed autoimmune disease. This is largely due to the fact that the diagnostic approach to SjS is rather complicated and must include multiple subjective and objective criteria. Thus, the purpose of the current studies was to determine if saliva contains biomarkers that can be used as diagnostic tools for SjS. **Methods.** A total of 27 SjS patients and 27 age-matched healthy controls were recruited for these studies. Unstimulated submandibular glands saliva was collected from the Wharton's duct using a suction device. Two ml of salvia were processed for mass spectrometry analyses using a prOTOF 2000 matrix-assisted laser desorption/ionization orthogonal time of flight (MALDI-O-TOF) mass spectrometer. Raw data were analyzed by Predictive Diagnostics Inc. who utilizes proprietary bioinformatics tools to identify biomarkers. **Results.** Data analysis resulted in several classification models built and several biomarkers were identified. A model based on 7 putative biomarkers yielded a sensitivity of 97.5%, specificity of 97.8% and an accuracy of 97.6%. Biomarkers identified were detected at mass to charge (*m/z*) values 902.48, 2,407.30, 2,912.56, 3,655.78, 3,803.38, 4,281.14, and 5,942.00. Another set of 7 putative biomarkers was identified in another classification model with a sensitivity of 85.0%, specificity of 91.3%, and an accuracy of 88.1%. One biomarker (*m/z*=3803.38) is a doubly charged ion and was present exclusively in SjS samples. This peptide when purified by HPLC and subjected to Edman degradation was identified as a fragment of human salivary proline rich proteins. **Conclusion.** Biomarkers detected in saliva by high-resolution mass spectrometry offer the potential to serve as diagnostic/prognostic tools for SjS.
(Supported by NIH grant R01 EY12383).

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