XXV ANNUAL MEETING OF THE EUROPEAN EYE BANK ASSOCIATION

Zagreb, Croatia, 18/19 January 2013

Under the auspices of the Croatian Academy of Sciences and Arts

Special Eye Hospital “Svjetlost”, Department of Ophthalmology, School of Medicine in Rijeka, University of Rijeka

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Dear Colleagues and Friends,
We are happy to welcome you to the XXV Annual Meeting of the European Eye Bank Association in the capital of Croatia, Zagreb. Our goal is to bring the latest topics and controversies in eye banking and corneal transplantation, to enable passionate and productive discussions as well as exchange of knowledge and skills.
Organizer of this Meeting is Special Eye Hospital “Svjetlost”, Department of Ophthalmology, School of Medicine in Rijeka, University of Rijeka and Croatian Society for Cataract and Refractive Surgery; and we will do our best to present you a quality program with all the novelties from the world of eye banking and corneal transplantation. Our key-note lectures will bring you expert knowledge from around the globe. There will be plenty of opportunities for constructive discussions and networking among participants.
We will introduce our beautiful city of Zagreb to our dear guests and colleagues. Very central location of Conference venue, Westin, will guarantee you a wonderful time. Zagreb is a city of great history and it will welcome you with its open heart.
Above all, this Conference is a chance to meet colleagues from all over the world, who much alike are striving to increase their abilities. We hope that you will have unforgettable days here in Zagreb! We warmly welcome you all,

Prof. Nikica Gabrić, MD, PhD
Head, Special Eye Hospital “Svjetlost”, Department of Ophthalmology, School of Medicine in Rijeka, University of Rijeka

Prof. Iva Dekaris, MD, PhD
President, European Eye Bank Association
Medical Director, Special Eye Hospital “Svjetlost”, Department of Ophthalmology, School of Medicine in Rijeka, University of Rijeka
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Special Eye Hospital “Svjetlost”, Department of Ophthalmology, School of Medicine in Rijeka, University of Rijeka, Croatia

Prof. Nikica Gabrić MD, PhD
Special Eye Hospital “Svjetlost”, Department of Ophthalmology, School of Medicine in Rijeka, University of Rijeka, Croatia

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Prof. Jesper Hjortdal MD, PhD
Danish Cornea Bank, Dept. of Ophthalmology Aarhus, University Hospital, Aarhus Denmark

Diego Ponzin MD, PhD
Fondazione Banca degli Occhi del Veneto Onlus, Venice Italy

Special Eye Hospital “Svjetlost”, Department of Ophthalmology, School of Medicine in Rijeka, University of Rijeka, Croatia

Croatian Society for Cataract and Refractive Surgery

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Conference Venue

Hotel Westin Zagreb
Kršnjavoga 1
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Program Overview

Friday 18 January 2013

9.00-12.00
EEBA Board Meeting

14.45 Opening Ceremony

15.15-16.55
Eye Banking

17.15-19.10
Endothelial Keratoplasty

21.00 Gala Dinner

Saturday 19 January 2013

8.30-9.30
EEBA Business Meeting

9.30-11.35
Eye Banking

12.00-14.05
Keratoplasty Miscellaneous

10.00-14.30
WET LAB: DSAEK, DMEK

15.00-16.20
Stem Cells and Amniotic Membrane

16.30 Closing Ceremony

18.00
Croatian National Theatre: Ballet
OPTIMIZING CELL CULTURE AND BANKING FOR CORNEAL GRAFT

FEMTOLASER AND CORNEAL GRAFT
EEBA 2013 Annual Meeting, Zagreb

Friday, January 18

9.00 - 12.00  **EEBA Board Meeting**

9.30 - 14.00  **WET LAB: DSAEK, Ultra-Thin DSAEK, DMEK – pneumatic dissection**  
(Prof. dr. Massimo Busin, Dr. Diego Ponzin, Italy, Sponsored by Moria):
- 9.30 - 11.30  Clinicians
- 12.00 - 14.00  Technicians

**09.00 - 19.00  REGISTRATION**

13.30 - 14.30  Lunch break

**14.45 - 15.15  Opening Ceremony**

President of the Croatian Academy of Sciences and Arts, Prof. Zvonko Kusić, MD, PhD
Minister of Health, Prof. Rajko Ostojić, MD, PhD

**15.15 - 16.55  Eye Banking**

**Invited lecture:**
- **15.15 - 15.45  Thomas Reinhard** – Endothelial failure: When to perform PK, DSAEK or DMEK  
  (University Eye Hospital, Freiburg, Germany)

- **15.45 - 15.55  John Armitage¹, P. Ashford², P. Distler²** – Standardized coding and labeling for ocular tissue  
  (¹School of Clinical Sciences, University of Bristol, Bristol Eye Hospital, UK; ²ICCBBA, California, USA)

- **15.55 - 16.05  Angelo Ghirardini¹, D. Fehily¹, P. Di Ciaccio, M. Mareri¹, F. Vespasiano¹, A. Nanni Costa¹, P. Ashford², R. Benedek³** – Eurocet 128: supporting the implementation of the European Coding System for traceability of tissues and cells  
  (¹Italian National Transplant Centre, Italian National Institute of Health, Italy; ²ICCBBA, California, USA; ³Artman Technologies sro., Slovakia)

- **16.05 - 16.13  Julia Promesberger¹, C. Uhlig¹, R. Koch², G. Hirschfeld³** – Evaluation of medical influences on cornea donation  
  (¹Department of Ophthalmology, University of Muenster Medical Center; ²Institute for science technology, University of Muenster; ³Institute of psychology, University of Muenster, Germany)

- **16.13 - 16.21  Constantin E. Uhlig¹, R. Koch², J. Promesberger¹** – Mental attitudes concerning cornea donation in a non medically educated and a professional medical cohort  
  (¹Department of Ophthalmology, University of Muenster Medical Center; ²Institute for science technology, University of Muenster, Germany)

- **16.21 - 16.29  Remy Julienne, G. Thuret, P. Gain** – Worldwide Eye Banking (WEB) project: International survey of demand and supply  
  (Department of Ophthalmology, University Hospital Saint-Etienne, France)
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16.29 - 16.37 Christina Sanchez Miller – An evaluation of total blood and plasma volume calculations in eye and tissue banking
(International Sight Restoration Eye Bank, Florida, USA)

16.37 - 16.45 Laura Giurgola¹, R. Mistò², F. Pateri², C. Gatto¹, J. D’Amato Tothova¹ – Evaluation of the decontamination methods of donor cornea
(¹Research and Development Department of Alchimia S.r.l.; ²Azienda Ospedaliera San Gerardo, Italy)

16.45 - 16.53 John Armitage¹, M. Jones², I. Zambrano³, F. Carley³, D. Tole¹ – Use of 5-year corneal graft survival for the validation of eye bank quality standards
(¹School of Clinical Sciences, University of Bristol, Bristol Eye Hospital; ²NHS Blood & Transplant; ³CTS Manchester Eye Bank, Manchester Royal Eye Hospital, UK)

16.55 - 17.15 Coffee break / Exhibition / Posters

17.15 - 19.05 Endothelial Keratoplasty
Moderators: Massimo Busin, Juan Alvarez de Toledo, Iva Dekaris

Invited lecture:
17.15 - 17.45 Massimo Busin – Ultrathin DSAEK: The present Status
(Professor, Società Italiana Trapianto di Cornea Villa Serena Hospital, Forli, Italy)

17.45 - 17.53 Anders Ivarsen, S. Kerathanathan, K. Nielsen, J. Hjortdal – Precutting of donor corneas for posterior lamellar keratoplasty
(The Danish Eye Bank, Department of Ophthalmology, Aarhus University Hospital, Denmark)

17.53 - 18.01 Mor Dickman¹, F. W. van Marion², Y. Schuchard², P. Steijger-Vermaat², R.M.M.A. Nuijts¹ – Predictability of Pre-cut Single-pass Ultra-thin DSAEK lamellae thickness with a novel operator-independent hands-free mechanical microkeratome system
(¹University Eye Clinic Maastricht, Maastricht University Medical Center; ²Euro Cornea Bank Beverwijk; The Netherlands)

18.01 - 18.09 Aurelien Bernard¹, C. Mauclair², E. Audouard², G. Thuret¹, M. Peoc’h¹, J. M. Dumollard¹, J. Granier³, H. Soder³, P. Gain¹ – Study of stromal femtosecond laser ablation for deep corneal cut optimization
(¹Corneal Graft Biology, Engineering and Imaging Laboratory, EA2521, Federative Institute of Research in Sciences and Health Engineering, Faculty of Medicine, Jean Monnet University; ²Laboratoire Hubert Curien; ³IMPULSION SAS, Pôle Optique Vision, France)

18.09 - 18.17 Mor Dickman¹, M. P.F.H.L. van Maris², T. J.T.M. Berendschot¹, R. M.M.A. Nuitjs¹ – Surface topography and 3-dimensional optical profiling of femtosecond and novel mechanical microkeratome dissected posterior human corneal discs for DSAEK
(¹University Eye Clinic Maastricht, Maastricht University Medical Center; ²Eindhoven University of Technology, Department of Mechanical Engineering, Materials Technology, Multi Scale Lab; The Netherlands)

(Special Eye Hospital Svjetlost, Department of Ophthalmology, School of Medicine in Rijeka, University of Rijeka, Croatia)
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18.25 - 18.33 Juan Alvarez de Toledo, L. Fernández-Vega Cueto, M. F. de la Paz, P. Sauvageot - Beneria – Initial endothelial cell loss after DSAEK using a novel donor inserter
(Centro de Oftalmología Barraquer, Spain)

18.33 - 18.41 Agnieszka Bielinska, E. Rakowska, D. Haszcz, T. Żarnowski – Posterior lamellar keratoplasty-postoperative results after modification of surgical technique
(Department of Ophthalmology, Lublin, Poland)

18.41 - 18.49 Esther Groenevald–van Beek¹,², L. Ham¹,³, K. van Dijk¹,³, I. Dapena¹,³, G. R.J. Melles¹,²,³ – Mid-term results on visual acuity and stability after Descemet membrane endothelial keratoplasty (DMEK)
¹(Netherlands Institute for Innovative Ocular Surgery; ²Amnitrans EyeBank Rotterdam; ³Melles Cornea Clinic Rotterdam, The Netherlands)

(Netherlands Institute for Innovative Ocular Surgery, The Netherlands)

18.57 - 19.05 Sonja Heinzelmann, S. Hüther, D. Böhringer, P. Maier, T. Reinhard – The role of the donor in DMEK-surgery
(University Eye Hospital Freiburg, Germany)

21.00 Gala Dinner
Hotel Westin, 17th floor

Saturday, January 19

8.30 - 9.30 EEBA Business Meeting

10.00 - 14.30 WET LAB: DSAEK, DMEK
(Dr. Phillip Maier, Andrea Gareis-Lok, CEO, CEBT, MTA, Germany, Sponsored by Gebauer & Geuder):
10.00 - 12.00 Clinicians
12.30 - 14.30 Technicians

9.30 - 11.35 Eye banking
Moderators: Donald Tan, François Majo, Jesper Hjortdal

Invited lecture:

9.30 - 10.00 Donald Tan – Eye banking challenges in Asia – Can they be overcome?
(President of Association of Eye Banks of Asia, Singapore National Eye Centre, Singapore)

10.00 - 10.08 James Eide MacPherson¹, J. Klokk Slettedal¹, J. Hjortdal², K. Nielsen² – Electrolyte composition of four eye bank media during corneal preservation
¹(Department of Ophthalmology, Oslo University Hospital, Norway; ²The Danish Eye Bank, Department of Ophthalmology, Aarhus University Hospital, Denmark)
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10.08 - 10.16 François Majo¹, M. Deprez², M. Nicolas¹ – Integrity of human corneal epithelium maintained in organ-cultured using CorneaMax®
(¹Jules-Gonin Eye Hospital, Switzerland; ²University of Liege, Belgium)

10.16 - 10.24 Maja Pauk-Gulić, S. Lukačević, N. Milići, N. Drača, M. Ratković, I. Dekaris – Prospective clinical evaluation of hypothermic vs organ cultured corneal grafts
(Special Eye Hospital Svjetlost, Department of Ophthalmology, School of Medicine in Rijeka, University of Rijeka, Croatia)

10.24 - 10.32 Diego Ponzin¹, M. Parekh¹, E. Negura¹, G. Salvalaiol, S. Ferrari¹, C. Albrecht², D. Fortier², M.-C. Amoureux² – A novel objective method to evaluate the overall quality of corneal tissue used for comparative study between two hypothermic preservation media
(¹The Veneto Eye Bank Foundation, Italy; ²Eurobio, France)

10.32 - 10.40 Gilles Thuret¹, Z. He¹, A. Bernard¹, S. Piselli¹, N. Campolmi¹, B. M. Ha Thi¹, J. M. Dumollard², M. Peoc’h², N. Delesalle³, P. Gain¹ – European study on reliability assessment of endothelial cell count in eye banks: The Euro-Keratotest study
(¹Corneal Graft Biology, Engineering and Imaging Laboratory, EA2521, Federative Institute of Research in Sciences and Health Engineering, Faculty of Medicine, Jean Monnet University; ²Department of Pathology, University Hospital of Saint-Etienne; ³Agence Nationale de Sécurité du Médicament et des produits de santé (ANSM), France)

10.40 - 10.48 V. M. Borderie – Evaluation of donor corneas during storage by full-field optical coherence tomography
(Centre de Recherche Institut de la Vision, UMR S 968 Inserm / UPMC, UMR 7210 CNRS, France)

10.48 - 10.56 Nicolas Michael¹, A. Pipparelli¹, G. Thuret², P. Gain², F. Majo¹ – ROCK inhibitor enhances adhesion and wound healing on human corneal endothelial cells ex vivo and in vitro
(¹Jules-Gonin Eye Hospital, Switzerland; ²Faculty of Medicine, University of Saint Etienne, France)

10.56 - 11.04 Clotilde Jumelle¹, N. Campolmi¹, A. Bernard¹, S. Piselli¹, C. Mauclair², E. Audouard², J. Granier³, H. Soder³, P. Gain¹, G. Thuret¹ – Delivery of molecules into corneal endothelium using nanoparticles activated by femtosecond laser pulses: proof of concept
(¹Corneal Graft Biology, Engineering and Imaging Laboratory, EA2521, Federative Institute of Research in Sciences and Health Engineering, Faculty of Medicine, Jean Monnet University; ²Laboratoire Hubert Curien; ³IMPULSION SAS, Pôle Optique Vision, France)

11.04 - 11.12 Kristine Ustgård-Andersen¹, K. Haug¹, A. Azqueta², B. Nicolaissen¹, A. R. Collins¹ – DNA damage in donor corneal endothelium upon transfer from Optisol GS to Organ Culture
(¹Norwegian Cornea Bank, Center for Eye Research, ²Department of Ophthalmology, Oslo University Hospital, Ulleval and University of Oslo; Department of Nutrition, Institute for Basic Medical Sciences, University of Oslo, Norway)

11.12 - 11.20 Mahmood Farazdaghi¹, T. E Askew², S. Farazdaghi² – VisionGraftR Sterile cornea - a new phenomenon in ocular surgery
(¹International Federation of Eye & Tissue Banks; ²Tissue Banks International, USA)

11.20 - 11.28 Laura Giurgola¹, R. Mistò¹, F. Pateri², C. Gatto¹, J. D’Amato Tothova¹ – False negative results in tissue banking: The corneal tissues
(¹Research and Development Department of Alchimia S.r.l.; ²Azienda Ospedaliera San Gerardo, Italy)
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11.28 - 11.33 Patricia Dahl - Emergency Preparedness
(The Eye Bank for Sight Restoration, Inc., USA)

11.35 - 12.00 Coffee break / Exhibition / Posters

12.00 - 14.05 Keratoplasty: Miscellaneous
Moderators: Marian Macsai, Petja Vassileva, Ante Barišić

Invited lecture:
12.00 - 12.30 Marian Macsai - History of EBAA and Project Notify
(Immediate Past Chair, Eye Bank Association of America (EBAA) NorthShore University Health Systems, Glenview, USA)

12.30 - 12.38 Beata Rymgałło-Jankowska¹, A. Bielinska¹, E. Rakowska¹, D. Haszcz², G. Płaszczewska², M. Skowronek² – Management in non-traumatic corneal perforations
¹(Department of Ophthalmology, Lublin; ²Eye Bank Lublin, Poland)

(Centro de Oftalmologia Barraquer, Spain)

12.46 - 12.54 Ante Barišić, V. Glavota, I. Dekaris, N. Gabrić – Cataract and Fuch’s Dystrophy: DSAEK and PHACO or staged procedure?
(Special Eye Hospital Svjetlost, Department of Ophthalmology, School of Medicine in Rijeka, University of Rijeka, Croatia)

12.54 - 13.02 Neven Miličić, M. Pauk Gulić, N. Gabrić, I. Dekaris – Penetrating keratoplasty or Descemet Stripping Automated Endothelial Keratoplasty over multifocal iol: which is better?
(Special Eye Hospital Svjetlost, Department of Ophthalmology, School of Medicine in Rijeka, University of Rijeka, Croatia)

(Centro de Oftalmologia Barraquer, Spain)

13.10 - 13.18 Petja Vassileva, N. Surchev, M. Moutaftchieva - Donor risk factors for graft survival after PK
(University Eye Hospital “Akad. Pashev”, Bulgaria)

(Special Eye Hospital Svjetlost, Department of Ophthalmology, School of Medicine in Rijeka, University of Rijeka, Croatia)

13.26 - 13.34 Sandra Sekelj¹, T. Balog², I. Mahovne¹, E. Kondza Krstonijevic³, Z. Janjetovic¹, Z. Vukovic Arar⁴, I. Dekaris⁴ – Prediction of corneal graft failure due to preoperative measurement of VEGF in recipient cornea
¹(General Hospital Dr J. Bencevic; ²Institute Ruder Bošković; ³Health center Zagreb East; ⁴Special Eye Hospital Svjetlost, Department of Ophthalmology, School of Medicine in Rijeka, University of Rijeka, Croatia)
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Invited lecture:

13.34 - 14.04 Kevin Corcoran – Eye Bank Association Collaboration and Outreach
(President and CEO, Eye Bank Association of America (EBAA))

14.05 - 15.00 Lunch break

15.00 - 16.20 Stem cells and amniotic membrane
Moderators: Thomas Fuchsluger, Andrea Gareiss Lok, Philip Maier

Invited lecture:

15.00 - 15.30 Thomas Fuchsluger – Artificial cornea
(Department of Ophthalmology, Düsseldorf University Hospital, Dusseldorf, Germany)

15.30 - 15.38 Binh Minh Ha Thi¹, Z. He¹, N. Campolmi¹, S. Piselli¹, P. Gain¹, M. Peoc’h², J. M. Dumollard², S. Acquart³, O. Garraud³, G. Thuret¹ – Identification of label-retaining endothelial cells in adult human corneas: a new clue for the existence of endothelial stem cells
¹(Corneal Graft Biology, Engineering and Imaging Laboratory, EA2521, Federative Institute of Research in Sciences and Health Engineering, Faculty of Medicine, Jean Monnet University; ²Department of Pathology, University Hospital of Saint-Etienne; ³Eye Bank, French Blood Centre, France)

15.38 - 15.46 Mirna Tominac Trcin¹, T. Dolenec¹, M. Sokol¹, E. Zdraveva², B. Mijović², M. Pauk-Gulić³, I. Dekaris³ – Viability of human limbal epithelial cells cultured on different types of scaffolds
¹(University Department of Traumatology, Sestre Milosrdnice Hospital Center; ²Faculty of Textile Technology, University of Zagreb; ³Special Eye Hospital Svjetlost, Department of Ophthalmology, School of Medicine in Rijeka, University of Rijeka, Croatia)

15.46 - 15.54 Eva Martinez Conesa, M. Pérez, N. Otero, N. Nieto-Nicolau, E. Agusti, A. Vilarrodoneda, E. Trías, R. P. Casaroli-Marano – Epithelial and progenitor cell markers of ocular surface in mesenchymal stem cells from human adult adipose tissue
(Transplant Services Foundation (TSF), Hospital Clinic, Spain)

15.54 - 16.02 Henning Thomasen, K. P. Steuhl, D. Meller – Validation of an automated test system for sterility controls of amniotic membrane for clinical applications
(Cornea Bank Essen, Department of Ophthalmology, University Hospital Essen, Germany)

16.02 - 16.10 Henning Thomasen, D. Meller, K. P.Steuhl – Influence of storage conditions of placental tissue on sterility and histologic properties of amniotic membrane
(Cornea Bank Essen, Department of Ophthalmology, University Hospital Essen, Germany)

16.10 - 16.18 Jurica Predović¹, I. Dekaris², T. Balog³, S. Sobočanec³, A. Šarić³ – VEGF164 antibodies delay corneal vascularization after alkali burn
¹(Ophthalmology Clinic, Clinical Hospital Sveti Duh; ²Special Eye Hospital Svjetlost, Department of Ophthalmology, School of Medicine in Rijeka, University of Rijeka; ³Ruđer Bošković Institute, Croatia)

16.30 - 17.00 Closing Ceremony

18.00 Croatian National Theatre: Ballet
An evening of German authors:
Marco Goecke - Johann Sebastian Bach SUITE SUITE SUITE
Uwe Scholz - Robert Schumann SECOND SYMPHONY

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Posters

Khadizhat D. Tonaeva, S. A. Borzenok, N. A. Onishenko, Yu. A. Komakh, A. A. Zeltonozko, Z. I. Moroz, O. V. Kravchuk – Results of morphological examination of the corneas stored under the cryogenic preservation
(Eye tissue bank of the S.Fyodorov Federal State Institution IRTC “Eye Microsurgery”, Russia)

(Special Eye Hospital Svetlost, Department of Ophthalmology, School of Medicine in Rijeka, University of Rijeka, Croatia)

Dean Šarić, V. Lasmanovic, I. Petric, Z. Mandic – Keratoplasty “a chaud”
(Eye Clinic, Clinical Hospital "Sisters of Charity", Croatia)

Marko Vlašić, Z. Tomić, N. Miličić, R. Lazić, I. Dekaris – Penetrating keratoplasty combined with pars plana vitrectomy
(Special Eye Hospital Svetlost, Department of Ophthalmology, School of Medicine in Rijeka, University of Rijeka, Croatia)

Stefan Lang, D. Böhninger, T. Reinhard – Cell-by-cell alignment of repeated specular microscopy
(Umversity Eye Hospital Freiburg, Germany)

(Special Eye Hospital Svetlost, Department of Ophthalmology, School of Medicine in Rijeka, University of Rijeka, Croatia)

Anja Grunert, M. Klüppel, J. Hausser, T. Reinhard, R. Sundmacher, T. Fuchsluger*, G. Geerling* – Rigid gas-permeable contact lens correction of infant aphakia following congenital cataract surgery
(Dusseldorf University Hospital, Germany)

Adis Pašalić, N. Drača, M. Pauk Gulić, A. Biščević, I. Dekaris – Intrastromal voriconasol treatment for fungal infection after penetrating keratoplasty
(Special Eye Hospital Svetlost, Department of Ophthalmology, School of Medicine in Rijeka, University of Rijeka, Croatia)

Dean Šarić, V. Lacmanovic, I. Petric-Vickovic, Z. Mandic – Deep anterior lamellar keratoplasty vs penetrating keratoplasty for herpes simplex keratitis scar
(Eye Clinic, Clinical Hospital "Sisters of Charity", Croatia)

Renata Gržetić Lenac – Transplantation of amniotic membrane in corneal ulcers and persistent epithelial defects
(Department of Ophthalmology, Institute Ruđer Bošković, Clinical Hospital Center Rijeka, Croatia)

Morena Gavrić, A. Biščević, N. Miličić – Correction of post – keratoplasty asitgmatism with contact lenses
(Special Eye Hospital Svjetlost, Department of Ophthalmology, School of Medicine in Rijeka, University of Rijeka, Croatia)

Ana Čović, A. Barišić, I. Dekaris, N. Gabrić – Management of astigmatism in cataract surgery with toric IOL in a patient with keratoconus: A case report
(Special Eye Hospital Svetlost, Department of Ophthalmology, School of Medicine in Rijeka, University of Rijeka, Croatia)
Accommodation & Traveling

Zagreb

The capital of the Republic of Croatia, is one of the oldest European cities and is yet one of Europe’s youngest metropolises. It is often called the city of museums, as there are many of them, approximately fifty museums and galleries, as well as private art collections and about twenty theatres and musical venues, which does not mean there is nothing else for you to do.

Despite being a Central European city in geography, culture and baroque architecture, in many ways, Zagreb has a Mediterranean way of life. Thanks to its many influences, the city has a special charm and hospitable feel generated by its open-hearted people. You can, according to a local custom, have a coffee (“popiti kavu”), which translated from Croatian means: “sit and watch the world go by”, as Miroslav Krleža, a famous Croatian writer used to do, or immerse in other rich contents this city offers to its visitors.

Zagreb is famous for its green areas. You will find charming parks in the city center and for those of you who are park lovers there is the Maksimir park, one of the biggest city parks in Eastern Europe.

A walk through Zagreb is an interesting and pleasant journey that encapsulates both history and modern day life. Ilica, the longest street in Zagreb, divides the city into the old romantic Upper Town and the young, busy and business orientated Lower Town. The oldest areas, Gradec and Kaptol, from which Zagreb arose, are considered to be one of the most preserved and beautiful European city centers built in the Art Nouveau style. The Upper and Lower Towns are connected through the Kamenita vrata (Stone gate), yet another recognizable Zagreb tourist attraction that is linked to many legends and beliefs, as well as to faith and peace.

For some, the most recognizable place in Zagreb is its neo-gothic Cathedral situated at Kaptol. Although it took many centuries to build, the Cathedral that stands today was completed at the end of the 19th century.
Accommodation

The following hotels have reduced prices for EEBA Meeting participants:

**Hotel Westin Zagreb (*****)**

- Centrally located in the very hearth of Zagreb
- Easy walking distance to central square
- 378 guest rooms

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[www.hotelwestinzagreb.com](http://www.hotelwestinzagreb.com)

For EEBA 2013 congress bookings:

Breakfast and VAT is included, City tax is € 1,00 per day.

**Hotel International (****)**

- 10 min by tram from Hotel Westin
- 20 min walk from Hotel Westin

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For EEBA 2013 congress bookings:

Breakfast and VAT is included, City tax is € 1,00 per day.
How to get to Zagreb

Special Visa requirements can be checked at http://www.mvep.hr/.

By Air

Zagreb airport is located 17km from the centre of the city, or 20-25 minutes by bus. Information on flights can be obtained by calling + 385 1 6265 222. The Zagreb Airport bus terminal (bus stop) is at the Central Bus Station on Marin Drzic Avenue. For more information on bus schedule visit www.plesoprijevoz.hr.

By Train

The Main Railway Station is located in the centre of the City (at Kralj Tomislav Square 12, a ten minutes walk from the central city square). Information on arrivals and departures can be obtained by dialing 060-333-444. Information on arrivals and departures can also be obtained at the travel agency “Croatia Express”, Telephone: +385 1 457 3253.

By Car

Main international roads are:
- Trieste - Ljubljana - Zagreb
- Graz - Maribor - Zagreb
- Klagenfurt - Ljubljana - Zagreb
- Budapest - Varazdin - Zagreb

By Bus

The Central Bus Station is located on Marin Drzic Avenue, a few minutes by tram (line number 6) from the central city square. Information can be obtained by calling: +385 60 340 340. Information on arrivals and departures: +385 60 313-333. Bookings for domestic lines can be made by calling +385 60 313-333. For international lines bookings can be made by calling +385 1 6008-631. Traffic office telephone number: +385 1 6008-645.
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INVITED LECTURE: THOMAS REINHARD, GERMANY – ENDOTHELIAL FAILURE: WHEN TO PERFORM PK, DSAEK OR DMEK

Chairman and professor, Eye Hospital at the Albert-Ludwigs University in Freiburg
Chairman, Section for Tissue Transplantation and Biotechnology
Board Member, EuCornea

Biography: Professor Thomas Reinhard was born in Ahrweiler, Germany in 1962. He graduated from RWTH Aachen in 1989. From 1989 to 1992 Professor Reinhard was awarded a fellowship at the Eye Hospital of the Heinrich-Heine University in Düsseldorf. Since 1996 he holds a habilitation at Heinrich-Heine University. In 1994 the helped establish the LIONS Cornea Bank North Rhine Westfalia and he acted as head of it until 2003. From 2003 to 2005 he acted as head of LIONS Cornea Bank Baden – Württemberg. In 2003 Professor Reinhard became the chairman of the Eye Hospital at the Albert-Ludwigs University in Freiburg. He has been a Board Member of the German Ophthalmological Society (DOG) since 2008, and in 2009 he became an Executive Board Member. Within the DOG he has acted as a Board Member in the Cornea Section since 2001. In 2008 he became the Chairman of the Section for Tissue Transplantation and Biotechnology. He has been Vice President of the DOG two times (from 2009 to 2010 and from 2011 to 2012) and President once (from 2010-2011). Since 2009 Professor Reinhard has acted as a Council Member of the European Foundation for Tissue Banks and he has been a Board Member of EU Cornea from 2009 to 2013. Professor Reinhard has performed more than 23,000 surgical procedures. His surgical specialties include corneal transplantation, penetrating and lamellar procedures, limbal stem transplantation and amniotic membrane transplantation, as well as cataract and glaucoma surgery. He has written 307 papers (of these 251 articles in journals with review system) and has given 740 oral presentations (invited lectures, courses, posters, videos).
Purpose: Traceability is a key requirement for ocular tissues. Traceability is enhanced when documentation of the collection, processing, and distribution of ocular tissues can be performed using bar codes. However, for bar codes to be useful, they must be globally standardized so that disparate computer systems can interpret essential information in the same way. ISBT 128, an existing system designed to support traceability of substances of human origin, has been used for blood, cellular therapy products, and tissues around the world. The ISBT 128 system supports globally unique identification for each donation as well as standardized product coding. Adapting it to ocular tissue required the development of standardized ocular terminology.

Methods: Building a global system for labeling and coding of tissue products follows an organized process. First, terms describing products must be selected and defined through a global consensus process. Then, reference tables mapping the terminology to computer codes must be developed. Computer codes from the reference tables must then be formatted into standardized data structures allowing disparate computer systems to interpret the data. Finally, these data structures are incorporated into bar codes (or other electronic delivery mechanisms) and utilized on labels. Using an existing standard, such as ISBT 128, for encoding of information not only provides for quicker implementation, it also allows many substances of human origin to utilize the same system. Representatives from Association of Eye Banks of Asia; European Eye Bank Association; Eye Bank Association of America; Eye Bank Association of Australia and New Zealand; Eye Bank Association of India; Pan American Association of Eye Banks; and ICCBBA, as well as other technical experts, met by teleconference over a period of a year to select and define appropriate terminology. The draft of the terminology was submitted to eye bank societies and the public for comment, revisions were made, and terminology was finalized by the group.

Results: An international system for standardized coding and labeling of ocular tissue now exists. The terminology developed by this consensus process may be used within the ISBT 128 system to label ocular products with standardized bar codes enabling the electronic capture of critical data in the collection, processing and distribution of products.

Conclusions: The Boards of the above organizations confirmed their support for the international use of ISBT 128 in the coding of ocular tissue and encourage Eye Banks to:
Adopt this standard terminology for use in communications and in the labeling of ocular tissue grafts;
Implement ISBT 128 globally unique donation identification for ocular tissue grafts;
Move towards full implementation of ISBT 128 nomenclature, coding, and labeling in accordance with guidance published by the eye bank technical advisory group.
Following this process, ISBT 128 computer codes were assigned to various products and may now be used to label ocular tissues.
EUROCET 128: SUPPORTING THE IMPLEMENTATION OF THE EUROPEAN CODING SYSTEM FOR TRACEABILITY OF TISSUES AND CELLS

A. Ghirardini¹, D. Fehily¹, P. Di Ciaccio, M. Mareri¹, F. Vespasiano¹, A. Nanni Costa¹, P. Ashford², R. Benedek³

¹Italian National Transplant Centre, Italian National Institute of Health, Italy; ²ICCBBA, California, USA; ³Artman Technologies sro., Slovakia

Purpose: The primary aim of Eurocet 128 is to support the traceability of human tissues and cells intended for human application in patients in the European Union. From donation to transplantation, it is essential to maintain traceability so that the cells or tissues can always be linked back to the original centre where they were procured and received and, indeed to their original human origin. Traceability of these substances across the EU will be improved by the implementation of a single European Coding System as required by Directive 2004/23/EC.

Methods: The European Commission has awarded a contract to the Eurocet 128 consortium to deliver the tools required for the implementation of the Single European Code. The coding system implementation will be supported by the construction of two official public lists - the tissue establishment compendium and the product compendium - and the provision of an online code-translator. The first compendium will be a list of all authorized establishments that procure, receive, process, store and/or distribute tissues or cells for human application, with their EU identifying codes, a minimum data set and their real-time authorization status by activity and by tissue/cell type. The compendium is being constructed on the basis of the previous work of Eurocet (the European network of the competent authorities for tissues and cells) project, which collects information on authorized tissue establishments and their activities from the EU Competent Authorities. The second compendium will be a list of types of tissue and cell products with agreed descriptions and their associated codes. It will support the use of the international standard ISBT 128, EuroCode and existing national systems by mapping these systems to their corresponding European Generic Code. The online application will be an electronic code translator that will translate alphanumeric codes to textual information, as well as textual information to alpha-numeric codes.

Results: It will be mandatory for tissue and cell products distributed in the European Union to carry a European Identifying Code. The Single European Code is an alphanumeric code that includes information on the TE origin, the donation number, the product code, divisions and expiry date in a standardized format. The requirements to use the code will be established through an EU legal instrument, together with requirements on data to be recorded by each tissue establishment. It is anticipated that the Single European Code will be required from 2014; the exact date will be specified in the legal instrument. Through the online application, tissue and cell professionals, clinical users and regulators will be able to insert the European Identifying Code and obtains the information related to the tissue establishment and the product or vice versa.

Conclusions: At the completion of the full implementation of this work, all tissues and cells distributed for clinical use in the European Union will have a standard Single European Code on their label. This code will allow to rapidly identify the tissue establishment of origin and its status, and consequently to trace back the path followed by this tissue. It will also enable common understanding of the tissue or cell product type. Tissue Establishments should be preparing for the implementation of this new standard.
EVALUATION OF MEDICAL INFLUENCES ON CORNEA DONATION

J. Promesberger¹, C. Uhlig¹, R. Koch², G. Hirschfeld³

¹Department of Ophthalmology, University of Muenster Medical Center; ²Institute for science technology, University of Muenster; ³Institute of psychology, University of Muenster, Germany

Purpose: In Germany more patients are waiting for corneal transplantation than donor corneas are available. This study is evaluating medical factors that could have a negative or positive influence on cornea donation.

Methods: An anonymous, randomized questionnaire was created in collaboration with the institute of psychology and information technology. It was then distributed to a different social stratum and group of occupation (health care service, civil servants and factory employees). The distribution of the questionnaire was conducted either via paper version or electronically via email or website with the EFS (Encrypting file system) Survey program. The questionnaire was collecting demographic information, information about the attitude towards organ and tissue donation, the knowledge about the procedures regarding organ and tissue donation and also tested different possible influence factors on the willingness to donate. The analysis was organized with the "Predictive Analytics Software" (PASW 18).

Results: A total of 3043 questionnaires were included in the statistical analysis. 68% of all people participating in this questionnaire stated their willingness to donate their cornea. The analysis showed that there are definite medical factors influencing the willingness to donate organs and tissue. The understanding of the diagnosis 'brain death' influenced the willingness to donate. A significant higher percentage of the probands who understand brain death as irrevocable and definite (79,84%) declared their willingness to donate their corneas or other organs than people who doubted this diagnosis (42,11%). Another medical factor that influenced the willingness to donate was the individual health condition. Probands who stated their condition as very healthy showed a higher willingness to donate (70,24%) than participants in poor health condition (54,29%). A change of current life situation influenced the willingness to donate: A significant higher percentage of people who stated their refusal regarding cornea donation would agree with a corneal transplantation in case of their own blindness (17,4%) compared to people who stated their willingness to donate at first stage (14,2%). Other not statistically significant medical factors influencing the willingness to organ and tissue donation were determined in this study: explantation of the complete bulbus versus the corneoscleral complex and cosmetic outcome after donation.

Conclusions: The understanding of the diagnosis 'brain death', the individual health condition, certain changes of the current life situation and other medical factors seem to influence cornea donation. Respecting, these parameters to a greater extent might increase the vote for cornea donation in public.
MENTAL ATTITUDES CONCERNING CORNEA DONATION IN A NON MEDICALLY EDUCATED AND A PROFESSIONAL MEDICAL COHORT

C. Uhlig¹, R. Koch², J. Promesberger³
¹Department of Ophthalmology, University of Muenster Medical Center; ²Institute for science technology, University of Muenster, Germany

Purpose: To evaluate if medical education influences attitudes for postmortem cornea donation.

Methods: Prospective, randomized questionnaire distributed via internet-email cohorts to employees of a German university clinic (UE), or via homepage link to the employees of a large, industrial city in Germany (CE). The survey included 12 questions regarding baseline demographic characteristics of participants, and 21 questions concerning their knowledge, attitudes, and motives in respect to organ and cornea donation.

Results: 805 city employees (69.3 % female, 30.4% male, 40.6 catholic, 27.0% protestant) and 1499 medical employees (70.3% female, 29.7% male, 57.8% catholic, 26.2% protestant) responded to the questionnaire. Age distribution in CE was 8.2% (18-29 years), 51.5% (30-49), 40.2 (50-69) and in UE 20.2%(18-29), 56.6%(30-49), and 23.1%(50-69). Willingness for postmortem cornea donation was 77.3% in CE and 66.5% in UE. 16.0% of the CE in contrast to 7.7% (UE) are anxious that they would be medically treated worse if they possessed a donor card. 19.0% of the CE in contrast to 8.2% (UE) are afraid, that organ and tissues are commercially treated. In both groups, large majorities are in favour of a prohibition concerning any commercial treatments with organs and tissues (CE: 86.7%; UE: 84.5%). 24.9% (CE) and 19.4% (UE) do not want their personal donor attitude to become officially registered.

Conclusions: Employees without or with medical education express similar negative attitudes towards organ and tissue commerce and official registration of donor acceptance. Without medical education, participants were more open-minded in general cornea donation, but also more sceptical as regards postmortem medical treatment or commercial use of organs and tissues. Such anxiety should be reason to improve specific public information. The reasons that might decrease willingness for postmortem cornea donation in medical professionals should be investigated in further surveys.
WORLDWIDE EYE BANKING (WEB) PROJECT: INTERNATIONAL SURVEY OF DEMAND AND SUPPLY
R. Jullienne, G. Thuret, P. Gain
Department of Ophthalmology, University Hospital Saint-Etienne, France

**Purpose:** The whole eye banking (EB) process, from corneal retrieval to surgery, is improving. Paradoxically, corneal blindness worldwide is still increasing. Supply seems lagging far behind global demand but only partial data of the worldwide situation is available. We therefore launched an international survey on the balance of demand and supply, called the “Worldwide Eye Banking” (WEB) project. Our goal is to identify suitable solutions to countries willing to improve their corneal supply.

**Methods:** Design: descriptive epidemiological worldwide transversal study. A questionnaire was designed and e-mailed to EB staff and ophthalmologists involved in corneal grafts using mailing lists from local and international ophthalmological societies or by face to face interview during international ophthalmology, eye research or EB congresses.

**Results:** Significant disparities are highlighted. Developed countries tended to satisfy corneal demand. US EBs use short term storage and are exporters, while Europeans use long term organoculture and nearly satisfy local demand. Keratoplasty indications have 2 profiles: infections, mainly trachoma, for developing countries, keratoconus, endothelial dystrophy or iatrogenic edema in developed countries.

**Conclusions:** This demand/supply disparity, at this stage of the study (ongoing), is severe in most developing countries. Decreasing demand requires: 1) Trachoma fight in endemic zones 2) Iatrogenic edema prevention. Increasing supply requires: 1) Corneal donation politic dynamism 2) Local eye banking implantation in each country 3) Optimizing storage technique for better efficiency (retrieval/delivery graft ratio) 4) Bioengineering of endothelial graft.
AN EVALUATION OF TOTAL BLOOD AND PLASMA VOLUME CALCULATIONS IN EYE AND TISSUE BANKING

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International Sight Restoration Eye Bank, Florida, USA

Purpose: To evaluate the differences of weight-based total blood and plasma volumes compared to the Nadler Total Blood Volume Formula and a percentage-based plasma volume calculation.

Methods: Review of 58 adult donors and 1 pediatric donor from ISR Eye Bank. The gender, weight in kilograms, and height in meters was obtained from each donor. Each donor was classified as overweight and normal according to the Body Mass Index, with the exception of the pediatric case. The total blood volume was calculated for each donor using the standard weight-based (.07L/kg) total blood volume estimation and the Nadler Formula. The total plasma volume was calculated for each donor using the standard weight-based (.04L/kg) total plasma volume estimation and a percentage-based (55%) plasma volume estimation. The averages of the total blood and plasma volumes for all donors, overweight donors, and normal donors were calculated. The ranges of total blood and plasma volumes were determined for all donors, overweight donors, and normal donors.

Results: There were 41 overweight donors and 17 normal donors that comprised the study. The pediatric case was not classified as overweight or normal. The average total blood volume for all donors utilizing the weight-based calculation was 6.63L compared to the Nadler Formula which averaged 5.56L. The two calculations yielded an average difference of 1.07L. The average total blood volume for overweight donors utilizing the weight-based calculation was 7.35L compared to the Nadler Formula which averaged 5.91L. The two calculations yielded an average difference of 1.44L. The average total blood volume for normal donors utilizing the weight-based calculation was 4.92L compared to the Nadler Formula which averaged 4.72L. The two calculations yielded an average difference of 0.20L. The range of the weight-based calculation and Nadler Formula for total blood volume was 4.14L-14.28L and 3.43L-8.90L, respectively. The average total plasma volume for all donors utilizing the weight-based calculation was 3.79L compared to the percentage-based calculation which averaged 3.06L. The two calculations yielded an average difference of 0.73L. The average total plasma volume for overweight donors utilizing the weight-based calculation was 4.20L compared to the percentage-based calculation which averaged 3.25L. The two calculations yielded an average difference of 0.95L. The average total plasma volume for normal donors utilizing the weight-based calculation was 2.81L compared to the percentage-based calculation which averaged 2.60L. The two calculations yielded an average difference of 0.21L. The range of the weight-based calculation and percentage-based calculation for total plasma volume was 2.29L-8.16L and 1.89L-4.89L, respectively. The pediatric case yielded a total blood volume of 1.27L for the weight-based calculation and 1.19L for the Nadler Formula. The total plasma volume for the weight-based calculation was of 0.73L and 0.66L for the percentage-based calculation.

Conclusions: Among the classes of all and overweight donors, the weight-based calculation for total blood volume and total plasma volume was over-estimated. There was no significant difference among the normal donors. The Nadler Formula and the percentage-based calculation are best suited for estimating blood and plasma volumes for all adult donors, regardless of weight classification. All calculations for the pediatric case were not supported by literature. Calculations of blood and plasma volumes for pediatric donors need further evaluation. When calculating total blood and plasma volumes for plasma dilution algorithms, eye and tissue banks should use stricter formulas resulting in lower volumes. Lower volumes are less likely to result in false negatives due to plasma diluted samples.
EVALUATION OF THE DECONTAMINATION METHODS OF DONOR CORNEA
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Purpose: Our previous studies showed that standard corneal storage media do not guarantee efficient decontamination of donor cornea. The aim of the study was to determine the process conditions for effective decontamination of donor corneas.

Methods: Thirty donor corneas were procured by the Monza Eye Bank (Italy). Ten corneas were stored in a decontamination medium prototype A at 31°C for 20 days. Twenty corneas were decontaminated at 4°C overnight in a decontamination solution prototype B and subsequently stored either in Eusol-C at 4°C or Tissue-C at 31°C. The decontamination phase was skipped for 24 corneas used as control tissues, which were stored under organ culture conditions. Microbiological analyses were performed pre-processing and post-processing after removal of antibiotic residues with the ResEP™ device (ALCHIMIA, Italy). Endothelial cell density (ECD), endothelial morphology and mortality were monitored pre-processing, 24h post-decontamination and post-processing. Antibiotic residues in the corneal tissue were determined by HPLC after processing.

Results: Pre-processing, 50% of the tissues stored in decontamination medium A were contaminated (Staphylococcus spp, E. Coli, C. Albicans); all tissues resulted decontaminated at the end of the process. The corneas showed unvaried mortality till the end of the storage; an altered endothelial morphology and ECD reduction were observed starting from the 14th day as compared to controls. 54% of the tissues decontaminated at 4°C overnight with decontamination solution B and then stored either in Eusol-C or in Tissue-C was contaminated pre-processing (Staphylococcus spp.); all tissues resulted decontaminated at the end of the process. ECD, endothelial morphology and mortality rate resulted unvaried 24h after decontamination and at the end of the process both for organ culture and cold storage. Control tissues, which were stored under organ culture conditions, showed 75% of contamination pre-processing and 45% contamination at the end of the process. HPLC analysis showed the absence of antibiotic residues in all investigated tissues at the end of the process.

Conclusions: Overnight decontamination at 4°C using decontamination solution B allowed to eliminate all contaminants from donor corneas without tissue alteration. These process conditions resulted compatible both with organ culture and cold storage.
USE OF FIVE-YEAR CORNEAL GRAFT SURVIVAL FOR THE VALIDATION OF EYE BANK QUALITY STANDARDS

J. Armitage¹, M. Jones², I. Zambrano³, F. Carley³, D. Tole¹

¹School of Clinical Sciences, University of Bristol, Bristol Eye Hospital; ²NHS Blood & Transplant; ³CTS Manchester Eye Bank, Manchester Royal Eye Hospital, UK

Purpose: Analysis of the influence of donor and recipient factors on five-year graft survival for validation of the quality standards applied in the CTS Eye Banks in Bristol and Manchester.

Methods: Corneas stored by the CTS Eye Banks between April 1999 and March 2005 were included in the study. First, a logistic regression analysis was carried out to determine the influence of donor factors on the suitability of corneas for penetrating keratoplasty (PK). Only one cornea randomly selected from each donor was included in this analysis. For corneas in this cohort that were assessed as suitable and transplanted, the influence of donor and recipient factors on five-year graft survival of first PK was investigated. Survival data were analysed by univariate methods (Kaplan-Meier survival) and multiple regression (Cox proportional hazards), as appropriate.

Results: Suitability for PK (n=7107). Donor age (p<0.0001) and storage time in organ culture (p<0.0001) were the principal factors affecting suitability. Death to enucleation time and enucleation to processing time had little influence. Corneas from organ donors were more likely to be suitable for PK (p=0.0003). Five-year graft survival (n=3014). The only donor factor affecting graft survival was gender with a greater risk of failure associated with corneas from male donors (HR 1.3, 95% CI 1.1 to 1.5, p=0.008). Graft survival was predominantly influenced by the indication for PK. Kaplan-Meier five-year survival estimates ranged from 91% (95%CI 89 to 93) for keratoconus to 57% (95%CI 53 to 60) for bullous keratopathy. Among the other pre- and postoperative factors that had a significant impact, allograft rejection was a major risk factor for failure (HR 2.6, 95%CI 2.1 to 3.3, p<0.0001).

Conclusions: While donor factors, in particular age and storage time in organ culture, influenced the suitability of corneas for PK, these and other factors such as post-mortem times to enucleation and processing had no effect on five-year graft survival. Donor sex was the only donor factor found to influence graft survival. The indication for PK and other recipient factors (i.e., preoperative risk factors and postoperative complications) were the main predictors of graft failure. These data therefore support the donor and cornea selection criteria applied by the CTS Eye Banks in the UK; namely, no upper donor age limit, death to enucleation times up to 24 hours, storage by organ culture for up to four weeks, and a minimum endothelial cell density for PK of 2200 cells/mm².
INVITED LECTURE: MASSIMO BUSIN, ITALY: ULTRATHIN DSAEK – THE PRESENT STATUS
Chairman, Department of Ophthalmology at “Villa Serena-Villa Igea” Hospitals in Forlì
Professor, University Eye Hospital in Bon (Germany)
President, SITraC (Società Italiana Trapianto di Cornea)

Biography: Massimo Busin obtained his MD degree from the University of Ferrara (Italy) in 1980 and trained at the Ferrara Eye Hospital to become an ophthalmologist in June 1984. Between July 1984 and June 1986 he was fellow in “cornea and external diseases” with Herbert E. Kaufman at the LSU Eye Center in New Orleans (USA). He was a consultant at the University of Orange Free State in Bloemfontein (Rep. of South Africa) from July 1986 till December 1986. Since January 1987 he joined the Faculty of the University Eye Hospital in Bon (Germany), where he became Privat Dozent in 1989 and University Professor in 2001. Before moving back to Italy in 2006, he was granted the apl (ausserplanmaessiger) Professorship, which he still holds. Since January 1996 Professor Busin is the chairman of the Department of Ophthalmology at “Villa Serena-Villa Igea” Hospitals in Forlì (ITALY). Professor Busin is author of 106 peer reviewed articles, 21 chapters in books, as well as 1 book. He has been Associate Editor for Europe of the Refractive & Corneal Surgery (now Journal of Refractive Surgery) till 1997 and presently serves regularly as reviewer for all major ophthalmologic Journals, including Ophthalmology, AJO, Archives of Ophthalmology, JCRS, etc. Since 1984, Professor Busin has delivered over 400 lectures as invited speaker at all major meetings in the world, including AAO, DOG (Deutsche Ophthalmologische Gesellschaft), ASCRS (American Society of Cataract and Refractive Surgeons), ESCRS (European Society cataract and Refractive Surgeons), DOC (Deutsche Ophthalmologische Chirurgen), SOI (Società Oftalmologica Italiana), SFO (Société Français d’Ophtalmologie), etc. He has received the “Honor Award” of the AAO (American Academy of Ophthalmology) in 1993 and the “Senior Honor Achievement” award of the AAO in 2003, the Gold Medal “Maestro dell’Oftalmologia” of SOI in 2012, and will deliver the Chancellor’s Award Lecture in Neurosciences and Ophthalmology in January 2013 at the LSU Eye Center in New Orleans (USA). He has also received several prizes for both videos and lectures delivered at international meetings, among which the AAO, the ESCRS, the DOC, the ASCRS, etc. Professor Busin is presently President of SITraC (Società Italiana Trapianto di Cornea)

Summary: Ultrathin DSAEK: The Present Status
The lecture will present a complete review of Descemet Stripping Automated Endothelial Keratoplasty (DSAEK), focussing in particular on the development of the technique for the dissection of ultrathin (UT) donor tissue. History as well as basic principles necessary to understand the mechanisms of UT DSAEK will be introduced to the attendees. In setting the indication to UT DSAEK, the author will discuss the role of recipient corneal status, type of endothelial disease, concomitant eye disease (i.e. glaucoma), presence of clear lens and other preoperative factors. The different techniques of conventional DSAEK, UT DSAEK and Descemet membrane endothelial keratoplasty (DMEK) will be compared, pointing out the single steps, which are instrumental in facilitating surgery, while improving the final outcome. In case of multiple intervention, combined versus sequential procedures (e.g. phacoemulsification, IOL surgery, vitrectomy, etc.) will be discussed. The authors will highlight advantages and disadvantages of different surgical approaches. Possible implications for eye banking will also be addressed, with particular emphasis on preparation and storage of tissue for UT DSAEK and DMEK. Slides and videos of case studies will illustrate the most common complications (i.e. graft detachment, dislocation, failure, rejection etc.) as well as the appropriate solutions.
**PRECUTTING OF DONOR CORNEAS FOR POSTERIOR LAMELLAR KERATOPLASTY**

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The Danish Eye Bank, Department of Ophthalmology, Aarhus University Hospital, Denmark

**Purpose:** An increasing number of Eye banks offer precut corneas for posterior lamellar keratoplasty. At present there are no published studies on the outcome of DSAEK with precut organ-cultured tissue. This study compares surgeons-cut versus precut grafts.

**Methods:** In the Danish Eye-bank, precutting is performed with the Horizon DSAEK system. Corneas are de-swollen for 24-hours in transport medium, and the appropriate single-use cutting head is chosen from ultrasound pachymetry. After cutting, the cap is replaced and the cornea is distributed for use. Thirty-six grafts from the first six months of precutting were compared with 31 grafts from the last six months of surgeons-cut tissue using the Moria ALTK with a 350 head. Pentacam data was used to determine central and peripheral thickness of the lamellar graft and the endothelial count was determined using specular microscopy.

**Results:** Average follow-up was 338 ± 37 days and 214 ± 63 days for surgeons-cut and precut grafts, respectively. No difference in preoperative and postoperative endothelial cell count was observed. Average central thickness of the graft was similar in both groups, being 164 ± 52 µm for surgeons cut and 179 ± 47 µm for precut tissue, respectively. Peripheral thickness also was similar in both groups. There was no significant difference between surgeons cut and precut grafts with respect to cutting failures (2 versus 4), rebubbling rate (1 versus 2) or primary failures (1 versus 1).

**Conclusions:** Precut, organ cultured donor corneas for posterior lamellar keratoplasty appear to be comparable to corneas cut in the operating theatre.
PREDICTABILITY OF PRE-CUT SINGLE-PASS ULTRA-THIN DSAEK LAMELLAE THICKNESS WITH A NOVEL OPERATOR-INDEPENDENT HANDS-FREE MECHANICAL MICROKERATOME SYSTEM

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Purpose: To report our preliminary experimental results using the Gebauer SLc operator-independent hands-free mechanical microkeratome system for single-pass pre-cutting of ultra-thin DSAEK lamellae by eye bank technicians.

Methods: Twenty-five human donor corneas unsuitable for transplantation were obtained from the Euro Cornea Bank, Beverwijk, The Netherlands. Corneas were preserved in minimal essential medium (MEM) and transferred to a transport medium containing 6% Dextran 24 hours prior to dissection. Corneoscleral buttons were mounted on the artificial anterior chamber of the Gebauer SLc microkeratome system (Gebauer, Neuhausen, Germany) and pressure was adjusted to 65 mmHg. Following epithelial removal, central corneal thickness was measured by ultrasonic pachymetry (Corneo-Gage Plus, Cleveland, OH). The Gebauer SLc system equipped with either a 400, 450, 500 or 550-µm head, depending on the thickness of the cornea was used for lamellar dissection. For each cornea a new blade was used. Following dissection, the thickness of the cut anterior lamella was measured using the Vogel electrical micrometer (resolution 1 µm; Vogel GmbH, Germany).

Results: All lamellar dissections were completed successfully without complications. The mean (± SD) cutting depth using the Gebauer SLc microkeratome system was 392 ± 20 µm with the 400-µm head (n=5), 459 ± 19 µm with the 450-µm head (n=9), 505 ± 19 µm with the 500-µm head (n=6) and 552 ± 11 µm with the 550-µm head (n=5).

Conclusions: The Gebauer SLc operator-independent hands-free mechanical microkeratome system enables single-pass dissection of DSAEK lamellae with a low standard deviation allowing the cornea bank to safely provide the surgeon with precut tissue of any desired thickness. Further studies assessing endothelial cell viability and clinical outcomes of such grafts are needed.
STUDY OF STROMAL FEMTOSECOND LASER ABLATION FOR DEEP CORNEAL CUT OPTIMIZATION

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Purpose: Anterior and posterior stroma of human cornea present different biophysical characteristics, the later being more hydrated and collagen fibers less tightly packed. Our aim was to investigate interactions between femtosecond laser (FL) and stroma according to the depth of cut in order to optimize FL endothelial graft preparation.

Methods: Organ cultured human corneas were prepared with a mechanical microkeratome (Moria, France) by a lamellar cut from anterior side at two different depths: 50µm for the study of anterior stroma and 350µm for the study of posterior stroma. Grooves were then performed in the remaining anterior or posterior stroma with a 800nm, 150fs FL (Thales, France) with different processing configurations (Speed: 1900 to 10000µm/s; Power: 0.8 to 6mW). After treatment, corneas were observed by light and second harmonic generation (SHG) microscopy to compare ablation rates (AR) (in µm/pulse) and cut quality in anterior and posterior stroma.

Results: Preliminary results (n=4 corneas) showed no significant differences between posterior stroma AR (1.97±0.91µm/pulse) and anterior stroma AR (1.73±0.50µm/pulse). Using SHG microscopy, two different cutting types (with or without disruption) occurred, depending on processing configurations and independently of depth in the stroma.

Conclusions: These results suggest that there is no significant difference of AR between anterior and posterior stroma. Consequently, difficulties usually encountered to cut endothelial grafts with FL may not directly depend on a particular ablation rate of the posterior human corneal stroma, but rather on the optical scattering when FL passes through the stromal layers, already identify as a limiting factor.
SURFACE TOPOGRAPHY AND 3-DIMENSIONAL OPTICAL PROFILING OF FEMTOSECOND AND NOVEL MECHANICAL MICROKERATOME DISSECTED POSTERIOR HUMAN CORNEAL DISCS FOR DSAEK

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Purpose: To evaluate and compare the surface topography and roughness of human posterior corneal buttons for DSAEK dissected with a femtosecond laser (FS), a novel hands-free operator-independent mechanical microkeratome system (MK) and an operator dependent MK.

Methods: Eighteen human corneas unsuitable for transplantation were obtained from the Euro Cornea Bank (ECB) Beverwijk, The Netherlands. Posterior corneal buttons for DSAEK were dissected with either the Intralase FS60 laser (Abbott Medical Optics, Santa Anna, Ca.) using optimized settings, the Moria ALTK operator-dependant MK (Moria, Antony, France) or the Gebauer SLc operator-independent MK (Gebauer, Neuhausen, Germany). Dissection using the Gebauer SLc MK and the Moria ALTK MK was performed by experienced eye bank technicians and an experienced corneal surgeon, respectively. Following dissection, corneas were fixed in a 3.0% Glutaraldehyde solution and examined by a confocal profiler (Sensofar PLu2300, Sensofar, Terrassa, Spain) and by environmental scanning electron microscopy (ESEM). Linear Mixed Model analysis was used to quantify the difference in surface roughness between the different techniques.

Results: Lamellar dissection was successful in all cases but one, cut with the Moria ALTK MK, equipped with the 450-µm CB head, due to donor perforation. Confocal profiling was successful in all cases allowing quantitative surface roughness analysis and 3-dimensional reconstruction of the central 2x1.5 mm² area of the dissected grafts. Complimentary ESEM imaging allowed wide field analysis of all samples at lower magnification. Surface roughness of FS60 dissected tissues was significantly higher (P < 0.001) than that of both operator-dependent and independent mechanically dissected tissues, despite optimized laser settings. No significant difference in surface roughness was found between operator-dependent and independent dissection although different surface topographies were observed with both techniques.

Conclusions: Surface roughness of FS60 dissected corneal buttons for DSAEK was significantly higher than that of mechanically dissected tissues, despite optimized laser settings. No significant difference in surface roughness was found between operator-dependent and independent mechanical dissection. Confocal profiling, a novel imaging modality used in this study for the first time for quantitative stromal bed roughness analysis, enables non-contact submicron measurements and 3-dimensional reconstruction of large clinically significant areas. Future clinical use of this technology could be useful in the diagnosis and follow up of corneal pathologies.
ULTRA-THIN DECEMET STRIPPING AUTOMATED ENDOTHELIAL KERATOPLASTY-FIRST RESULTS
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Purpose: To present our results of Ultra-thin Descemet stripping automated endothelial keratoplasty (UT-DSAEK) in eyes with pseudophakic bullous keratoplasty (PBK) and compare these results to conventional DSAEK.

Methods: A prospective case series of 10 PBK eyes undergoing UT DSAEK (group 1) and 30 PBK eyes undergoing conventional DSAEK (group 2) for the treatment of PBK. Both surgeon-cut and “pre-cut” tissue obtained from certified eye banks was used. All patients underwent serial central graft thickness measurements with non-contact optical coherence tomography (Zeiss Visante™ AS-OCT) at various time points after surgery. The eyes in DSAEK group were subdivided into 3 subgroups based on a 1st day postoperative endothelial graft thickness: 2a) thin grafts (thickness:<180 μm), 2b) medium thick grafts (≥180≤ 250 μm) and 2c) thick (thickness:>250μm). Differences between the groups regarding best spectacle-corrected visual acuity (BSCVA) and endothelial cells density loss (ECD) were recorded.

Results: There was no statistically significant difference in age, sex, or preoperative BSCVA between groups. The median postoperative graft thickness in group 1 was 78±21.30 μm and in group 2 it was 190±47.61 μm. Postoperative follow-up was 3-18 months. UT DSAEK group showed better postoperative BCVA both in quantity (≥0.8) and speed of recovery (at 1 month) as compared to all conventional DSAEK groups. Thin DSAEK grafts (<180 μm) had best visual acuity among DSAEK subgroups and reached BCVA of 0.5 at 6 months postoperatively, while thick grafts never reached BCVA of UT or thin DSAEK grafts.

Conclusions: UT-DSAEK provides faster and more complete visual rehabilitation as compared to conventional DSAEK. Corneas with thicker grafts does eventually improve but not to the BCVA obtained with ultra-thin grafts. Moreover, big advantage of a „fast“ visual recovery is lost.
INITIAL ENDOTHELIAL CELL LOSS AFTER DSAEK USING A NOVEL DONOR INserter.
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Purpose: to measure and to evaluate the endothelial cell loss in the first 6 months after Descemet´s stripping automated keratoplasty (DSAEK) using a novel coaxial irrigating donor inserter (Endosaver®, Ocular Systems).

Methods: This is a retrospective study on 45 eyes where a standardized DSAEK procedure using a coaxial irrigating donor inserter through a 4.1 mm. clear corneal incision was performed. Fuchs dystrophy (24/45), bullous keratopathy (14/45) and late endothelial failure after previous penetrating keratoplasty (7/45) were the main preoperative surgical indications. Surgery was performed under peri-bulbar anaesthesia. In 18 cases, cataract extraction by standard phacoemulsification with a four haptics in-the-bag IOL implantation was performed at the same time, previous to the donor lenticle insertion. After standard descemetorrhexis performed under air or viscoelastic, corneal incision was enlarged to 4.1 mm. Donor posterior lamellar disk was obtained with the Moria 350 micron microkeratome head using the artificial anterior chamber. Pachymetry of the donor was routinely performed with an ultrasound pachymeter. Donor was cut with a disposable Barron´s punch trephine at the desired diameter. Donor lenticle was then placed onto the Endosaver holding spatula and folded inside it. The inserter was introduced in the anterior chamber and the donor was gently inserted while a coaxial irrigation was activated with the phacoemulsification irrigating system to avoid anterior chamber collapse. Donor was repositioned on the posterior corneal surface by a complete refilling of the anterior chamber with an air bubble, which was partially removed after ten minutes. Donor preoperative endothelial cell density (ECD) was measured in the eye bank with the Konan Cell-Check EB-04 RU specular microscope. In the postoperative period, non-contact specular microscopy (Topcon SP 2000-P) measurements of the donor endothelial cell density were performed after 1, 3 and 6 months post-op.

Results: Donor preoperative mean ECD was 2575 ± 219 (2132/3165) cells/mm2. After 1 month mean ECD was 2285 ± 542 (1971 – 3055 ) cells/mm2 indicating an 11,26% endothelial cell loss. After 3 months mean ECD was 2177 ± 564 (1741 – 2981 ) cells/mm2 , a 15,45 % of endothelial cell loss. After 6 months post-op mean ECD was 1979 ± 459 ( 1130 – 2941 ) cells/mm2 meaning a 23,14% endothelial cell loss. No intraoperative complications were observed. Postoperative complications occurred in 8 cases including graft dislocation (3/45:6,6%), graft exchange (2/45: 4,4%), conversion to PKP (1/45: 2,2%), pupillary block (1/45: 2,2%) and cystoid macular edema (1/45: 2,2 %).

Conclusions: endothelial cell loss using a novel irrigating donor inserter is similar to those described using other donor insertion techniques. The decreasing of the size of the incision to 4.1 mm. may not imply a significant endothelial cell loss compared to other insertion techniques. The simplicity of use of this inserter has made this technique as our procedure of choice.
POSTERIOR LAMELLAR KERATOPLASTY – POSTOPERATIVE RESULTS AFTER MODIFICATION OF SURGICAL TECHNIQUE.
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Purpose: Presentation of modified surgical technique and its impact on postoperative results.

Methods: Too high flap misalignment and lack of adhesion in early postoperative period led to modification of surgical treatment. Between January 2011 and June 2012 38 lamellar posterior keratoplasty were performed with changed technique. Charts of all patients were revised to analyze results.

Results: Among 38 procedures performed 4 grafts needed refixation with air injection into anterior chamber. Macular oedema, papillary block and persistent corneal erosion were postoperative complications. Postoperatively 39,5% of endothelial cell loss was observed within first 3 months, average ECC reached 1890 cells/mm². Early failure appeared in 4 patients, in three penetrating keratoplasty was performed, in the one case lamellar grafting was redone. BCVA in the follow-up was 0,6 (0,01-0,9)

Conclusions: In 89,5% of patients flap was properly fixated after modification of surgical technique. Concerning endothelial cell loss, BCVA and complications satisfied postoperative results were achieved.
Purpose: To report on the mid-term visual outcomes and stability after Descemet membrane endothelial keratoplasty (DMEK).

Methods: DMEK was performed for Fuchs endothelial dystrophy, bullous keratopathy or previous corneal transplant failure in 300 consecutive eyes. The best corrected visual acuity (BCVA) was documented before, and after surgery at 1, 6, and 12 months, and annually thereafter.

Results: 97.4% of eyes reached a BCVA of ≥20/40 (0.5) within the first months after surgery, which was sustained over 5 years. A BCVA of ≥20/25 (≥0.8) was reached by 77.6% at 6 months (n=228), and 87.5 at 5 years (n=8). A BCVA of ≥20/20 (≥1.0) was obtained by 45.6% at 6 months and 62.5% at 5 years. Furthermore, a BCVA of even ≥20/17 (≥1.2) was obtained by 14.5% at 6 months and 12.5% at 5 years.

Conclusions: DMEK provides a fast and often complete visual recovery. Furthermore these visual outcomes appear to remain stable for at least 5 years after surgery. Thus, DMEK surgery seems to surpass other EK techniques in visual rehabilitation and final visual outcome.
ENDOTHELIAL CELL DENSITY AFTER DESCemet MEMBRANE ENDOTHELIAL KERATOPLASTY (DMEK): 1 TO 6 - YEAR FOLLOW-UP
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Purpose: To assess the rate of decline in endothelial cell density (ECD) for patients up to 6 years after Descemet membrane endothelial keratoplasty (DMEK).

Methods: From a larger group of 300 consecutive patients who underwent DMEK for Fuchs endothelial dystrophy or pseudophakic bullous keratopathy, ECD measurements were available for 254 eyes with 6 months follow-up; for 234 eyes with 1 year follow-up; 130 with 2 years follow-up; 63 with 3 years follow-up; 25 with 4 years follow-up; 9 with 5 years follow-up, and 7 eyes with 6 years follow-up.

Results: Our findings show a 35% sharp decrease in ECD in the first 6 months after DMEK, followed by an annual decrease of approximately 9%.

Conclusions: The rate of endothelial cell loss in patients up to 6 years after DMEK closely resembles the published reports for patients after alternate forms of endothelial keratoplasty. This, combined with evidence that >¾ of patients achieve visual outcomes of ≥20/25 (≥0.8) at 6 months after surgery, may indicate that DMEK could become a preferred treatment method in corneal endothelial disease.
THE ROLE OF THE DONOR IN DMEK SURGERY
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Purpose: During the past years, there is enormous progress in lamellar endothelial keratoplasty techniques. Currently, it seems that Descemet membrane endothelial keratoplasty (DMEK) can lead to superior clinical outcomes. But the technique of graft preparation and surgical technique is not yet standardized and very sensible. Potential factors influencing duration and complications during surgery are investigated. We hypothesize that donor age may correlate with the size of the graft roll for DMEK.

Methods: In 28 DMEK procedures, graft preparation was performed by one experienced surgeon during surgery and a record was taken. The diameter of the enrolled DMEK-graft at the end of the preparation was measured. Correlation was drawn between size of the DMEK-roll and the unfolding time of the graft in the anterior chamber. Statistical analysis was performed using multifactor corrected regression analysis, respecting as well some more donor characteristics.

Results: There was statistical significant correlation of donor age and size of DMEK-rolls (p<0.01). Surprisingly, smaller rolls did not lead to longer unfolding times even though there are some statistical spikes.

Conclusions: In DMEK-surgery, not only surgical technique is essential, but also donor characteristics like age can influence the clinical outcome. Younger DMEK-grafts form smaller rolls that can be unfolded by an experienced surgeon. But looking at some statistical spikes, it seems advisable especially for young surgeons to first chose older donors for DMEK. Successful DMEK surgery starts with chosing of the donor.
INVITED LECTURE: DONALD TAN, SINGAPORE: EYE BANKING CHALLENGES IN ASIA – CAN THEY BE OVERCOME?

Medical Director, Singapore National Eye Centre (SNEC)
Chairman, Singapore Eye Research Institute (SERI)
Professor of Ophthalmology, National University of Singapore
Medical Director, Singapore Eye Bank
President, Association of Eye Banks of Asia

Biography: Donald Tan is the Medical Director of the Singapore National Eye Centre (SNEC), Chairman of the Singapore Eye Research Institute (SERI), Professor of Ophthalmology at the National University of Singapore, Chair of the Eye Academic Clinical Program at the Duke-NUS GMS, and Medical Director of the Singapore Eye Bank. Involved primarily in clinical and translational research in cornea, refractive surgery and myopia, he has published over 300 peer-reviewed articles (h index = 42), contributed 18 book chapters and holds 13 patents in stem cell culture, myopia prevention, refractive corneal implants and surgical devices for endothelial keratoplasty. He has trained 22 corneal fellows from 13 countries, and is the recipient of over 20 awards, which include the AAO 2006 Distinguished Achievement Award, the ISRS/AAO 2009 Casebeer Award, the Saudi Ophthalmological Society 2010 Gold Medal, the Australia and New Zealand Corneal Society 2011 Doug Coster Award, the Canadian Society of Ophthalmology 2011 W. Bruce Jackson Award, the 2012 EuCornea Medal, and the Portland, Oregon Arthur Devers 2012 Lecture. Professor Tan established the Asia Cornea Society in 2007 and the Association of Eye Banks of Asia in 2009, and is currently President of both societies. In 2012 he assumed the Presidency of the US-based Cornea Society, its first International President.

Summary: Eyebanking Challenges in Asia – can they be overcome?

Asia has the greatest burden of world blindness, representing 53% of the 285 million globally blind. Corneal blindness may represent only 4% of global blindness, but when combined with other forms of infections and ocular surface disease, such as trachoma and keratomalacia, is second in importance only to cataract. Whilst the majority of corneal blindness occurs in Asia, and in Africa, it is these same continents which have the least developed eyebanking structures, standards and corneal donors. Causes of corneal blindness in Asia are also significantly different from the West, with more severe inflammatory and infectious disorders and later stage presentations. The Asia Cornea Society (ACS) was formed in 2007 by corneal leaders in Asia, and is a professional society dedicated to provide educational initiatives and research into corneal and external diseases, and clinical networking amongst eyecare professionals. ACS initiated the Association of Eye Banks of Asia (AEBA) in 2009, a supranational organization bringing eye banking organizations throughout Asia under one roof, with the aim of enhancing, regularizing and unifying eye banking standards, providing educational support and advocacy for eye donation in Asian countries, and developing new sources of donor tissue, in the form of establishment of new model eye banks and initiation of tissue sharing initiatives, all geared towards helping to alleviate corneal blindness in Asia. The formation of the new National Eye Bank of Sri Lanka (NEBSL) represented a model AEBA eye bank, and is the result of a collaboration between a developing Asian country (Sri Lanka), and an industrialized Asian country (Singapore), represented by the Singapore Eye Bank (SEB). Launched in 2011, NEBSL currently procures around 600 corneas a year, transporting tissue of the highest quality standards, to several Asian countries, with the potential for exceeding 1000 corneas a year. The NEBSL-SEB alliance also initiated the first pre-cut donor tissue in Asia in 2012, the same year when draft medical standards for Asian eyebanking were developed by AEBA. Eyebanking challenges in Asia can be overcome, and progress is being made.
ELECTROLYTE COMPOSITION OF FOUR EYE BANK MEDIA DURING CORNEAL PRESERVATION

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Purpose: To compare four different eye bank media for electrolyte composition and changes to concentrations during corneal preservation. Potential implications of variations in electrolyte composition will be discussed.

Methods: 24 human donor corneas were preserved in either: Optisol at 4°C, Minimum Essential Medium (MEM) containing 2% Fetal Calf Serum (FCS) at 32°C, MEM with 8% FCS at 32°C or Stem Alpha (STA) serum-free organ culture medium at 31°C. Cornea-free control media for each group was also stored. Samples were drawn at 0, 3, 7, 14, 21 and 28 days of storage and analyzed for K+, Na+, Ca2+, Cl−, glucose and lactate using a blood gas analyzer. No change of media was performed during the storage period.

Results: Mean concentrations of K+ at day 0 were 3.58, 4.56, 5.70 and 4.22 mmol/l for Optisol, 2% and 8% FCS in MEM and STA culture media respectively. Concentrations of Na+ were 174.1, 126.6, 136.9 and 129.8 mmol/l, Ca2+ 0.70, 1.33, 1.34 and 1.13 mmol/l and Cl− 103.3, 101.4, 113.6 and 97.8 mmol/l. Comparing the concentrations at Day 0 against Day 28, no statistically significant difference was found, with the exception of Ca2+ in STA and Opt.

Conclusions: Concentrations of electrolytes in various eye bank media differ. Concentrations vary little though the preservation period. Some electrolyte concentrations are not within the physiologic range when compared to concentrations in aqueous humor or tear fluid. Optimizing electrolyte composition of eye bank media may be beneficial for the quality of donor tissue.
INTEGRITY OF HUMAN CORNEAL EPITHELIUM MAINTAINED IN ORGAN-CULTURE USING CORNEAMAX®

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Purpose: Development of medium for organ-culture during eye banking is based on endothelium integrity. Nothing is described in the literature about conservation of corneal epithelium with CorneaMax® during banking. Therefore, we wanted to examine the integrity of human corneal epithelium maintained in CorneaMax®.

Methods: All procedures conformed to the tenets of the Declaration of Helsinki for biomedical research involving human subjects. Human corneas, considered unsuitable for transplantation, were obtained from the Eye Bank in Lausanne and maintained in organ-culture in Corneamax® at 32°C. Average post-mortem time was 14 hours. Different time points were analysed from 0 to 35 days (N=5 for each time points). Epithelial integrity was evaluated by H-E staining and by immunostaining with antibodies against E-cadherin and ZO-1. Proliferation and apoptosis were assessed by immunostaining with antibodies against Ki67 and Caspase 3 respectively.

Results: During the first three days of culture the epithelium lost its adherence to the basal lamina of the cornea creating a large epithelial sheet. Some remaining limbal basal cells could be detected, allowing regeneration of the epithelium between day 2 to day 10. From day 10, the depth of the epithelium is reduced, consisting of only two to three cell layers. No change is observed in the distribution of E-cadherin and ZO-1. Ki 67 staining demonstrated that the whole cornea proliferated during the 35 days of organ-culture. Apoptosis was rarely detected in the corneal epithelium.

Conclusions: Corneas maintained in CorneaMax® showed a complete disappearance of the corneal epithelium during the two first days and a conservation of limbal basal cells in the limbal region. These remaining cells allowed a full regeneration of the tissue, leading to an atrophic epithelium, composed of only two to three cell layers. Following regeneration, adherens and tight-junctions proteins are detected, suggesting that the epithelium integrity is maintained. This study is a first step to develop medium in organ-culture in order to conserve corneal epithelial cells.
PROSPECTIVE CLINICAL EVALUATION OF HYPOTHERMIC VS ORGAN CULTURED CORNEAL GRAFTS
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Purpose: To compare clinical outcome of corneal transplantation patients using corneas preserved by hypothermic (group 1) or organ culture storage (group 2).

Methods: Prospective study included 180 patients who underwent perforating keratoplasty in Eye Clinic Svjetlost between March 2009 and April 2012. Patients were divided into two groups according to the donor corneal storage. Donor charts were reviewed for: death to preservation time (DP), condition of the epithelium in storage and preservation to surgery time (PS). Follow-up was 6-36 months and included graft transparency, best corrected visual acuity, endothelial cell loss and other complications. The outcomes in two groups were compared.

Results: In 75% transplanted patients donor corneas were preserved in hypothermic storage and 25% patients had their corneas stored in organ culture. Preoperative status of donor tissue from group 1 was: epithelial exposure in 28% of corneas, epithelial defect in 4%, epithelial sloughing in 24%, intact epithelium in 45% and DP time was 9,5 (±6) hours. In group 2 there was no epithelial defects and average DP time was 17 (±6.8) hours. Early epithelial defect (EED) occurred in 14,8 % of patients in group 1 immediately after PK, and none in group 2. EED have transferred to persistent epithelial defect in 23% of patients. In group 2, visual acuity was better at month 1, but after 6 up to 36 months there was no significant difference. Graft reaction occurred in 10,7% of patients in group 1 and 5,7 % in group 2. Overall corneal graft survival rate in patients in group 1 was 91,6%, in group 2 97,2% and in patients with PED 62,5%. Other complications such as postoperative astigmatism, intraocular pressure elevation and cataract formation did not differ at any time between the two investigated groups.

Conclusions: Organ cultured donor corneas have lower rate of epithelial problems in a recipient eyes. Final graft outcome, ECD loss and visual outcome are not significantly different between patients with or without EED but in case of PED graft rejection becomes higher.
A NOVEL OBJECTIVE METHOD TO EVALUATE THE OVERALL QUALITY OF CORNEAL TISSUE USED FOR COMPARATIVE STUDY BETWEEN TWO HYPOTHERMIC PRESERVATION MEDIA

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Purpose: To demonstrate and validate a new evaluation technique for calculating the overall quality of the cornea and to check its efficacy in a comparative study for corneas preserved in Optisol - GS and a new formulation Cornea Cold®.

Methods: 24 pairs of unsuitable corneas with intact epithelial layer and good morphology were selected for this study. 12 Right and 12 left corneas (and vice versa) were placed in each medium for a 4 week comparative study to reduce the bias. The study was divided into 4 phases, where phase 1 was open and the rest were blinded (masked observers). Endothelial cells were stained with trypan blue and were counted under a light microscope to check the mortality and cell density manually. The endothelial cell density and the mortality were taken in consideration together to measure the viable endothelial cell density. Difference between epithelial and endothelial layer was evaluated microscopically for thickness measurement which was also confirmed later using OCT measurements. A transparency device was used for calculating the degree of transparency. Epithelium was stained using trypan blue to check the integrity, morphology and viability. All the above subjective parameters were converted to objective values for determining the overall quality of the cornea. Statistical analysis was used to confirm the significant difference.

Results: The conversion to objective values from subjective analysis helped to evaluate the quality of corneas at different time intervals of preservation in different media. Students t-test showed statistically better results (p<0.05) from week 2 when thickness, transparency and overall quality were considered whereas statistical difference was observed from week 1 (p<0.05) for morphology and viable endothelial cell density for the corneas that were preserved in Cornea Cold® rather than in Optisol-GS. Epithelial quality was similar regardless of the medium.

Conclusions: The overall quality evaluation of cornea presented here is efficient, consistent and easy. This new technique could be useful for comparative studies and to value corneas for eye banks, biobanks and research or transplantation purposes. Cornea Cold® is a promising corneal preservation medium for hypothermic storage with slightly longer preservation time. This permits higher flexibility, evaluation accuracy, surgical manipulation and ease of transportation.
Purpose: In European eye banks, 15-20% of corneas are discarded for inappropriate ECD. Given the importance of a precise, robust and reproducible ECD, we organized an international survey of the quality of ECD determination.

Methods: The Euro-Keratotest study reproduced 2 surveys driven in 2003 and 2008 by our team in the 18 French eye banks (Transplantation2004), with substantial improvements: test slides (3rd generation keratotests) were fabricated with technologies employed in micro-optics, from images of real human corneas (Optics Letters2012). Twelve different mosaics with ECDs covering the usual range observed in eye banks, were created in a 8x8 mm quartz square. Keratotests, observable with transmitted light or specular microscopes were sent simultaneously to all volunteer eye banks (n=100). Each technician had to determine ECD and morphometry of the 12 mosaics with his/her standard counting method. Data were collected on a specific website.

Results: A first analysis of 120 technicians of 38 eye banks will be presented. It allowed identification of inter and intra bank variability and of bias likely involved (inappropriate counting strategy or wrong microscope calibration) and susceptible to be improved.

Conclusions: Participation of the eye banks to this survey using 3rd generation keratotests improves our knowledge on the reliability of cell counting methods in eye banks, and help standardize graft quality assessment. Keratotests are also perfect tools for the initial formation and continuous training of eye banks technicians, as well as for the eye banks certification. Grant: Interregional Hospital Clinical Research Project 2011, Ministry of Health, DIRC Rhônes-Alpes.
EVALUATION OF DONOR CORNEAS DURING STORAGE BY FULL-FIELD OPTICAL COHERENCE TOMOGRAPHY

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Assessment of the donor corneal stroma in eye banks is an important issue. In fact several corneal conditions, such as keratoconus, scars after infectious disease, or refractive surgery, are contraindications to the use of donor corneal tissue for penetrating or anterior lamellar keratoplasty. Current techniques do not fulfill this issue with the expected efficiency. Gross examination of the donor cornea can only detect severe opacities and slit-lamp examination of the donor tissue is difficult when corneas are retrieved by in situ excision. In addition post mortem corneal edema makes more difficult the fine evaluation of the stroma with a slit lamp. Full field optical coherence tomography (OCT) is a safe, non-invasive, and non-destructive process which permits optical in-depth biopsies of gross tissue within minutes with a 1-µm 2D and 3D histopathological resolution, and easy exploration, acquisition, and rendering in DICOM format. The donor tissue is examined immersed in its storage medium. En-face sections of the tissue are acquired and various 2-D or 3-D images can be obtained.

Analysis of human donor corneas, either normal or diseased, was performed with the Light-CT ScannerR. In normal donor corneas, the epithelium, Bowman layer, keratocytes, stromal lamellae, and Descemet's membrane could be clearly imaged. Corneas with impaired endothelium function featured stromal edema and subepithelial fibrosis. Stromal scars after infectious keratitis or ocular burns were easily distinguished from non-affected stroma. Application of a freeze probe on normal donor tissue resulted in loss of the superficial and mid epithelial layers. Defects in Bowman layer were easily detected.

Full-field OCT appears to be a useful tool to assess the donor corneal stroma during storage. This new technology is complementary to conventional assessment of the donor endothelium performed in eye banks.
ROCK INHIBITOR ENHANCES ADHESION AND WOUND HEALING ON HUMAN CORNEAL ENDOTHELIAL CELLS EX VIVO AND IN VITRO

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Purpose: Maintenance of corneal transparency is crucial for vision. When endothelial cell density falls below a critical threshold, the barrier and “pump” functions of the endothelium are compromised and this results in the formation of a corneal oedema and loss of visual acuity. The conventional treatment for such severe disorder is transplantation of cornea. Unfortunately, there is a worldwide shortage of donor corneas. Recently it was reported that the ROCK inhibitor Y-27632 promotes adhesion, inhibits apoptosis, increases the number of proliferating monkey corneal endothelial cells in vitro and enhance corneal endothelial wound healing both in vitro and in vivo in animal models. Here, we proposed to assess the potential of this compound to increase the number of corneal graft available for the clinic.

Methods: Using organ-culture human cornea (N=34), the effect of ROCK inhibitor was evaluated either in vitro or ex vivo. Toxicity, ECD, cell proliferation, apoptosis, cell morphometry, adhesion and wound healing process were evaluated by standard cell counting method, EdU labelling, Ki67, Caspase3, Zo-1 and Actin immunostaining.

Results: In our study, we demonstrated for the first time in human endothelial cells ex vivo and in vitro, that ROCK inhibitor did not induce any toxicity effect and did not alter cell viability. Compared to animal model, ROCK inhibitor treatment did not induce human endothelial cell proliferation. However, ROCK inhibitor significantly enhances corneal endothelial cell adhesion and wound healing.

Conclusions: The present study shows that Y-27632, a selective ROCK inhibitor, has no effect on human corneal endothelial cells proliferative capacities, but alters cellular behaviours. It induces a change in cell shape, increases cell adhesion and enhances wound healing ex vivo and in vitro. Even the results were promising in animal models; this inhibitor is not able to induce human endothelial cell proliferation of organ-culture human cornea.
DELIVERY OF MOLECULES INTO CORNEAL ENDOTHELium USING NANOposites ACTIVATED BY FEMTOSECOND LASER PULSES: PROOF OF CONCEPT

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**Purpose:** The so-called NanoFemtoTransfection (NFT) is an innovative and promising non-viral technique to transfer molecules into cells (Charatskyy. Nature nanotechnology 2011). It consists in temporarily permeabilizing cell membrane by a photoacoustic effect obtained by nanoparticles of black carbon activated by Ti-Saphir femtosecond laser (fsL) pulses. Calcein (622 Da), tagged bovine serum albumine (70 kDa) and one eGFP plasmid (5 MDa) were transfected into two non-adherent cell lines (DU145 prostate-cancer and GS-9L rat gliosarcoma). Our aim was to adapt the NFT to adherent human corneal endothelial cells (HCEC).

**Methods:** We tested the NFT of calcein in vitro on the HCEC line HCEC-12 seeded at 1500 cells/mm² in 6 wells plates and ex vivo on whole human organ cultured corneas. A matrix of experiments comprising 4 exposition times, 6 fluences and 2 fsL beam movements was performed in order to obtain transfection with minimal toxicity. After exposition to fsL, nuclei were counterstained with Hoechst33342 and transfection efficiency was determined by observation on a fluorescence inverted microscope (IX81, Olympus, Japan) and further quantified by flow cytometry (FACSCalibur, BD, CA). Viability was assessed by Trypan blue staining.

**Results:** In HCEC-12, a fluence of 100 mJ/cm² and a laser beam movement of 3.5 mm/s gave a transfection of 17% and a viability of 97%. In whole corneas, with the same parameters, transfection was detectable in disseminated EC.

**Conclusions:** We obtained the POC of the NFT in HCEC. Further optimization is ongoing to increase the transfection rate while maintaining minimal toxicity, especially for bigger molecules, like plasmids.
DNA DAMAGE IN DONOR CORNEAL ENDOTHELIUM UPON TRANSFER FROM OPTISOL GS TO ORGAN CULTURE
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Purpose: The upper limit for storage of donor corneas in Optisol GS is relatively short. Transfer of such tissue to an Eye Bank Organ Culture (EBOC) system may increase the life span, reduce the number of donor corneas discarded due to expired shelf life, and increase the overall pool of donor tissue. We have recently examined the limbal epithelium and shown that such transfer is compatible with a maintained regenerative potential and expression of key morphological characteristics (Haug K et al. 2012). We here examine the endothelium on donor corneo-scleral rims for DNA damage after primary storage in Optisol GS and after a subsequent incubation for 1 week in Eye Bank Organ Culture (EBOC).

Methods: Comet assay (single cell gel electrophoresis) was used to measure DNA damage in corneal endothelial cells before (n=7) and after (n=5) transferring from cold storage to EBOC. Electrophoresis of lysed and enzyme (FPG, Endo III and T4 Endo V)-treated samples results in structures resembling comets, observed by fluorescence microscopy. The intensity of the comet tail relative to the head reflects the frequency of DNA altering lesions (Collins AR & Azqueta A 2012).

Results: The level of strand breaks was relatively low in both cold-stored tissue and in the tissue incubated in EBOC for 1 week. Samples retrieved after EBOC showed a significant decrease in FPG-sensitive sites reflecting less oxidized purines (mainly 8-hydroxy guanine). Accumulation of dimers induced by UV-light are indicated by the net T4 endo V-sensitive sites, which is observed to be quite high in both groups. The amount of oxidized pyrimidines, given by digestion of Endo III is however significantly decreased upon EBOC incubation.

Conclusions: We have found that enzyme-sensitive sites relevant for DNA damage in corneal endothelial cells seem to be decreased using a two-step storage procedure. Our findings may be explained by a detachment of more severely damaged cells, by less infliction of cellular damage, and also by an activation of DNA repair mechanisms in the more physiological micro-environment afforded by the EBOC system.
VISIONGRAFT® STERILE CORNEA – NEW OPTIONS IN OCULAR SURGERY

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VisionGraft® sterile cornea (Tissue Banks International, Baltimore, Maryland, USA) is a clear, acellular, terminally-sterilized, gamma irradiated allograft with a two year shelf life at room temperature. This novel graft provides a new alternative to addressing ocular surgeries such as corneal ulcers with micro-perforation, keratoprosthesis – associated corneal melt, limbal mass, pterygium, chemical burns, chronic ulcerative keratitis, glaucoma drainage tube coverage, glaucoma filtration surgery, refractory glaucoma, etc. Traditionally, treatments of these conditions were managed using fresh corneal tissue, glycerin preserved corneas, sclera, pericardium, amnion or tissue adhesives. VisionGraft® sterile cornea can be used in corneal procedures that do not require a viable endothelium.

Histopathology and electron microscopy studies demonstrate similar mean interfibrillar distance of collagen fibrils between fresh corneal tissue and VisionGraft® corneas. Light transmission microscopy shows that VisionGraft® corneas are mostly similar to that of fresh corneal tissue. Suture pull studies demonstrate comparable tensile strength between the fresh and VisionGraft® corneas.

Gamma irradiation offers additional patient safety and virtually eliminates the risk of bacterial or fungal disease transmission. In addition, gamma irradiation inactivates antigen-presenting cells; functionally eliminating an immune stimulating allogenic response and reducing rejection potential.

VisionGraft® sterile cornea can be used to cover glaucoma draining devices. The tissue remains durable and clear providing for visualization of the drainage tube, therefore provides a treatment window for correction of blockage, if necessary. At the same time, it is cosmetically more appealing to the patient compared to traditionally used sclera and pericardium.

This processing method enables use of corneas with low endothelial cell counts thus increasing tissue availability and utilization of corneas worldwide. VisionGraft® Sterile cornea provides an excellent alternative to use of tissues like, sclera and pericardium and is readily available for trauma and emergency cases. Its use does not require a facility with an eye bank or support from a local eye bank as the tissue can be stored prior to use.
FALSE NEGATIVE RESULTS IN TISSUE BANKING: THE CORNEAL TISSUES
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Purpose: The use of antibiotic cocktails during corneal processing can lead to an antibiotic carry-over effect, which in turn can generate false negative results in microbiological analysis. To investigate the impact of antibiotic residues on microbiological analyses of organ cultured donor corneas.

Methods: Twenty-four corneal tissues were retrieved by the personnel of the Eye Bank of Monza (Italy) and transported to the bank in Eusol-C (AL.CHIMI.A., Italy). Tissues were transferred to Tissue-C (AL.CHIMI.A., Italy), stored for 12-14 days at 31°C and then placed in the deswelling/transport medium Carry-C (AL.CHIMI.A., Italy) at room temperature for 24 h. Microbiological analyses were performed pre-processing on Eusol-C and post-processing on Tissue-C and Carry-C by the Eye Bank of Monza, according to bank standard procedures using BacTEC plus aerobic/anaerobic and, in parallel, by AL.CHIMI.A. with sterility test according to the European Pharmacopeia, after removing potential interfering antibiotics with the ResEP™ device (AL.CHIMI.A., Italy).

Results: Pre-processing microbiological analysis of the media, after removing potential interfering antibiotics with ResEP™ device, showed that 75% of the samples were contaminated (Staphylococcus spp.). 25% of such contaminations were not detected by BacTEC, thus yielding false negative results. 45% of the samples remained positive (Bacillus spp., Candida spp. Staphylococcus spp.) at the end of the process. None of these contaminations was detected by BacTEC.

Conclusions: Removal of antibiotic residues from corneal storage media with the ResEP™ device resulted in a significant number of false negative results in the microbiological analyses of the media. The presence of contaminants in the media at the end of the storage process indicates that standard corneal storage media do not guarantee efficient decontamination of donor corneas.
EMERGENCY PREPAREDNESS

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The Eye Bank for Sight Restoration, Inc., USA

Emergency preparedness -- the plan everyone needs and hopes never to use. Are eye banks prepared to meet the needs during an emergency without having to experience a crisis first-hand? In the past 12 years, The Eye-Bank for Sight Restoration has had to rely on perseverance and resourcefulness to combat the effects of two physical disasters -- an act of terrorism in September, 2001 and Superstorm Sandy in October, 2012. Both crises disrupted the services The Eye-Bank provides to donor families, surgeons and recipients in the greater New York metropolitan area. Following Superstorm Sandy in October, The Eye-Bank office was closed and without electricity, phone and Internet service for two and a half weeks. This presentation will offer a view of coping from remote locations during the aftermath of a physical disaster while maintaining standard operating procedures specified by regulatory authorities. The goal of this presentation will be to encourage eye banks to build contingency plans into their SOP manuals that can be utilized in the event of a power emergency.
INVITED LECTURE: MARIAN MACSAI, USA: HISTORY OF EBAA AND PROJECT NOTIFY

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Biography: Marian S. Macsai, MD is the immediate past Chair of the Eye Bank Association of America and presently holds the position of Secretary/Treasurer of the Cornea Society. She is also Chief of the Division of Ophthalmology at NorthShore University HealthSystem and Professor of Ophthalmology at the University of Chicago Pritzker School of Medicine. She also serves as Medical Director of the Eye & Vision Center located at Glenbrook Hospital. Dr. Macsai is a Board Certified Ophthalmologist with fellowship training in cornea and refractive surgery. Her areas of specialties include corneal transplants, refractive and cataract surgery, as well as medical and surgical treatment of diseases of the external eye. Dr. Macsai serves as an examiner for the American Board of Ophthalmology. She is a member with thesis of the American Ophthalmologic Society. Dr. Macsai is nationally recognized as an expert in the fields of refractive and corneal surgery and has served as a guest speaker at numerous academic institutions, both in the United States and Worldwide. She has been the recipient of numerous honors and awards including the Paton Award, Distinguished Contributions in Medicine Award, and other outstanding teaching/education awards. Dr. Macsai has served on the Food and Drug Administration Ophthalmic Devices Panel and currently serves on the Advisory Committee on Blood and Tissue Safety and Availability (ACBTSAs). Dr. Macsai also serves on the World Health Organization Biovigilance and Surveillance Committee.

Summary: History of EBAA and Project Notify
The Eye Bank Association of America was founded in 1961 by a small group of corneal transplant surgeons. It has developed into an international organization that meets the needs of patients in providing safe tissue in a fair and equitable manner. The establishment of Medical Standards and a Medical Advisory Board has set a standard that is recognized as an authority by the FDA and numerous international organizations. Over the past few years in conjunction with the WHO and international transplant organizations, the need for global vigilance and surveillance of transplantation has been recognized. Project Notify is the first tool developed to help members of the transplant community find information about serious adverse events involving transplantation worldwide. This is the first tool of its kind that is the result of global cooperation to further the advancement in knowledge and prevention of disease transmission through transplantation.
MANAGEMENT IN NON-TRAUMATIC CORNEAL PERFORATIONS

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Materials: In January 2010-July 2012 twenty-two patients with non-traumatic corneal perforations or descemetocoele were admitted to our department. Dry eye syndrome in patients with rheumatoid arthritis was diagnosed in 10 cases. Bacterial (8) and viral (4) ulcer was diagnosed in 10 cases.

Methods: Non-surgical steps included: corneal and conjunctival microbiological culture, topical medications: antibiotics (fluoroquinolones), atropine, artificial tears, ointment with vitamin A, contact lens application, lacrimal silicone plugs implantation. Surgical procedures: Amniotic membrane transplantation covering the whole surface of the cornea by amniotic membrane, placing amniotic membrane in previously performed corneal pocket in the anterior part of the cornea. Keratoplasty full-thickness graft (7-8 mm diameter of trephination), full-thickness mini-graft (4-5 mm), anterior graft, posterior mini-graft. Tectonic graft (epi-keratoplasty) covering with full-thickness corneo-scleral graft. Blepharorraphy as a procedure supporting corneal healing kantorrhaphy or blepharorrhaphy, temporary or permanent.

Results: In 21 cases corneal perforation was successfully healed. In 10 patients visual acuity improved. In 8 cases during two-years follow-up additional surgical treatment was needed, including tectonic corneo-scleral graft in two patients, keratoplasty with full-thickness graft after previous amniotic-membrane treatment and exchanging corneal button in two patients. In one case, treated non-surgically, mini-keratoplasty was performed due to progressive corneal melting that occurred in 6 months after initial treatment. In one patient enucleation was performed due to severe corneal melting and loss of light perception.

Conclusions: Non-traumatic perforations or severe descemetocoele diagnosed in many anterior segment pathologies might lead to the loss of the eyeball if untreated. Topical treatment, contact lens application and tissue glue are among non-surgical therapeutic options. In progressive corneal melting resistant to that kind of therapy application of amniotic membrane, keratoplasty, tectonic corneo-scleral graft or blepharorrhaphy are possible treatment procedures. Choosing appropriate method depends on surgeon’s experience and tissue availability in the Eye Bank. Long-term and complex treatment is needed concerning general disorders that might have influence on corneal perforation.
ANATOMICAL AND FUNCTIONAL RESULTS OF PENETRATING QUERATOPLASTY IN PATIENTS WITH ACANTAMOEBA-KERATITIS
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Purpose: to describe the anatomical and functional outcomes of patients who underwent penetrating keratoplasty for acanthamoeba keratitis.

Methods: This is a retrospective study on 16 eyes of 16 patients who underwent penetrating keratoplasty for acanthamoeba keratitis at our eye center from 1995-2009. Data on preoperative factors like use of contact lenses, visual acuity and treatment, as well as post-operative data like graft diameter, intraocular pressure, complications and treatment were evaluated. Graft survival was evaluated with the Kaplan-Meier life table analysis and comparisons were performed using Log-rank.

Results: 68% of the patients were women, and mean age was 35.5 (+/- 12.6, range: 13-56) years. 68% reported use of contact lens prior to infection. 6.8% reported ocular trauma as a possible cause of the keratitis. In the post-operative follow-up, 56% developed glaucoma, 75% developed cataract, 12.5% had retinal detachment and 6.2% ended up in endophthalmitis. 9 patients required a second penetrating keratoplasty due to graft failure, 1 patient required a third keratoplasty. 43% maintained a clear graft during the first year post-operation. 2 patients had recurrence of acanthamoeba keratitis onto the graft.

Conclusions: Penetrating keratoplasty is a viable treatment option in patients with acanthamoeba keratitis who do not respond to medical treatment. However, graft survival has guarded prognosis due to the aggressiveness of the infection, as well as the medical treatment which has to be continued in the post-operative period. Post-operative glaucoma and cataract are post-operative complications which may condition the anatomical and functional success of the graft.
CATARACT AND FUCH’S DYSTROPHY: DSAEK AND PHACO OR STAGED PROCEDURE?
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Purpose: Patients with corneal Fuchs dystrophy and cataract present a challenge for surgeon. Sometimes it is difficult to determine the best treatment option, cataract surgery alone or cataract surgery combined with keratoplasty. Combined surgery, especially with Descemet Stripping Automated Endothelial Keratoplasty (DSAEK) is becoming popular among many surgeons. Correct calculating of intraocular lens can be a problem in combined surgery. In our hospital up to now we prefer to perform phacoemulsification (PHACO) alone and DSAEK if needed. In this retrospective study we show our results with cataract surgery in patients with corneal Fuch’s dystrophy.

Methods: PHACO was performed in 38 eyes of 23 patients with moderate cataract and corneal Fuch’s dystrophy. Ozil torsional PHACO and dispersive viscoelastic were used. Mean preoperative endothelial cell density (ECD) was 1118±220 cell/m². We prospectively followed up all patients for six months after surgery and measured ECD preoperatively, 1, 3 and 6 months after PHACO, visual acuity, corneal thickness (Visante anterior OCT) and need for lamellar keratoplasty.

Results: Mean ECD loss after 6 months was 17.7%. Mean best corrected distant visual acuity (BCDVA) was 0.52 and mean corneal thickness was 0.64 mm. Four eyes of 38 (10.52%) underwent DSAEK 4-6 months after cataract surgery.

Conclusions: Patients with corneal Fuchs dystrophy can obtain satisfying visual acuity after cataract surgery. If necessary, DSAEK is recommended as secondary procedure.
Purpose: To present two patients with implanted multifocal intraocular lenses (MFIOL) who underwent corneal transplantation for PBK.

Methods: In last 4 years we have done 2 PK and 1 DSAEK in eyes with MFIOL. In a first case 64 year old women underwent penetrating keratoplasty (PK) for her left eye, and two years after DSAEK for her right eye. In a second case, 77 year old women underwent PK for her right eye. In follow up time of 2 years we compared visual recovery, BCVA, postoperative astigmatism, endothelial cell loss, and graft outcome.

Results: The eye that underwent DSAEK procedure showed better postoperative BCVA, both in quantity and speed of recovery as compared to other 2 that underwent PK. DSAEK eye achieved BCVA 0.9 in less than 1 month postoperatively, while other two eyes needed longer recovery time, more than 4 months to reach BCVA of 0.6. There were significant difference in postoperative astigmatism; in DSAEK it was 1 and in PK 3.5 dicyl. Endothelial cell loss in DSAEK eye was 39% and in PKP eyes was 37% in two years follow up. All grafts were clear. Patient's satisfaction was much higher in DSAEK-eye as compared to PK.

Conclusions: DSAEK is preffered procedure when dealing with bullous keraopathy since it provides faster visual rehabilitation as compared to PK. Low amount of induced astigmatism seems to be especially important in PBK eyes with already implanted multifocal IOL.
RECURRENT OF ANTERIOR CORNEAL DYSTROPHIES AFTER KERATOPLASTY

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Purpose: To describe the rate of simple recurrence (SR) and/or clinically significant recurrence (CR) of different kinds of anterior dystrophies in donor corneas after keratoplasty.

Methods: Retrospective case series of patients who underwent keratoplasty for anterior dystrophies from 1954 to 2008 with a minimum follow-up time of three years. Kaplan Meier survival curves were calculated for both parameters (SR and CR) to demonstrate recurrence through time.

Results: A total of 109 eyes of 66 patients were included: 8 cases of Bowman’s dystrophy (6 eyes with CDRB/Reis Bucklers and 2 eyes with CDTB/Thiel Behnke), 19 cases of CDG/Granular, 53 cases of CDL/Lattice and 29 cases of CDM/Macular. The median follow-up time for each kind of dystrophy varied from 75 months (6.25 years) to 180 months (15 years). The survival function for simple recurrence was significantly higher (376.2 months/31 years) for cases of CDM, as compared to those dystrophies related to the keratoepithelin gene: CDRB = 177.6 months, CDL = 168 months and CDG = 89.2 months. Clinically significant recurrence (CR) was noted predominantly in cases of CDL and CDG.

Conclusions: Keratoplasty (whether penetrating or lamellar) is an effective treatment for cases of anterior dystrophies, when visual axis is affected or when other less invasive treatment modalities fail. The statistically significant difference in survival time of recurrence after keratoplasty for the different kinds of anterior dystrophies coincides with literature and may help us in prognosticating the best treatment option for each individual case.
DONOR RISK FACTORS FOR GRAFT SURVIVAL AFTER PENETRATING KERATOPLASTY
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**Purpose:** To assess the impact of donor factors on graft survival after penetrating keratoplasty (PK).

**Methods:** Review of donor records from all PK performed at our hospital during the period 2005-2010. Data on donor parameters and current graft status were collected and statistically evaluated for possible relationship to graft failure by means of Cox regression analysis.

**Results:** Increasing donor age appears to have a weak negative effect on graft survival (HR=1.036, 95% CI 1.002-1.072). This relation was not confirmed when comparing survival of corneas from donors aged 65 or less vs. older ones. Other donor cornea parameters were not significantly associated with graft failure.

**Conclusions:** Donor factors do not seem to impact significantly PK graft survival when within the approved corneal bank parameters.
USE OF ANTI-VEGF (BEVACIZUMAB) AFTER PENETRATING KERATOPLASTY (PK) FOR HERPETIC KERATITIS

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Purpose: To improve corneal graft survival rate after PK for herpetic keratitis by bevacizumab treatment combined with conventional therapy.

Methods: Prospective study of 15 eyes with postherpetic corneal scar which underwent PK: 6 male caucasian eyes (average age 48,67 years), and 9 female caucasian eyes (average age 54,45 years). All patients underwent PKP surgery ended by subconjunctival bevacizumab injection (25 mg/ml), local and systemic acyclovir and conventional postgraft treatment. Grafts were prospectively (average 20 months; range:6-36) examined for clearance and presence of neovascularization (NV).

Results: 93.3% of corneal grafts remained clear at the end of the observation period. BCVA improvement was from 0.062 (range: light perception – 0.25) to 0.62 (range 0.3 – 1.0); 28% of eyes had high postoperative astigmatism (> 5 diopter, range 5 – 10), 7% IOP elevation, 14,2% had recidivant herpes infection on transplant, 21% had cataract formation, 21% had graft reaction.

Conclusions: Addition of subconjunctival bevacizumab injection to convencional therapy after PK for herpetic keratitis improves the survival rate in our practice up to 93.3%.
PREDICTION OF CORNEAL GRAFT FAILURE DUE TO PREOPERATIVE MEASUREMENT OF VEGF IN RECIPIENT CORNEA
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Purpose: To estimate the probability of corneal graft reaction (or failure) due to preoperative measurement of vascular endothelial growth factor (VEGF) in recipient cornea.

Methods: Twenty-five patients were operated from October 2006 till March 2007 for penetrating keratoplasty. Recipient corneal button were examined for the presence and quantity of VEGF using ELISA (R&D System, USA). All the patients were postoperatively treated with topical steroid treatment. Patients were followed up for 3 years. Control group for evaluation of value of VEGF in cornea were cadaver’s corneas.

Results: All rejection episodes and most graft failures occurred during the first postoperative year. Eight grafts showed one of the signs of graft rejection in a first six months after surgery, some with reversible effect after enhanced steroid treatment. All 6 grafts which were finally rejected, despite the fortified treatment, had significantly higher amount of VEGF in their corneas at time of the surgery (mean 354.5 pg/ml, range 289.31-425.17 pg/ml) as compared to controls having VEGF of 142.28 pg/ml (range: 49.31-281.72 pg/ml).

Conclusions: Increased VEGF value in the recipient cornea at the time of PK may predict higher amount of corneal graft reactions and final graft rejections in grafted eyes.
INVITED LECTURE: KEVIN CORCORAN, USA: EYE BANK ASSOCIATION COLLABORATION AND OUTREACH

President & CEO, Eye Bank Association of America
Board of Directors, ASAE Business Services, Inc.

Biography: Kevin Corcoran is the president and chief executive officer of the Eye Bank Association of America. Since joining EBAA in January 2012, he has initiated a new strategic planning process, an outreach program to foster more member collaboration and engagement, the development of a legislative and regulatory advocacy program and enhancements to the association’s communications efforts. Kevin has spent over 20 years in non-profit management, serving as the CEO of the National Association of Health Underwriters, where he transformed an organization on the verge of bankruptcy, doubling revenue and increasing membership by 50% in eight years; and the American Chiropractic Association, where he led the chiropractic profession’s first comprehensive collaboration, ultimately encompassing over 25 organizations. He also founded a successful consulting practice serving the non-profit sector. Kevin holds a Bachelor of Science degree in Business Administration from Georgetown University and the Certified Association Executive designation, granted by the American Society of Association Executives (ASAE). He serves on the Board of Directors of ASAE Business Services, Inc. and is a chapter author of ASAE’s Membership Marketing handbook.

Summary: Eye Bank Association Collaboration and Outreach

The Eye Bank Association of America is undergoing a significant transition as it welcomes it its first new CEO in over 20 years and responds to changes in the American healthcare system and within the U.S. eye banking community. As a result, EBAA has developed a comprehensive strategic plan that reflects a new perspective on how it can serve its members and the profession, and what it can offer, and learn from, the global eye banking community.
INVITED LECTURE: THOMAS FUCHSLUGER, GERMANY: ARTIFICIAL CORNEA

Head, LIONS Cornea Bank in North Rhine-Westphalia
Consultant, Department of Ophthalmology at the University Hospital Düsseldorf
Chair, Cornea Section, European Association for Vision and Eye Research

Biography: Thomas Fuchsluger, MD FEBO MSc, is a clinician-scientist with an own research group located at the Dept. of Ophthalmology at Heinrich-Heine-University in Düsseldorf, Germany. He is head of the Lions Eye Bank at Düsseldorf University Eye Hospital. His research focus is gene and cell therapy to corneal cells using viral vectors and nanoparticles and ocular surface reconstruction by bioengineered, biocompatible tissues. Since scientific stays in Kyoto/Japan and Harvard Medical School, Boston MA, USA, he gained particular expertise in studying and regulating cell death pathways of corneal cells. His group is working on translational approaches relating to corneal transplantation, both during corneal storage and after engraftment. Dr Fuchsluger is associate editor with Acta Ophthalmologica and the Open Journal of Ophthalmology. Recently, he was elected Chair of the „Cornea & Ocular Surface“ section of the European Association for Vision and Eye Research (EVER), the leading ophthalmological research association in Europe which covers all areas of ophthalmology and the visual sciences.

Summary: Artificial cornea
Artificial corneas present an alternative to conventional corneal transplantation using corneal tissue. The field can be divided into „classical“ keratoprostheses and „biomimetic“ scaffolds. The presentation will give an overview over different strategies and recent developments in both groups.
IDENTIFICATION OF LABEL-RETAINING ENDOTHELIAL CELLS IN ADULT HUMAN CORNEAS: A NEW CLUE FOR THE EXISTENCE OF ENDOTHELIAL STEM CELLS

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**Purpose:** The lack of self-renewal capacity of human corneal endothelial cells (EC) in vivo was explained by cell cycle arrest in the G1-phase due to cell contact inhibition, TGF-beta signaling, and stress induced premature senescence. Nevertheless, their residual ability to divide in primary culture suggests the existence of progenitor cells, probably located at the endothelial periphery (Whikehart, MolVis2005; He.StemCells2012). Stem cells are slow-cycling cells characterized by their quiescent state in niches and their ability to retain for a long time markers of S-Phase like BrDU or EdU. Aim: to search for the presence of label-retaining EC in human corneas.

**Methods:** Label retaining EC were searched by 5-Ethynyl-2’-Deoxyuridine (Click-it EdU) incorporation during long-term culture: 30 days for organ cultured human corneas (n=10) or 15 days for in vitro primary cultured EC (n=5), both followed by a 30-day culture without EdU. Flat-mounted corneas and EC cultures were observed with an inverted fluorescent microscope.

**Results:** Label-retaining EC were observed in the peripheral area of all OC corneas, varying from 1 to 50. Numerous label-retaining EC were also present in all primary cultures, always attached to Descemet membrane fragments.

**Conclusions:** The presence of label-retaining EC constitutes a new clue for the existence of corneal endothelial stem cells in human. Their apparent scarcity is consistent with the inability of the human corneal endothelium to repair in vivo, but isolation and expansion of these endothelial stem cells or progenitors could allow development of bioengineered endothelium.
VIABILITY OF HUMAN LIMBAL EPITHELIAL CELLS CULTURED ON DIFFERENT TYPES OF SCAFFOLDS
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Purpose: Limbal stem cell deficiency has been treated for the last 14 years more or less successfully using in vitro expansion of autologous or allogenic cells isolated from small bioptate of limbal epithelium. There are various techniques of culturing human limbal epithelial cells. Some of them are using mouse 3T3 cells as a feeder layer according to Rheinwald &Green method for keratinocyte cultivation. In addition, several cell scaffolds have been used for this purpose like: amniotic membrane, fibrin glue or contact lenses. In the field of tissue engineering, new types of scaffolds made from nanofibers offer favourable 3D environment for cell attachment and growth thanks to interconnected pores and a very large surface-to-volume ratio. It was our intention to test new scaffolds made from nanofibers for limbal cell proliferation and viability.

Methods: Nanoscaffolds were prepared from solutions of polyurethane and polycaprolactone polymers using electrospinning method. Prior to the cell seeding they were cut into 14 mm diametre circles, disinfected with UV-light and hydrated by soaking into 70% 50%, 25%(v/v) ethanol, sterile distilled water and Hank’s balanced salt solution. Fibrin glue scaffolds were prepared from commercial fibrin glue kit (Tisseel, Baxter) diluted with aprotinin and 1.1% NaCl and 1mM CaCl2 solution. Cryopreserved human amniotic membrane was obtained from our tissue bank and defrosted in the cell culture medium. Cells were seeded on an intact basal side. Surgical excess of human limbal epithelium from 8 donors were treated with 0.25% trypsin. Trypsinized suspensions of limbal cells were seeded on an irradiated mouse 3T3 feeder cells in 2:1 ratio. Cells were cultured until 80% of confluence and frozen. Defrosted cells were seeded with 3T3 feeder cells in 24 well plates: cell culture plastic gel alone, amniotic membrane, fibrin, polyurethane and polycaprolactone nanoscaffolds and contact lenses (Focus Night&Day, lotrafilcon A, CIBA Vision, Dublin). Viability of cells was determined fluorimetrically using Fluoroskan II (Labsystems) with CellTiter-Blue (Promega, Madison, WI, USA) reagents. Presence of CK3, CK12 and p63 markers was determined by immunofluorescence using a confocal microscope (Leica, TCS SP2 AOBS). Cell cultures were photographed by Scanning Electron Microscope as well.

Results: Cultured limbal epithelial cells showed the best viability when cultured on plastic and fibrin gel compared to other tested scaffolds. A statistically significant difference in viability of cultured limbal cells was found between cultures on fibrin gel and cultures on all other scaffolds. Immunofluorescence staining showed the presence of p63 marker of limbal stem cells and CK3 and CK 12 markers of differentiated corneal cells in cultures on all tested scaffolds. SEM photographs of nanoscaffold surfaces showed good cell attachment and colony spreading on both – polycaprolactone and polyurethane scaffolds.

Conclusions: All tested scaffolds showed good cell viability and colony spreading of seeded limbal cells and could be used as a method of cell delivery for therapeutical purposes. Nanoscaffolds had lower cell viability compared to fibrin and plastic gel. Although high porosity and large surface promised superior cell attachment and spreading, this was not the case. The reason could be in hydrophobic properties of nanoscaffold surface. Modifications of these surfaces with chemicals such as NaOH that could enhance their wettability and hydrophilicity should be tested against limbal cell cutivation.
EPITHELIAL AND PROGENITOR CELL MARKERS OF OCULAR SURFACE IN MESENCHYMAL STEM CELLS FROM HUMAN ADULT ADIPOSE TISSUE
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Purpose: Analyze the expression pattern of cytokeratins and progenitor cell markers in mesenchymal stem cells from human adult adipose tissue (ADS).

Methods: We carried out a qualitative and quantitative analysis expression of different cytokeratins (CK) in ADS cells by immunocytochemistry (IFI), Western blot (WB) and real-time PCR (qRT-PCR). Limbic sclerocorneal epithelial cells (LSC) and corneal epithelium cells (CO) were used as controls.

Results: ADS cells expressed a set of progenitor cell markers, including p63 and ABCG2. Furthermore, CK expression (CK12, CK76 and CK1/5/10/14) was observed in ADS cell cultures by IFI and WB, which demonstrate potential and capability to acquire epithelial-like cell characteristics. The presence of CK12 was also confirmed by PCR amplification. DNA fragment was amplified, purified, sequenced and compared with the human CK12 mRNA sequence. Finally, we observed comparative differences in the expression of progenitor cell markers between LSC, CO and ADS by qRT-PCR.

Conclusions: Adult ADS cells could be a potential source for cell therapy in ocular surface regeneration. The expression of putative stem cell markers and CK supports the hypothesis that ADS cells have self-renewal capacity and intrinsic plasticity that enables them to acquire some epithelial-like characteristics.
Purpose: Cryopreserved amniotic membrane (AM) is widely used in ophthalmology because of its beneficial clinical capabilities. For clinical use a careful testing for microbial contamination is essential to ensure a safe application. Up to now there has been no validated automated test system according to EU regulations to perform this task. In our study we evaluated the use of the BacT/Alert® test system for the screening of microbial growth in AM.

Methods: Sections of fresh AM which were cleaned of placental blood and tissue residues, cryopreserved AM (each about 5 cm²) were injected separately in BacT/Alert culture media test bottles. BacT/Alert I-AST® bottles were used to evaluate the growth of fungi and aerobic bacteria and BacT/Alert i-NST® bottles to evaluate anaerobic bacteria. Several bacterial and fungal test strains according to European Pharmacopoeia monograph 2.6.27 were applied to test the performance of the system (Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Candida albicans, Aspergillus brasiliensis, Propionibacterium acnes and clostridium sporogenes). About 10 to 100 colony forming units of each germ were applied on the samples prior to injection in the corresponding test bottles. For each strain about four bottles with AM samples were inoculated, two additional bottles only contained the germ to verify its growth while bottles with samples lacking germs served as negative controls. Bottles were incubated at 37°C for a period of seven days. In case of microbial growth within a test bottle the germ was subcultivated and identified.

Results: Growth of the test strains was detected in all inoculated samples within the seven days incubation period. Neither non-processed nor cryopreserved AM inhibited the growth of the germs, despite the standardised application of antibiotics in cryopreserved AM.

Conclusions: Since all applied test strains were detected, we conclude that the BacT/Alert® in combination with the i-NST and i-AST bottles system is suitable for testing of the microbial safety of amniotic membrane in clinical practice according to EU regulations.
INFLUENCE OF STORAGE CONDITIONS OF PLACENTAL TISSUE ON STERILITY AND HISTOLOGIC PROPERTIES OF AMNIOTIC MEMBRANE

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Purpose: Cryopreserved amniotic membrane (AM) is a common tool for the treatment of several ophthalmologic applications because it provides beneficial clinical effects. The preparation of AM needs to follow stringent standard operating procedures to ensure, quality and safety of the tissue for clinical application. Part of the SOPs is to define how long and under which conditions donor placentas can be stored without compromising clinical efficacy of the prepared tissue. Our aim was to evaluate the impact of storage time and temperature on clinical important features of AM.

Methods: After informed consent placentas were obtained and stored for different periods of time at various temperature conditions. In total there were six groups, each n=3, specified by the storage times and conditions: 1h at 8°C, 20°C and 40°C, 6h at 8°C, 1h at 40°C followed by 5h at 20°C and 1h at 20°C followed by 5h at 8°C. Afterwards AM was prepared from the placentas and examined in regard of tissue sterility, histologic appearance and the basement membrane composition. Sterility controls were performed at the department of microbiology. The AM was stained with hematoxilin/eosin and the presence of the basement membrane components Laminin, fibronectin, collagen IV and collagen VII was analyzed by means of immunohistochemistry.

Results: Only tissue from the group of samples which was stored for 1h at 40°C showed microbiological contamination with the bacterium Ralstonia pickettii whiles all other samples were negative for microbial growth. The histologic appearance of the tissue did not differ within or between the particular groups. Neither the general staining, nor the immunohistologic staining displayed a recognizable difference.

Conclusions: The detected contamination is most likely an artifact which resulted from bacterial contamination of the water bath used to hold the temperature of 40°C. There was no link between the examined storage conditions of the placenta prior to preparation and microbial growth on the tissue. The examined storage conditions also did not seem to have a significant effect on the histological structure of AM. Therefore we conclude, that storing the placenta up to 6h at these temperatures before preparation is tolerable for tissue quality of the AM.
VEGF\textsubscript{164} ANTIBODIES DELAY CORNEAL VASCULARIZATION AFTER ALKALI BURN

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**Purpose:** The purpose of the study is to determine if subconjunctival monoclonal antibodies to Vascular Endothelial Growth Factor (VEGF) 164 isoform can prevent corneal vascularisation and opacification after corneal burn in mice. Decreasing corneal opacification could preserve visual acuity and thus avoid corneal grafting. Preventing corneal vascularisation may additionally increase corneal grafting success rate. Next aim is to define if corneal burn or additional VEGF164 subconjunctival treatment affects the expression of VEGF, interleukin 1β (IL-1β) and matrix-metalloproteinase 9 proenzyme (proMMP-9) in mice cornea.

**Methods:** Control animals were not treated. Other mice were divided in three groups and underwent corneal burn with 1N NaOH. Unlike group 1, group 2 and group 3 were treated with anti-VEGF164 antibodies subconjunctivally, 1st day after burn and 1st, 3rd and 5th day after burn, respectively. Corneal opacification and vascularisation assessment and VEGF, IL-1β and proMMP-9 were followed during 3 weeks after burn.

**Results:** Groups 2 and 3 had lower grades of corneal opacification compared to group 1 during first five days after corneal burn. Corneal vascularisation appeared 5th day after burn in all groups and was significantly lower in groups 2 and 3 compared to group 1; 5th and 11th day in group 2 and from 5th to 21st day after burn in group 3, respectively. IL-1β, proMMP-9 and VEGF concentrations increased after burn and were not significantly affected by anti-VEGF164.

**Conclusions:** Using anti-VEGF164 subconjunctivally in different strategies (single injection 1st day and repeated injections 1st, 3rd and 5th day after corneal burn in mice) we decreased corneal opacification and delayed onset of vascularisation.
RESULTS OF MORPHOLOGICAL EXAMINATION OF THE CORNEAS STORED UNDER THE CRYOGENIC PRESERVATION

Eye tissue bank of the S. Fyodorov Federal State Institution IRTC “Eye Microsurgery”, Russia

Eye tissue bank of the S. Fyodorov Federal State Institution IRTC “Eye Microsurgery” procured 56 cryopreserved donor corneas, 43 of them were utilized in clinic for urgent meliorative and tectonic keratoplasties. Donor corneas in native condition were exposed to cross-linking procedure with subsequent cryopreservation under t -80°C. Corneas were investigated with light and scanning electron microscopy (SEM) after 1, 6 and 12 months of cryopreservation.

Light and scanning electron microscopy data revealed ultrastructure integrity of the corneal stroma and more dense structure of the interfibrillar space of the collagen fibers at all follow-up periods compared with corneas not exposed to cross-linking procedure. Yet we found apparent endothelial cell loss up to 60-78% compared to baseline on follow-up 12 months of cryostorage.

Corneas exposed to cross-linking on follow-up 12 months of cryostorage completely retain natural structure of the connective tissue however do not preserve enough endothelial cell density. Such corneas might be recommended for long-term donor reserve establishment and conducting urgent meliorative and tectonic keratoplasties.
Purpose: Typical ocular problems associated with Stevens-Johnson (SJS) include conjunctivitis, scarring of the conjunctiva, iritis, corneal blisters and perforation, which can potentially lead to permanent vision loss. The visual rehabilitation in such patients is difficult and often frustrating for both the patient and the physician. Patients with persistent corneal opacity may be operated by lamellar or penetrating keratoplasty, but with a very high risk of tissue rejection. We present a case report where in our highly motivated SJS patient we performed combined corneal grafting with transplantation of amniotic membrane (AMT) and intraoperatively and postoperatively administered bevacizumab (Avastin).

Methods: Case report. 79 years old female patient came to us with bilateral symblepharon, vascularized corneal leucoma, complete corneal conjunctivalization and complicated cataract. BCVA prior to surgery was light perception to both eyes. The patient underwent combined surgery: symblepharolysis, phacoemulsification (PHACO) with posterior chamber intraocular lens implantation (PCIOL), penetrating keratoplasty (PKP) and AMT. Avastin (2.5mg/1mg) was given subconjunctivaly at the end of the surgery. Postoperatively patient was treated with topical steroid/antibiotic, bevacizumab (throughout 4 months) and artificial tears drops and cyclosporine systemically. The transplant was obtained from a donor aged 64 years, with endothelial cell count of 2564 cells/mm2. At each visit BCVA was measured and a full eye examination was performed. Follow-up period was 24 months.

Results: The graft remained clear in early postoperative period; eye movements were normal in all directions. At 6th week of follow up neovascularization (NV) reached recipients bed and AMT was repeated together with subcobnjuctival bevacizumab injection. In a following 3 months the graft remained transparent, and no significant NV was observed, so local bevacizumab treatment was stopped. BCVA of 0.3 was achieved. No adverse reactions and no graft rejection was reported till 18th month postop when graft rejection occured, did not respond to the treatment, and the graft finally failed.

Conclusions: Corneal graft in a highest risk cases, such as SJS, can be additionaly proteced with the use of topical and subconjunctival anti-VEGF treatment added to the conventional one, at least on a short term basis. However, the side effects of a prolonged use of anti-VEGF treatment are not know yet, and it remains to be discovered whether even longer use of anti-VEGF could have prevented final graft failure.
KERATOPLASTY “A CHAUD”
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**Purpose:** To present and analyse our results in urgent keratoplasty

**Methods:** Retrospective study during two and a half years of 32 urgent keratoplasties

**Results:** Out of 32 surgeries 24 were successful, 2 cases ended in enucleation, 2 urgent reoperations, and 4 reoperations needed

**Conclusions:** Study results show that some undesirable outcomes could be potentially avoided by avoiding of overuse of steroids and timely referral of patients who are not responding to initial treatment.
Purpose: To present our results of penetrating keratoplasty (PK) combined with pars plana vitrectomy (PPV) being one of the most difficult surgical procedures in ophthalmology. In Eye clinic “Svjetlost” we performed 9 PK combined with PPV procedures out of total 270 corneal transplantations in period from 2009 to 2012. In most cases the corneal pathology was due to previous vitreoretinal surgery (silicone oil keratopathy) which was performed in other medical institutions. Indications for prior vitreoretinal procedures were diabetic retinopathy and retinal detachment. All vitreoretinal procedures were done with silicone oil tamponade. Because of reduced visibility of fundus there was no information about macula and optic nerve condition. However, there was enough will in patients to go through this complex surgical procedure.

Methods: The combined procedure was performed using temporary keratoprosthesis. PK was done by the corneal surgeon and PPV by the retinal specialist. The use of silicone oil tamponade was necessary in all 9 cases.

Results: The success of surgery was defined as clear corneal graft maintenance (6 out of 9 corneal grafts remained transparent), retinal attachment (retina remained attached in 8/9 patients) and normal intraocular pressure (average 16 mmHg ± 2.2 mmHg).

Conclusions: In patients with simultaneous corneal and vitreoretinal pathology PK combined with PPV is used to restore normal anatomical integrity of the eye with some maintained visual functionality.
CELL-BY-CELL ALIGNMENT OF REPEATED SPECULAR MICROSCOPY IMAGES
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Purpose: Modern specular microscopes (SM) can robustly depict the same central area of the corneal endothelium with the help of a built-in fixation light. However, repeated image acquisitions are slightly shifted and rotated because of variable positions in the chin and forehead rest. This effectively forecloses the manual tracking of individual corneal endothelial cells (CECs). We herein devise and validate an automated image registration algorithm that moves and rotates a SM image until the CECs coincide, given some overlap with the other image.

Methods: We selected 27 same-eye image pairs for the à priori presence of some overlap. We applied our registration method to capture the overlap in each pair. Two observers independently validated the registration results for correctness by means of alternation flicker. In order to assess the robustness of our tracking method, we also repeatedly applied our registration method on random image pairs (not from the same eye).

Results: All automated registrations of the same-eye image pairs turned out correct. However, one image incorrectly matched twice in 81 registration attempts between images not from the same eye. As it turned out, this image depicted only 73 cells. The average of cells dotted in all images was 253 (range 73-393).

Conclusions: Tracking of individual CECs is possible in non-contact SM images. However, at least around 100 CECs need to be identified on each image for adequate specificity. Our method can be used e.g. to robustly assess endothelial stability in clinical trials.
SURGICAL TREATMENT OF ACANTHAMOEBA KERATITIS – CASE REPORT
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Introduction: Acanthamoeba keratitis (AK) is a serious, debilitating, and intensely painful infection of the cornea caused by parasites of the genus Acanthamoeba. If it is not diagnosed early and treated aggressively, extensive ocular damage can occur. At present, diagnosis of the disease is not straightforward, and treatment of AK is very demanding. Despite the aggressive treatment, occasionally, the disease fails to respond. AK, originally associated with trauma with vegetative matter and exposure to contaminated water, in recent literature has been most closely linked to soft contact lens use, although it can occur even in the absence of contact lenses. The incidence of contact lens-related AK is still unclear, but it has been estimated that one in 300-1500 contact lens wearers may develop some form of AK over a 30-year period of contact lens wearing.

Methods: Case report.

Results: A 52-year-old woman, soft contact lens wearer was referred to our Clinic with cloudy vision, photophobia and a red, painful right eye. Swab corneal sample was taken and Acanthamoeba was proved. The patient was treated with chlorhexidine, brolene, atropine, ciprofloxacin and vigamox topically. 1 month after starting with therapy, the patient developed persistent epithelial corneal defect and secondary glaucoma. Visual acuity of the right eye was light perception. Combination of timolol and dorzolamid drops was included in therapy and amniotic membrane transplantation was performed. As no improvement occur, therapeutic penetrating keratoplasty was recommended. Triple procedure (phacoemulsification with posterior chamber intraocular lens implantation and penetrating keratoplasty) and anterior chamber lavage with voriconazol was the treatment of choice. Postoperative, the patient was treated with tobramycin, dexamethasone, voriconazole, chlorhexidine, combination of timolol and dorzolamid, brimonidin topically and voriconazole orally. The eye pressure was high despite the treatment with topical antiglaucoma drugs so the implantation of mini glaucoma shunt was necessary. Finally, after one year, the eye pressure was stabilized to normal level and corneal graft remained clear.

Conclusions: AK is a potentially blinding corneal infection which is often misdiagnosed. Early definitive diagnosis of AK and the prompt initiation of appropriate therapy are essential for a favorable clinical prognosis. Therapeutic penetrating keratoplasty is a method of choice when medical treatment fails.
RIGID GAS-PERMEABLE CONTACT LENS CORRECTION OF INFANT APHAKIA FOLLOWING CONGENITAL CATARACT SURGERY

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Purpose: To evaluate the functional outcome and safety of rigid gas-permeable contact lens wear for treating aphakia in congenital cataract infants following cataract surgery.

Methods: Outcomes of 75 infants treated with rigid gas-permeable contact lenses (CL) following cataract removal between 1987 and 2011 were evaluated. The infants were stratified into different groups: bilateral aphakia (Group I), monolateral aphakia with early (Group II) or late (Group III) surgery, aphakia with additional pathology (Group IV). Main parameters of analysis were visual acuity, keratometric power and keratometric astigmatism.

Results: As first analyses show, rigid contact lenses were tolerated very well by (small) infants. Handling was unproblematic following training of the parents. Visual acuity was dependent on the group: Whereas bilateral cases (Group I) obtained visual acuities up to 1.0, the results decreased with increasing time before cataract removal (Group II > III). Group IV showed the least postoperative values. Refractive CL power to compensate lens removal decreases along time necessitating frequent prescriptions of adapted CL.

Conclusions: To our knowledge, this is the largest patient collective yet analysed. Rigid gas-permeable contact lenses are a safe tool to avoid amblyopia in children with congenital cataract. Provided good collaboration with the parents, these rigid lenses are a favourable alternative to soft lens therapy or intraocular lens implantation.
INTRASTROMAL VORICONASOL TREATMENT FOR FUNGAL INFECTION AFTER PENETRATING KERATOPLASTY

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Background: Decompensated keratoconus is one of the most common indications for Penetrating keratoplasty (PKP). Success of the procedure as well as the visual rehabilitation in such patient is often satisfying for both patient and surgeon, but still there are some risk for graft rejection as well as infections due to prolonged steroid treatment. We present a case report of fungal keratitis after PKP. Treatment of post-graft infections can be challenging, especially in case of fungal keratitis.

Methods: Case report. 44 years old female patient presented with preperforation of the cornea due to decompensated keratoconus grade IV and underwent PKP in our institution. The transplant was obtained from a donor age of 56 years, with endothelial cell count of 2937 cells/mm². Postoperatively patient was treated with topical steroid/antibiotics and artificial tears drops. At each visit BCVA was measured and full eye examination was performed.

Results: 6 months following PKP the graft was clear and transparent and the patient obtained the best BCVA of 0.8. Eleven months postoperatively, patient presented with persistant erosion of the transplant and asymptomatic white infiltrate at the graft-host interface. BCVA decreased to 0.25. Microbiological testing of her corneal swab revealed Candida species. Patient was treated with topical and systemic voriconasols, ciprofoxacin drops and chloramphenicol ointment which led to improvement of clinical picture but not to a full regresion of the infiltrate. At 22nd day of treatment patient recived intrastromal voriconasol injections after which complete resolution of corneal infiltrates occured and visual acuity increased to 0.9.

Conclusions: Fungal infections are rare infections seen after PKP. The asymtomatic and nonspecific clinical picture may postpone the diagnosis and consequently the treatment. Therefore, in peristent erosion of the transplant after PKP, or in cases of infiltrates on the transplant, one should consider fungal keratitis.
DEEP ANTERIOR LAMELLAR KERATOPLASTY VS. PENETRATING KERATOPLASTY FOR HERPES SIMPLEX KERATITIS SCAR
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Purpose: To compare outcomes of deep lamellar keratoplasty with penetrating keratoplasty for treating corneal scars caused by herpes simplex keratitis

Methods: Included were patients with corneal scarring caused by herpes simplex keratitis who underwent primary deep lamellar keratoplasty or penetrating keratoplasty and completed at least 12 months postoperative follow-up. There was no significant difference of corneal scarring and vascularization between the 2 groups before surgery. Excluded were patients with a past history of corneal perforation, nonprimary graft, non-herpes simplex-related corneal scars, and failure to complete a minimum of 12 months of postoperative follow-up. 8 eyes of 8 patients in the deep lamellar keratoplasty group and 18 eyes of 18 patients in the penetrating keratoplasty group met the inclusion criteria. Two groups were compared due to postoperative managements, recurrence of herpes simplex keratitis, graft rejection and graft survival rate

Results: Due to small number of patients in the study it was impossible to use serious statistical analysis. Penetrating keratoplasty group had more frequent episodes of herpes simplex recurrent keratitis, more graft rejection episodes and also graft failures. The clear graft survival rate in the deep lamellar keratoplasty group was higher than that in the penetrating keratoplasty group.

Conclusions: Deep lamellar keratoplasty seems to be preferable to penetrating keratoplasty for treating herpes simplex keratitis-induced corneal scarring with relatively healthy endothelium and with no history of perforation.
TRANSPLANTATION OF AMNIOTIC MEMBRANE IN CORNEAL ULCERS AND PERSISTENT EPITHELIAL DEFECTS
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Introduction: Corneal epithelium is known for quick process of auto renewal, in the case of minor injury or inflammation. When the normal healing of corneal epithelium is disabled, pathological conditions are manifested as persistent epithelium defects, corneal ulcers and recurring erosions, which can lead to significant vision loss. The most common indications for transplantation of amniotic membrane are persistent epithelial defects.

Methods: The prospective study included 21 patients with corneal ulcer (n=18) or recurring erosions (n=3) unresponsive to conventional treatment. We used corneal cells surrounding the defects and part of the amniotic membrane used for transplantation. As a control group we used cells of healthy donor corneas unsuitable for transplantation. The corneal cells and rest of transplanted amniotic membrane were cultivated at 370°C for 24 hours, then stored at -80°C in organ culture (Cornea Max). The concentration of IL-1α, TNFα and VEGF were measured using commercial available ELISA system. The concentration of IL-1ra, sTNF and VEGF-R were also measured in supernatant of amniotic membranes.

Results: In corneal cell samples we have detected 3.51 +/- 1.79 pg/ml of IL-1α, 64.27 +/- 31.53 pg/ml TNFα and 209.07 +/- 201.82 pg/ml of VEGF. Levels of all 3 investigated cytokines were significantly higher as compared to controls (p ≤ 0.005). Clinically all ulcers and erosions healed. Visual acuity improved in 15 patients. Amniotic membrane contained 775.69 +/- 613.98 pg/ml of IL-1ra, 0.036 +/- 0.033 pg/ml of sTNF and 175.01 +/- 166.63 pg/ml of VEGF-R.

Conclusions: The use of AM in patients with corneal ulcers and persistent epithelial defects, in whom conservative treatment had failed, seems a reasonable option, having in mind that IL-1α, TNFα and VEGF levels are increased in such eyes, and AM secretes their natural antagonists IL-1ra, sTNF and VEGF-R.
CORECTION OF POST-KERATOPLASTY ASTIGMATISM WITH CONTACT LENSES

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Purpose: To describe the fitting of patients with high or irregular astigmatism following penetrating keratoplasty with Rose K2 Post Graft contact lenses and to answer the question whether or not contact lenses with special back surface design can improve visual acuity in complex cases after penetrating keratoplasty.

Methods: 23 eyes were included. There were thirteen female and ten male patients. They were fitted with Rose K2 Post Graft contact lenses a special back surface that was designed for optical rehabilitation after penetrating keratoplasty. The patients were followed up for an average period of 12.3 months. Lens tolerance and corrected visual acuity were evaluated and compared with that corrected with spectacles.

Results: Mean patient age was 28±7 years (range: 16 to 41 years). Mean postoperative spherical equivalent was 3.50±2.2 D and mean refractive cylinder was 4.20±2.50 D. Average postoperative BSCVA was 0.45. The visual acuity with Rose K2 Post Graft contact lenses was significantly improved in nearly all eyes with an average increase of 4.7 lines accompanied by good contact lens tolerance and satisfactory contact lens fit. No noticeable complications were observed.

Conclusions: Rose K2 Post Graft with special back surface design can improve visual results and lens tolerance, and minimise problems in contact lens fitting. This is in favour of contact lenses as an alternative to surgical procedures for correction of high or irregular astigmatism after penetrating keratoplasty.
MANAGEMENT OF ASTIGMATISM IN CATARACT SURGERY WITH TORIC IOL IN A PATIENT WITH KERATOCONUS: A CASE REPORT
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Purpose: Keratoconus generates irregular corneal astigmatism and myopia. We report a case of a 68-year-old man with cataract and stable keratoconus on both eyes where the cataract surgery with implantation of a toric monofocal intraocular lens (IOL) was a method for correction of high myopic astigmatism and spherical ametropia.

Methods: 68-year-old man with cataract, clear central cornea and stable keratoconus grade 2 on both eyes (Pentacam, Oculus) underwent phacoemulsification and implantation of toric IOL, AT LISA 809M. The second eye was operated 3 months after the first one. Preoperative manifest refraction was -1.0D of sphere and -3.00/130°Dcyl on right eye and -1.0D of sphere and -2.50/160°Dcyl on the left eye. Postoperative visual outcomes, IOL position and corneal topography were evaluated over 6 months.

Results: The distant uncorrected visual acuity increased from 0.3 on right eye and 0.4 on left eye to 0.8 and 0.9, while best corrected visual acuity increased from 0.5 and 0.6 to 1.0, with correction of +0.50 sphere on both eyes, respectively six months after surgery. Refractive astigmatism was completely corrected on both eyes. No postoperative vision-threatening complications or IOL misalignment occurred during follow-up time.

Conclusions: Cataract surgery with implantation of toric IOL in patients with stable keratoconus and clear central cornea is reasonable and safe treatment option for correction of spherical and cylindrical errors and improvement of vision.
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